

Review

Nanotransducers for wireless neuromodulation

Xiuying Li,¹ Hejian Xiong,¹ Nicholas Rommelfanger,^{2,4} Xueqi Xu,¹ Jonghae Youn,¹ Paul A. Slesinger,³ Guosong Hong,^{4,5,*} and Zhenpeng Qin^{1,6,7,8,*}

SUMMARY

Understanding the signal transmission and processing within the central nervous system is a grand challenge in neuroscience. The past decade has witnessed significant advances in the development of new tools to address this challenge. Development of these new tools draws diverse expertise from genetics, materials science, electrical engineering, photonics, and other disciplines. Among these tools, nanomaterials have emerged as a unique class of neural interfaces because of their small size, remote coupling and conversion of different energy modalities, various delivery methods, and mitigated chronic immune responses. In this review, we will discuss recent advances in nanotransducers to modulate and interface with the neural system without physical wires. Nanotransducers work collectively to modulate brain activity through optogenetic, mechanical, thermal, electrical, and chemical modalities. We will compare important parameters among these techniques, including the invasiveness, spatiotemporal precision, cell-type specificity, brain penetration, and translation to large animals and humans. Important areas for future research include a better understanding of the nanomaterials-brain interface, integration of sensing capability for bidirectional closed-loop neuromodulation, and genetically engineered functional materials for cell-type-specific neuromodulation.

A grand challenge in neuroscience is to understand signal transmission and processing in the nervous system. This has attracted significant interest of researchers from diverse disciplines such as genetics, materials science, engineering, and imaging, and has led to significant advances in developing new tools to address this challenge in the past decades. These advances include making smaller and more flexible electrodes to implant in local brain regions,^{1,2} developing optogenetics to optically address individual neurons,³ and creating pharmacological approaches such as caged compounds to selectively stimulate signaling in defined cell populations.⁴ These techniques come with their unique advantages and disadvantages, such as cell-specific neuron stimulation or inhibition but limited light penetration for optogenetics and caged compounds, reliable electrical modulation of local brain circuits but immune response and scar formation from large metal electrodes,⁵ and the requirement of genetically encoded non-native proteins for optogenetics. An ideal brain modulation technique would allow non-invasive control of neuron activities in target areas of the brain with high spatiotemporal resolution, and it should be able to translate into safe and effective use in large mammals, non-human primates, and humans.^{4,6} Such neuromodulation techniques with high spatiotemporal resolution, deep brain penetration, minimal invasiveness, and negligible inflammatory response are still highly desired.

Progress and potential

In the last 3–5 years, there have been significant interest and advances in the transformative potential of nanotransducers for neuromodulation. Many nanotransducer-based neuromodulation techniques have been developed recently, including sono-optogenetics enabled by mechanoluminescent nanoparticles and semiconducting polymer nanoparticles-mediated photoelectrical neuromodulation. Nanotransducers have demonstrated their clinical potentials, such as Au nanorods for restoring light sensitivity and alleviating ventricular arrhythmias. This review provides the current state of the art for nanotransducer-enabled neuromodulation and discusses the latest major advances and debates in using nanotransducers to modulate and interface with the nervous system. Future directions include a better understanding of nanomaterials-brain interface and development of the next generation of nanotransducers with sensing ability to bidirectionally communicate with local environment.

Nanomaterial transducers (nanotransducers) have emerged as a unique neuromodulation interface with the brain in the past 5–10 years, in order to overcome limitations of current neuromodulation techniques. The major advantages of nanotransducers is their small size and promise to greatly reduce immune response compared with large electrodes.⁷ Nanotransducers have the capability to wirelessly transduce external electric fields, light, magnetic fields, or ultrasound waves into a local signal (light, thermal, mechanical, electrical, or chemical) in the region of interest (Figure 1A). For example, light can be converted into chemical signal,⁸ heat,⁹ mechanical force,¹⁰ and electric current or voltage by optical nanotransducers,¹¹ such as semiconducting polymer nanoparticles. Similarly, magnetic nanotransducers can locally convert a magnetic field into electric current or voltage,¹² heat,¹³ or mechanical force.¹⁴ The local stimulation leads to changes in the neuron activity through various mechanisms, including stimuli-responsive ion channels, G protein-coupled receptors (GPCRs), or transient membrane capacitance changes. The capability of nanotransducer-enabled neuromodulation is largely dependent on the nanotransducer properties and the interaction of nanotransducers with the brain (nano-brain interface).

In this review, we provide an overview for nanotransducer-enabled neuromodulation by focusing on two important aspects: innovations in nanotransducers for wireless neuromodulation and the nano-brain interface. Briefly, we will summarize recent advances in nanotransducer-enabled neuromodulation, mainly including optogenetic, mechanical, thermal, electrical, and chemical neuromodulation. The nanotransducer design, the working principle, and pros and cons of each approach will be covered. We will also discuss molecular and nanomaterials sensors for neurotransmitters and imaging of nanotransducers in the brain. Next, we will discuss the nano-brain interface, including approaches for delivery of nanomaterials to the central nervous system (CNS), the interaction between nanomaterials and neurons/glia cells, and nanomaterials transport and clearance in the CNS. Finally, we will outline current challenges toward developing the next generation of neuromodulation techniques and discuss some promising new nanotransducers for neuromodulation. Advances in nanotransducer-enabled neuromodulation techniques will contribute to understanding the signaling transmission and processing within the CNS and treatment of CNS disorders.

INNOVATIONS IN NANOTRANSDUCERS FOR WIRELESS NEUROMODULATION

There are five main working mechanisms for nanotransducer-enabled neuromodulation, including optogenetic, thermal, mechanical, electrical, and chemical neuromodulation (Figure 1 and Tables 1 and 2). The overarching idea is that nanomaterials transduce external energy in the form of light, magnetic field, or ultrasound into local energy that may be either the same or a different modality as the external energy input. The transduced energy directly interfaces with intrinsic or engineered molecular mechanisms to produce a cellular response and modulate neuron activity. These mechanisms include light-gated ion channels for optogenetic modulation, temperature-gated ion channels or temperature-dependent membrane capacitance change for thermal modulation, mechanosensitive ion channels or altered membrane capacitance for mechanical modulation, voltage-gated ion channels for electrical modulation, and ligand-gated ion channels and GPCRs for chemical modulation. For each mechanism, we will discuss the working principle and nanotransducers that have been reported in the last 5–10 years. We will compare the nanotransducer design, invasiveness, spatiotemporal precision, cell-type

¹Department of Mechanical Engineering, The University of Texas at Dallas, Richardson, TX 75080, USA

²Department of Applied Physics, Stanford University, Stanford, CA 94305, USA

³Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

⁴Wu Tsai Neurosciences Institute, Stanford University, Stanford, CA 94305, USA

⁵Department of Materials Science and Engineering, Stanford University, Stanford, CA 94305, USA

⁶Department of Bioengineering, The University of Texas at Dallas, Richardson, TX 75080, USA

⁷Department of Surgery, The University of Texas at Southwestern Medical Center, Dallas, TX 75080, USA

⁸The Center for Advanced Pain Studies, The University of Texas at Southwestern Medical Center, Dallas, TX 75080, USA

*Correspondence: guosongh@stanford.edu (G.H.), zhenpeng.qin@utdallas.edu (Z.Q.)

<https://doi.org/10.1016/j.matt.2021.02.012>

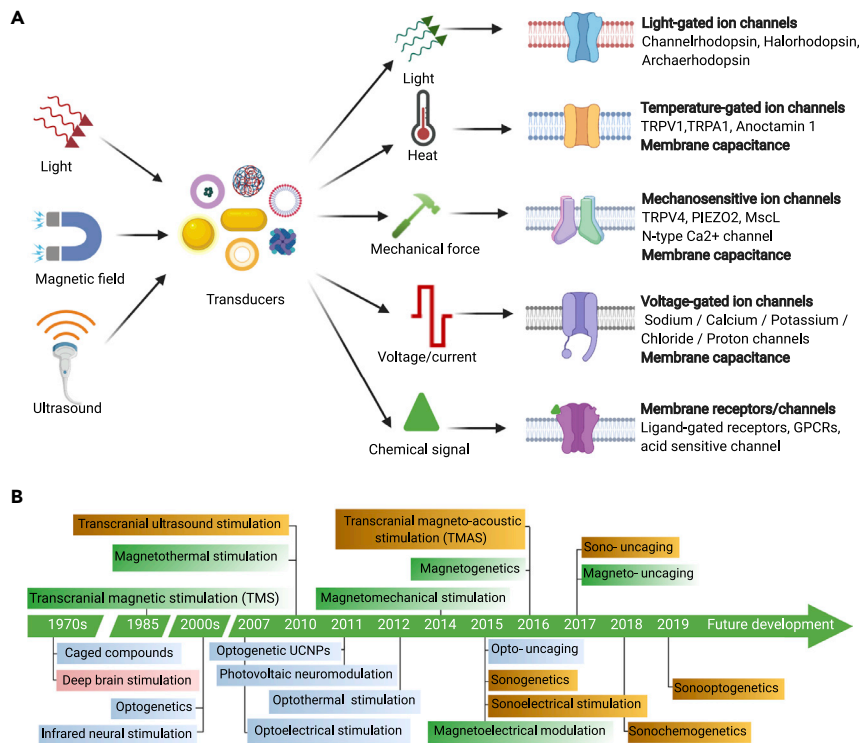


Figure 1. Working principles and evolution of nanotransducer-enabled wireless neuromodulation

(A) Working principles of nanotransducers for neuromodulation and (B) representative developments in nanotransducer-based and related neuromodulation techniques. Yellow for sono-neuromodulation, green for magneto-neuromodulation, cyan for optical neuromodulation, and pink for electrical neuromodulation. References for each technique: caged compounds,¹⁵ deep brain stimulation,¹⁶ transcranial magnetic stimulation,¹⁷ optogenetics,^{18,19} infrared neural stimulation,²⁰ optoelectrical stimulation,²¹ magnetothermal modulation,²² transcranial ultrasound stimulation,²³ optogenetics using upconversion nanoparticles (UCNPs),²⁴ photovoltaic neuromodulation,²⁵ optothermal stimulation,²⁶ magnetomechanical stimulation,²⁷ opto-uncaging,²⁸ sonogenetics,²⁹ sonoelectrical stimulation,³⁰ magneto-electrical modulation,¹² magnetogenetics,^{31,32} transcranial magneto-acoustic stimulation,³³ magneto-uncaging,³⁴ sono-uncaging,³⁵ sonochemogenetics,³⁶ sono-optogenetics.³⁷

specificity, brain penetration, and potential translation to large animals and humans. We will also discuss a closely related topic on molecular and nanomaterials sensors for neurotransmitters and imaging of nanotransducers in the brain.

Optogenetic neuromodulation

Working principle

First demonstrated in 2005, optogenetics has since transformed neuroscience research and entered clinical translation.¹⁸ Optogenetics uses light to control genetically engineered neurons that express light-sensitive ion channels and pumps (e.g., channelrhodopsin, halorhodopsin, and archaelhodopsin). These light-sensitive channels and pumps allow charged ions to flow across the cell upon illumination in order to stimulate or inhibit neuron activity. Taking advantage of genetic techniques to transfect specific cell types, optogenetics has allowed cell-specific modulation in freely moving mammals. Optogenetics also offers high spatiotemporal resolution, where light can be focused onto specific cells using one photon or two-photon techniques with millisecond control of neuron stimulation or inhibition.³ Despite advances in the development of light-sensitive ion channels and pumps,

Table 1. Summary of recent advances in nanotransducers for wireless neuromodulation

External stimulus	Local stimulus	Brain interface	Nanotransducers	Pros (+) and cons (-)	References
Light	light	ectopic opsins	UCNPs	(+) high spatiotemporal resolution (+) improved tissue penetration (-) required genetically encoded non-native proteins (-) invasiveness (-) poor water solubility of UCNPs	24,38,39
		ectopic opsins	mechanoluminescent nanoparticles	(+) high spatiotemporal resolution (+) through intact scalp and skull (+) deep tissue penetration (-) required genetically encoded non-native proteins	37
	heat	membrane capacitance	Targeted GNPs, Au nanorods, 3D fuzzy graphene	(+) does not require genetically encoded non-native proteins (+) high spatiotemporal resolution (-) limited tissue penetration	40,41
		TRPV1 channel	targeted Au nanorods, semiconducting polymer nanobioconjugates	(-) requires genetically encoded non-native proteins (+) high spatiotemporal resolution (-) limited tissue penetration	9,42,43
		PAR-2	targeted GNPs	(+) does not require genetically encoded non-native proteins (+) nanoscale localized heating (+) high spatiotemporal resolution (-) limited tissue penetration	44
	mechanical force	membrane capacitance	azobenzene compound (Ziapi2)	(+) high spatiotemporal resolution (+) does not require genetically encoded non-native proteins (-) limited tissue penetration	10
	electrical signal	voltage-gated sodium channel	QDs, metal and semiconducting organic nanocrystals, organic electrolytic photocapacitors, semiconducting polymer nanoparticles, GNP-decorated titania nanowire arrays, silicon	(+) high spatiotemporal resolution (+) does not require genetically encoded non-native proteins (-) limited tissue penetration	11,45–47
	chemical signal	neurotransmitter/neuropeptide receptors	Au nanoshell tethered liposomes, DNA nanocages, polypyrrole nanoparticles, azobenzene	(+) high spatiotemporal resolution (+) does not require genetically encoded non-native proteins (+) can be extended to different biomolecules	48
		inositol trisphosphate receptor	GNP-coated liposomes and mechanosensitive nanovesicles	(-) passive cargo leak in vivo	8,49

(Continued on next page)

Table 1. Continued

External stimulus	Local stimulus	Brain interface	Nanotransducers	Pros (+) and cons (-)	References
Magnetic field	heat	TRPV1	superparamagnetic ferrite nanoparticles, MNPs, magnetogenetic actuators	(+) deep tissue penetration (+) allows for chronic stimulation (-) limited spatiotemporal resolution (-) potential tissue damage upon long-term exposure (-) slower temporal dynamics compared with optothermal modulation (-) restricted motion of the animals due to magnetic coil	7,13,22,50
		TMEM16A	membrane-bound MNPs		51
	mechanical force	TRPV4/ PIEZO2	magnetite nanodiscs, ferritin protein fused to TRPV1/TRPV4	(+) deep tissue penetration (+) non-invasiveness (-) limited spatiotemporal resolution (-) restricted motion of the animals due to magnetic coil	31,52,53,54
		N-type mechanosensitive Ca^{2+} channel	MNPs		14
	electrical signal	Ca^{2+} and Na^{+} voltage-gated channels	$CoFe_2O_4$ - $BaTiO_3$ nanoparticles	(+) deep tissue penetration (+) non-invasiveness (+) does not require genetically encoded non-native proteins (-) limited spatiotemporal resolution (-) restricted motion of the animals due to magnetic coil	12
Ultrasound	chemical signal	dopamine D2 receptor	magnetic hydrogels	(+) lower dose of nanotransducers compared with magnetothermal modulation (+) deep tissue penetration (+) does not require genetically encoded non-native proteins (-) slower release of cargo compared with opto-uncaging (-) restricted motion of the animals due to magnetic coil	55
		TRPV1 / acid-sensing ion channels	iron oxide MNPs, magnetoliposomes		34,56,57
	mechanical force	engineered TRPV4, Prestin or MscL	N. A	(+) deep tissue penetration (+) moderate spatiotemporal resolution (-) requires head-mounted ultrasound transducer (-) May induce neuronal activity change via indirect mechanism	29,58–60
		TRPV4	semiconducting polymer nanoparticles		61
	electrical signal	Ca^{2+} and Na^{+} voltage-gated channels	Piezoelectric barium titanate nanoparticles	(+) deep tissue penetration (+) does not require genetically encoded non-native proteins (+) non-invasiveness (+) moderate spatiotemporal resolution (-) requires head-mounted ultrasound transducer	30,62
	chemical signal	GABA _A receptor	PFCs containing nanoemulsions, nanodroplets with microbubble contrast agent, and PFB gas	(+) deep tissue penetration (+) does not require genetically encoded non-native proteins (+) non-invasiveness (-) requires head-mounted ultrasound transducer (-) slower release of cargo compared with opto-uncaging	4,36,63

Table 2. Quantifying nanotransducer-based neuromodulation

Main Category	Parameters		Quantity	Refs
Nanotransducers	size	Liposomes	100–800 nm	4,8,49,56
		GNPs/GNRs	10–80 nm	9,64,65
		polymeric nanoparticles	25–800 nm	61,66
		magnetic nanoparticles	10–1000 nm	7,13,22,51,67
		Microbubbles/nanodroplets	200–2000 nm	29,63
		Quantum dots	2–10 nm	45
	diffusion coefficient	0.3% agarose	cortex	68
		35 nm QD	$1.9 \times 10^{-7} \text{ cm}^2/\text{s}$	$1.7 \times 10^{-9} \text{ cm}^2/\text{s}$
		70 kDa dextran (~14 nm)	$4.7 \times 10^{-7} \text{ cm}^2/\text{s}$	$6.5 \times 10^{-8} \text{ cm}^2/\text{s}$
		3 kDa dextran (~3 nm)	$2.22 \times 10^{-6} \text{ cm}^2/\text{s}$	$5.3 \times 10^{-7} \text{ cm}^2/\text{s}$
	retention time	polystyrene nanoparticles	>3 weeks	69
		magnetic nanoparticles	1 month	7
	energy conversion	upconversion quantum efficiency	UCNPs: 0.1%–9%	70
	penetration depth in brain	light	blue-green light, 0.5 mm; red light, 1.5 mm	71
			near infrared, 3.5 mm	
		magnetic field	>1 cm, frequency dependent	72
		ultrasound	>1 cm, frequency dependent	73
Brain interface	synapse	number (human)	$\sim 10^{13}\text{--}10^{15} (\approx 109/\text{mm}^3)$	74
		synaptic vesicle volume	$\sim 10^{-5} \mu\text{m}^3$	74
		width of synaptic cleft	20 nm	75
		membrane thickness	~4 nm	74
	CSF	volume	human	125–150 mL
			mouse	0.035 mL
		turnover time	human	4.8 h
			mouse	1.8 h
	extracellular space	space	50–1,000 nm, heterogeneous	78
		main composition	hyaluronan, heparan sulfate, chondroitin sulfate, collagen, fibronectin, laminin, tenascin-R	79
Optogenetics	opsin	activation wavelength	470–630 nm	80
		activation threshold	0.4–1 mW/mm ²	80
		time to ON (ms)	50–200 ms	81
		Time to peak (ms)	300–2,000 ms	81
		transducer concentration	NaYF ₄ :Yb/Tm@SiO ₂ UCNPs	200 mg/mL (5×10^{11} particle/dose)
		per dose	ZnS:Ag,Co@ZnS nanoparticles	8 mg/mL (10^{13} particle/dose)
			β -NaYF ₄ /Yb/Er@ β -NaYF ₄ UCNPs	25 mg/mL (5×10^{11} particle/dose)

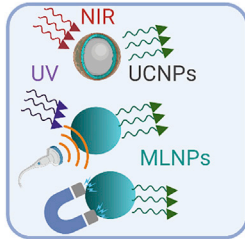
(Continued on next page)

Table 2. Continued

Main Category	Parameters		Quantity		Refs
Thermal modulation	TRPV1	activation threshold	~40°C		83
		rise time	0.75 ms		83
		transducer dose	superparamagnetic nanoparticles	10 nM	22
	Ano1/TMEM16A		magnetic nanoparticles	8×10^{11} particles/dose	7
		activation threshold	~29°C		84
		time to ON	250 ms		85
Mechanical modulation	TRPV4	transducer dose	magnetic nanoparticles	10 µg/mL	51
		activation time	0.1–1 s		61
		transducer dose	photoacoustic nanoparticles	1 mg/mL	61
	PIEZO2	activation threshold	5–10 mmHg		86
		inactivation time	~10 ms		87
	MscL	activation threshold	~97 mmHg		59
Electrical modulation	transient voltage		20–110 mV		88
	time constant		50 µs to 5 ms		88
Chemical modulation	release time		<1 ms to mins		8
	chemical dose	GABA _A receptor agonist (propofol)	1 mg/mL (8×10^{15} particle/dose)		4
		DREADD agonist (clozapine N-oxide, or CNO)	5 mg/mL		56
		D ₁ receptor antagonist (SCH-23390)	2.5 mg/mL		56
		intracellular second messenger (IP ₃)	0.31 mg/mL (concentration inside liposomes)		8
		GABA _A receptor agonist (pentobarbital)	0.022 ± 0.03 mg/mL		63

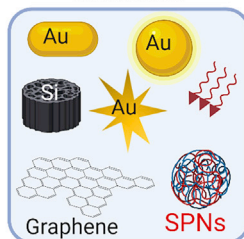
A Optogenetics

UCNPs and MLNPs

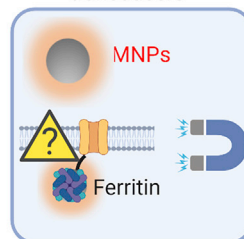


B Thermal modulation

Optothermal transducers

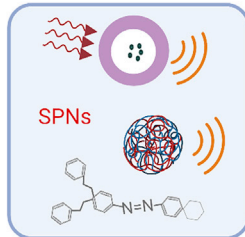


Magnetothermal transducers

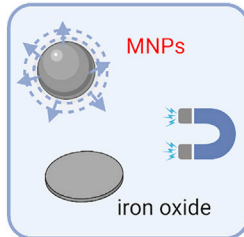


C Mechanical modulation

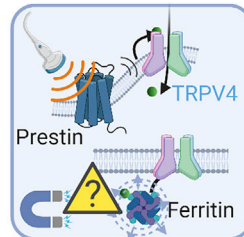
Optomechanical transducers



Magnetomechanical transducers

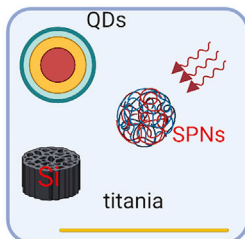


Genetically encoded transducers



D Electrical modulation

Optoelectronic transducers



Magnetoelectric transducers

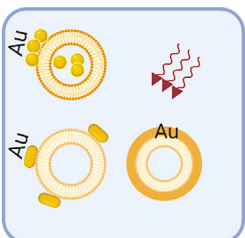


Piezoelectric transducers

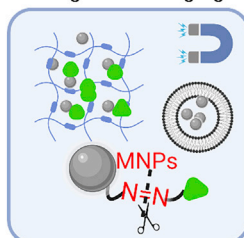


E Chemical modulation

Opto- uncaging



Magneto- uncaging



Sono- uncaging

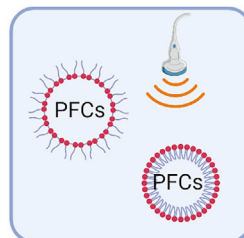


Figure 2. Collection of recently reported nanotransducers for neuromodulation

(A) Nanotransducers for optogenetics;^{38,39} (B) optical^{9,40–44,64} and magnetic transducers^{7,13,22,50,51} for thermal modulation; (C) nanotransducers for mechanical modulation (left, optomechanical transducers^{10,61,92}; middle, magnetomechanical transducers;^{14,52} right, genetically encoded transducers^{31,32,53,54,93}); (D) nanotransducers for electrical modulation (left, optoelectronic transducers^{11,45–47,94,95}; middle, magnetoelectric transducers^{12,96}; right, piezoelectric transducers^{30,62}); (E) nanotransducers for chemical modulation (left, transducers for opto-uncaging^{8,48,49}; middle, transducers for magneto-uncaging^{34,55–57}; right, transducers for sono-uncaging^{4,35,36}). MLNPs, mechanoluminescent nanoparticles; SPNs, semiconducting polymer nanoconjugates; MNPs, magnetic nanoparticles; PFCs, fluorocarbons (e.g., perfluorobutane [PFB] and perfluoropentane [PFP]).

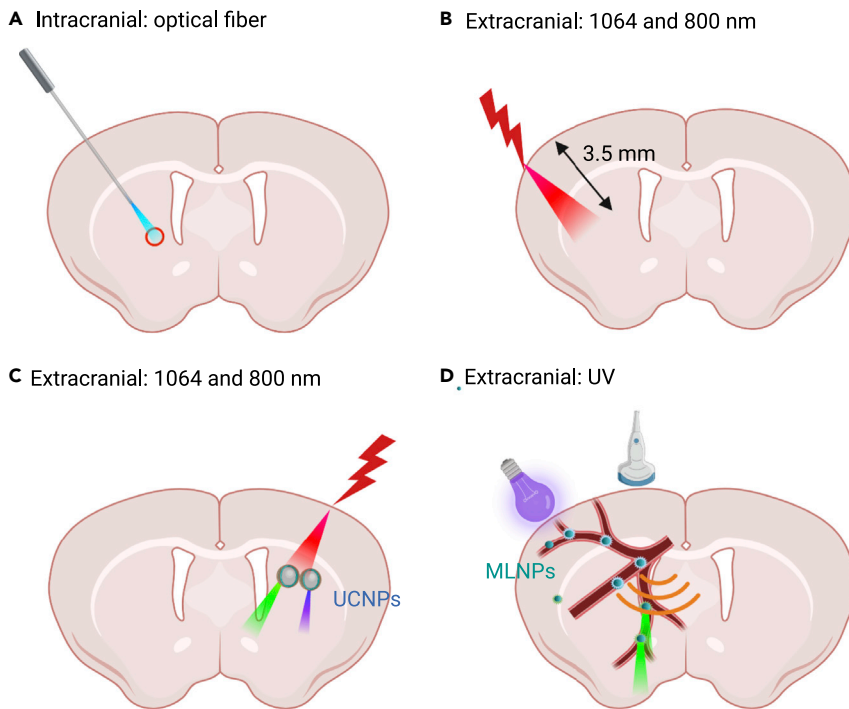


Figure 3. Light delivery for neuromodulation

(A) Intracranial light delivery via optical fiber; (B) extracranial delivery of NIR light, the penetration depth of NIR light in the brain is ~ 3.5 mm⁹⁷; (C) UCNPs convert NIR light to visible light; (D) MLNPs convert UV light into green light upon ultrasound activation.

such as the development of red-shifted opsins,⁸⁹ the potential of optogenetics is limited by the tissue penetration of visible light that is required to activate the engineered opsins. Opsins vary in light sensitivity (~ 1 mW/mm²)⁸¹ and kinetics (millisecond), and mutations that increase the light sensitivity of opsins often negatively affect the kinetics of the channel.⁹⁰ For example, channelrhodopsin-2 shows peak responses with a light intensity of 1.10 mW/mm², almost five times lower than another channelrhodopsin variant, ChETA. However, the channelrhodopsin-2 engineered channels open within 1.21 ms, slower than ChETA engineered channels, which open within 0.86 ms.⁹⁰

Nanotransducers

Recently, two promising nanotransducers have been developed to overcome the limitations of visible light penetration into tissue (Figures 2A and 3). The first technique involves the use of upconversion nanoparticles (UCNPs), which convert infrared or near-infrared (NIR) wavelengths into the visible light spectrum. NIR light encounters reduced scattering and absorption in tissue compared with visible light, enabling deeper tissue penetration. UCNP-enabled neuromodulation was first proposed in 2011²⁴ and was later used to stimulate neurons in culture and in living animals. Chen et al.³⁸ reported that channelrhodopsin-2-expressing neurons released dopamine in the ventral tegmental area (~ 4 mm depth), upon NIR irradiation of NaYF₄ nanocrystals doped with Yb³⁺/Tm³⁺. While inducing theta oscillations via activating inhibitory neurons in the medial septum, the released dopamine also silenced seizure through inhibiting hippocampal excitatory cells. However, several limitations of UCNPs remain. The upconversion quantum efficiency remains low (0.1%–9%)⁷⁰ and often requires a high NIR laser fluence. This high laser fluence

may introduce additional effects such as brain heating that may alter neural activity.⁹¹

Another advance for non-invasive optogenetic neuromodulation is the development of mechanoluminescent nanoparticles. Mechanoluminescent nanoparticles use tissue-penetrating focused ultrasound to trigger light emission and subsequent optogenetic modulation, called sono-optogenetics.³⁷ These nanoparticles can be injected systemically and charged by 400-nm light during circulation in superficial blood vessels. Afterward, nanoparticles can be activated by 1.5-MHz focused ultrasound stimulation to emit 470-nm light repetitively in the brain for optogenetic modulation. This approach combines the non-invasiveness and the tissue penetration of ultrasound stimulation with the high spatiotemporal control of optogenetics. With further work in materials development for mechanoluminescence, this concept may provide a clinically viable route for non-invasive neuromodulation in deep brain regions. Specifically, by preparing mechanoluminescent materials with a sensitive response to higher-frequency ultrasound, spatially precise sono-optogenetic neuromodulation with a higher resolution may be achieved. In addition, materials with different wavelengths of mechanoluminescence may provide multiplexed and spatiotemporally resolved excitation and inhibition patterns in the same animal's brain via a non-invasive interface.

Thermal neuromodulation

Working principle

Local temperature changes can modulate neuron activity by changing membrane capacitance or activating temperature-gated ion channels, such as the transient receptor potential cation channel subfamily V member 1 (TRPV1), the transient receptor potential cation channel ankyrin 1 (TRPA1), and the temperature-gated chloride channel anoctamin 1 (TMEM16A). These temperature-gated ion channels change their open probabilities within different temperature ranges to detect temperature from 29°C to 40°C (Table 2).⁸⁴ TRPV1 responds to temperature change within milliseconds and can be modeled by a two-state model.⁸³

Nanotransducers

Nanotransducers have been reported to generate thermal energy from external light and magnetic fields (Figure 2B). The first type involves optothermal nanotransducers that convert light into heat, such as plasmonic nanoparticles,²⁶ semiconducting polymer nanoconjugates,^{66,98,99} and silicon nanomaterials. The stimulation mechanisms of optothermal nanotransducers can be categorized into three different groups. The first mechanism involves thermally sensitive ion channels, such as TRPV1. There are several reports that show heating neurons by Au particles will inhibit/excite neurons.^{9,64,65} Surface-engineered plasmonic nanoparticles, such as Au nanorods, can activate single neuronal cells upon light illumination.⁹ Highly localized heat generated by Au nanoparticles (GNPs) induced Ca^{2+} influx by activating TRPV1, a thermosensitive channel. Other than NIR-responsive plasmonic nanoparticles, organic materials with higher biocompatibility (e.g., semiconducting polymer-based nanobioconjugates⁶⁶) have also been explored for thermal neuromodulation. Semiconducting polymers have large delocalized π -conjugated backbones that efficiently absorb NIR light with high optothermal conversion efficiency. Lyu et al.⁴² developed a series of NIR-responsive semiconducting polymer nanobioconjugates with higher optothermal conversion efficiency than that of Au nanorods. When excited by an 808-nm laser, targeted SPN_{sbc} specifically and rapidly activated the TRPV1 in a hybrid mouse neuroblastoma/rat dorsal root ganglion neuron cell line (ND7/23 cells), inducing significant intracellular Ca^{2+} influx. Built upon the

semiconducting polymer nanobioconjugates, macromolecular infrared nanotransducers for deep brain stimulation have been demonstrated to enable through-scalp neuromodulation in freely moving mice.¹⁰⁰ Owing to the minimized tissue attenuation of 1064-nm NIR-II irradiation, macromolecular infrared nanotransducers for deep-brain stimulation (MINDS) have enabled neuromodulation in the motor cortex and ventral tegmental area of naturally behaving mice with wide-field NIR-II illumination at a low power density, in contrast to a fiber-tethered interface required for conventional optogenetic neuromodulation. This approach can remotely apply light to stimulate individual animals in the same arena, such as the IntelliCage, thus may enable simultaneous neuromodulation of multiple socially interacting animals. In addition to exciting neurons, optothermal transducers can also inhibit neurons via a transmembrane thermosensitive potassium channel; e.g., TREK-1. Thermal stimuli activates TREK-1 to allow hyperpolarizing K⁺ currents, thus reducing neuron excitability.¹⁰¹ Light irradiation of Au nanoparticles generated heat, and inhibited the electrical activity, which was fully restored when stimulus light was removed. The degree of inhibition was precisely modulated by tuning the laser intensity.⁶⁵ Ye et al.⁴⁰ showed that neuron-targeted Au nanorods inhibited the neural activity of the left stellate ganglion, alleviating myocardial ischemia-induced ventricular arrhythmias in a canine model.^{40,65} This approach is promising for restoring normal firing of a hyperactive neuronal network and providing therapeutic effects for some brain disorders, such as epilepsy and Parkinson disease.

The second stimulation mechanism involves thermally driven membrane capacitance change. Shapiro et al.¹⁰² demonstrated that rapid infrared heating of water can excite cells by altering the electrical capacitance of cell membrane and generating depolarizing capacitive currents. This mechanism leads to infrared neural stimulation *in vivo*, which similarly uses pulsed infrared light (1,400–2,000 nm) to create transient temperature increase in neurons and induce neuron firing.¹⁰³ Several applications of infrared neural stimulation have been reported in recent years, including altering GABAergic neurotransmission⁹⁷ and activating visual cortex¹⁰⁴ and auditory neurons.¹⁰⁵ In addition to leveraging the heating of water alone, optothermal transducers that convert pulsed light into heat (such as Au nanoparticles) can also change membrane capacitance, depolarizing the cell and eliciting an action potential. Fuzzy graphene⁴¹ has also been explored as an organic optothermal transducer for nongenetic stimulation. When used for thermal neuromodulation, silicon nanowire-templated 3D fuzzy graphene requires activation laser energies lower than 100 nJ.⁴¹ Although more *in vivo* studies are needed to validate the efficiency of these organic optothermal nanotransducers for nongenetic thermal neuromodulation, these studies demonstrate promising alternative nanotransducers for optothermal neuromodulation.

The last mechanism involves a new technique called molecular hyperthermia, which uses plasmonic Au nanoparticles to target specific proteins, such as endogenous membrane receptors, and generates intense nanoscale heating upon nanosecond pulse excitation to inactivate targeted protein molecules. As a test case, molecular hyperthermia was demonstrated to transiently inactivate protease-activated receptor 2 (PAR2), an important G protein-coupled receptor for chronic pain sensitization.⁴⁴ Molecular hyperthermia with high spatiotemporal resolution can selectively and remotely manipulate protein activity and cellular behavior. This technique with a timescale of nanoseconds and a length scale of nanometers is different from traditional hyperthermia and did not induce global tissue heating. The photo-inactivation of membrane receptors is transient and lasts 6–8 h, because cells

have a mechanism to recycle inactivated receptors and synthesize new membrane receptors.

In addition to optothermal nanotransducers, the second type of thermal neuromodulation involves magnetothermal nanotransducers, which generate heat when exposed to alternating magnetic fields.⁶⁷ Magnetic hyperthermia has been studied since the 1960s to remotely treat cancer by local injection of magnetic nanoparticles and stimulation using external magnetic fields, and several companies, including MagForce, are actively pursuing clinical translation. The idea of magnetothermal stimulation of neurons expressing TRPV1 was first demonstrated in 2010²² and subsequently shown to allow wireless deep brain stimulation in behaving mice.¹³ Short 10-s magnetic field stimulation pulses heated magnetic particles, enabling a rapid rise in median temperature up to 43°C with a maximum increase to 45°C. During the subsequent 50-s rest time, the tissue cooled back down to 37°C.⁶⁴ This short intermittent exposure to a magnetic field induced neural activation and prevented harmful extended heating. Magnetic nanoparticles bound to TRPV1 on the neuronal membrane activated neurons expressing TRPV1 upon alternating magnetic field stimulation with high temporal control.¹³ The magnetothermal stimulation applied in the striatum resulted in rotation around the body axis in freely moving mice.¹³ The duration of the behavior was highly correlated with the duration of alternating magnetic field application. The same technique has also been used to silence targeted neurons *in vitro* with the chloride channel anoctamin 1.⁵¹ These studies suggest that magnetothermal nanotransducers are promising *in vivo* tools for remote, transient neuronal silencing for both therapeutic application and fundamental research. It is worth noting that magnetothermal modulation involves collective heating of injected magnetic nanoparticles that result in local brain tissue heating, rather than localized heating of individual nanoparticles, as demonstrated in multiple physical analyses.¹⁰⁶

To further advance thermal modulation into the next stage of development and translation, there are several key questions to be addressed. First, it remains unclear whether the repeated heating up to 43°C–45°C, although short in duration (several seconds), causes any deleterious effects on neurons and local brain tissue for long-term use. As there is a small temperature window for physiological function and there are many processes dependent on temperature (e.g., blood flow, protein function), potential deleterious effects must be carefully examined. Second, off-target heating may occur as a result of heat diffusion, which may trigger associated circuit (off-target) neural responses.⁹¹ Therefore, this technique requires methods to confine the heat in the localized regions.

Mechanical neuromodulation

Working principle

Local mechanical force can modulate membrane capacitance or activate mechanosensitive channels, including transient receptor potential cation channel subfamily V member 4 (TRPV4), piezo-type mechanosensitive ion channel component (PIEZO) channels, N-type mechanosensitive Ca²⁺ channel, and mechanosensitive channel of large conductance (MscL) (Figure 2C). PIEZO1 can be activated in about 10 ms⁸⁷ with a threshold of 97 mm Hg,⁵⁹ while TRPV4 responses to mechanical stimuli are within 0.1–10 s⁶¹ (Table 2).

Nanotransducers

Generation of local mechanical force can be introduced by external magnetic, ultrasound, and optical energy. Transcranial activation of photoacoustic transducers is an

emerging strategy for neuromodulation.⁹² Huang et al.⁶¹ used targeted photoacoustic nanotransducers based on semiconducting polymer nanoparticles to stimulate neural activities. Nanosecond laser pulse in the second NIR region (3 ns, 3.3 kHz, 21 mJ/cm²) of photoacoustic nanotransducers induced localized acoustic waves to activate neurons. Upon light stimulation, photoacoustic nanotransducers were able to modulate brain activities in freely behaving mice upon injection into brain cortex. This technique exhibited a temporal resolution in the millisecond range, a spatial resolution in the sub-millimeter range, and no harmful temperature accumulation.

External magnetic field stimulation can generate mechanical forces on nanoparticles (magnetomechanical transducers). This mechanical force in the piconewton range is strong enough to activate mechanosensitive channels, including TRPV4 and PIEZO2 in primary dorsal root ganglion neurons.¹⁰⁷ Nanomagnetic force (0.1–1 nN) stimulation of ferromagnetic nanoparticles opened N-type mechanosensitive Ca²⁺ channels, inducing Ca²⁺ influx within *in vitro*-grown cortical neural networks.¹⁴ The approach was then further applied for chronic stimulation of a fragile X syndrome (FXS) neural network model.¹⁴ FXS is the most common cause of intellectual disability and autism, and it shows an off-balance ratio of excitatory to inhibitory ion channels/receptors, including increased N-type Ca²⁺ channels and decreased GABA receptors. Chronic magnetic stimulation lowered the expression of N-type Ca²⁺ channels in FXS neurons to the normal level and increased the expression of GABA_A receptors, thus restoring the ion channel equilibrium. Magnetic nanotransducers may chronically modulate the expression of endogenous ion channels in neural circuits to investigate pathological mechanisms of many CNS diseases. Compared with other technologies, magnetomechanical neurostimulation is less likely to create deleterious responses, such as tissue heating during long exposure time, and is thus promising for long-term applications.

Genetically encoded transducers can link directly with mechanosensitive channels and can be stimulated externally with magnetic field or ultrasound. A major development in the area is referred to as magnetogenetics; i.e. use of the paramagnetic protein ferritin fused to TRPV4 for non-invasive neuromodulation³¹ (called Magneto). The TRPV4 channel could be opened and closed by activating ferritin fusion protein tethered to TRPV4 with an external magnetic field. Wheeler et al.³¹ reported that Magneto remotely controlled neural firing rates and behavior on a rapid and physiologically relevant timescale upon magnetic stimulation in both zebrafish and mice. While a few studies by other authors have validated this approach using the same or similar actuators,^{108,109} at least three independent studies showed that magnetic stimulation failed to electrophysiologically activate neurons expressing this magnetogenetic actuator.^{53,54,110} Although different stimulation efficiency of Magneto may result from experimental conditions, including alternating magnetic field versus constant magnetic field, different viruses used, and different virus expression levels,⁹³ the efficiency of the magnetogenetic actuator is still controversial. The underlying reason is that the proposed mechanisms, either heating or magnetic force introduced by individual ferritin protein, are 5–10 orders of magnitude lower than the threshold to activate the TRPV1 and TRPV4 channels.¹¹¹ Recently, it has been suggested that reactive oxygen species (ROS) may play an important role.¹¹² Specifically, radio-frequency waves activate ferritin tagged channels via iron-induced lipid oxidation, suggesting a biochemical mechanism^{113,112} (Figure 4).

Ultrasound can directly stimulate mechanosensitive ion channels to modulate neuronal activities. Transcranial focused ultrasonic stimulation is a clinically used neuromodulation technique based on this mechanism. This technique combines

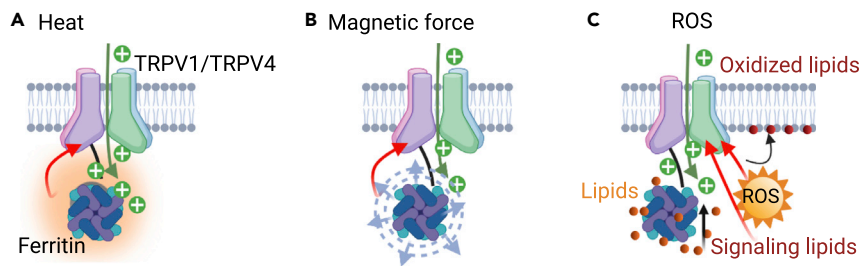


Figure 4. Proposed working mechanisms of magnetogenetics

Magnetic field stimulation of the ferritin protein directly coupled to TRPV1/TRPV4 induces calcium transients through a mechanism based on heat¹¹⁴ (A), force^{31,32,114} (B), or (ROS)^{113,112} (C).

non-invasiveness with high spatial resolution even in deep brain regions,¹¹⁵ enabling exploration of the role of specific brain regions in behaviors and neurological disorders. Compared with transcranial magnetic or electric stimulation, transcranial ultrasound stimulation can reach deeper brain regions and shows a higher spatial precision. However, the mechanism of ultrasonic stimulation is not yet fully understood. The proposed mechanisms include modulation of mechanosensitive ion channels to mediate transmembrane currents¹¹⁶ and opening of temperature-gated ion channel TRPA1.¹¹⁷ Besides activating the endogenous ion channels with ultrasound, sonogenetics involves the delivery of transgenes into neurons for expressing mechanosensitive ion channels, followed by ultrasonic stimulation of these neurons. Huang et al.⁵⁸ designed an ultrasound-responsive prestin (mPrestin) to modulate cellular activities.^{29,58} Focused ultrasound stimulation (0.5 MHz FUS, 0.5 MPa, 10 Hz PRF, 3 s duration) activated calcium signaling in neurons transfected with mPrestin, even in deep brain. Other mechanosensitive channels, such as MscL^{59,118} and sound-responsive opsin protein,¹¹⁹ have also been explored to enable sonogenetics in neurons. Ultrasound stimulation can activate cortical activity via indirectly activating auditory pathways instead of directly modulating neurons at the ultrasound stimulation sites.^{120,121} This indirect auditory mechanism for ultrasonic neuromodulation suggests that careful consideration is required when developing new ultrasonic neuromodulation techniques for brain research.

Azobenzene structures can convert light to molecular conformation changes or mechanical force to modulate neuronal activities. Recently, a plasma membrane-bound light-sensitive azobenzene compound (Ziapi2) was developed to modulate neuronal activity at high spatial-temporal resolution.¹⁰ *Trans*-dimerization of Ziapi2 in the dark thins the plasma membrane, increasing membrane capacitance at steady state. Millisecond pulses of visible light triggered *trans*→*cis* isomerization of Ziapi2, thickened the plasma membrane, decreased the membrane capacitance, and then induced action potential firing without affecting ion channels or local temperature. Action potentials can be evoked for up to 7 days, suggesting that Ziapi2 is promising for long-term applications.

Electrical neuromodulation

Working principle

Nanotransducers convert light, ultrasound, and magnetic field into a localized electric voltage or current, which can modulate voltage-gated ion channels, including sodium, potassium, calcium, chloride, and proton channels (Figure 2D). These channels respond to electrical stimulus within microseconds to milliseconds and operate at millivolt ranges.⁸⁸

Nanotransducers

Three classes of nanotransducers generate electrical output upon external stimulation, including optical, ultrasound, and magnetic energy. Optoelectronic transducers convert light into voltage or current and have been explored to remotely control neuron electrical signals. Semiconductor quantum dots (QD) are of great interest for electrical neuromodulation because of their broad absorption, narrow emission spectra, and large extinction coefficients.⁴⁵ Jalali et al.⁴⁵ incorporated type-II indium phosphide/zinc oxide core/shell QDs into a photoelectrode structure. Upon visible light exposure, this electrode induced a hyperpolarizing bioelectrical current and triggered the neuron firing. Visible light at $4 \mu\text{W mm}^{-2}$, 26-fold lower than the ocular safety limit for continuous exposure, is strong enough to activate the electrode. Organic pigment photocapacitors⁴⁶ and silicon-based materials⁹⁴ have also been explored as nanotransducers for electrical neuromodulation. Jiang et al.⁹⁵ developed a series of silicon-based materials that can behave as freestanding devices to modulate brain activities and simple animal behaviors. These silicon-based materials use light as a trigger; therefore, excessive wiring is not required, and the location of stimulation is determined by the location of light. Thus, optical stimulation of silicon-based materials has high flexibility and spatial resolution, and it can implement multiplexed and patterned stimulations. In another example, silicon nanowires with atomic Au on their surfaces elicit action potentials in neurons through a primarily atomic Au-enhanced photoelectrochemical process.¹²² An exciting and emerging application for nanotransducer-enabled electrical neuromodulation is vision restoration. Conjugated polymer nanoparticles injected into the subretinal space mediated light-evoked stimulation of retinal neurons and persistently rescued visual functions in a rat model of retinitis pigmentosa.¹¹ No effects on photoreceptor degeneration or retinal inflammation were observed upon light stimulation.

Piezoelectric transducers, such as barium titanate nanoparticles, also show potential for wireless electrical neuromodulation.^{30,62} Piezoelectric nanotransducers can efficiently generate electricity upon ultrasound stimulation to activate voltage-gated membrane channels, induce Ca^{2+} influx, and thus wirelessly modulate neuronal activity deep in the brain.

Magnetoelectric transducers have also been explored for wireless electrical neuromodulation. Magnetoelectric nanoparticles are composed of multiferroic materials that can efficiently convert external magnetic fields into local electric fields for modulating cell activities. Magnetoelectric nanoparticles were first proposed as a technique to non-invasively stimulate the brain of a patient with Parkinson disease in a computational study,⁹⁶ and further work is required to demonstrate the feasibility of this approach. Guduru et al.¹² showed that $\text{CoFe}_2\text{O}_4\text{-BaTiO}_3$ 30-nm nanoparticles can be excited by external magnetic field to modulate neuronal activities in the deep brain regions, and further characterizations are needed to better understand this mechanism.

Chemical neuromodulation

Working principle

Chemical neuromodulation uses neuromodulatory agents such as drugs or neurotransmitters that bind to ionotropic or metabotropic receptors and modulate neuron activity. This allows receptor-specific actions in local brain regions. These neuromodulatory agents can modulate either endogenous membrane receptors or channels (ligand-gated channels, GPCRs, and acid-sensitive channels), or designer receptors exclusively activated by designer drugs (DREADDs), a class of chemogenetically engineered proteins that allow spatial and temporal control of G protein signaling *in vivo*.

Nanotransducers

Three types of nanotransducers release molecules to modulate brain activity in local regions. First, neuromodulators can be released locally in the brain by light stimulation (opto-uncaging), offering high spatiotemporal resolution to modulate brain activity. NIR picosecond laser pulses induce transient nanobubbles around Au nanoparticle-coated liposomes. The collapse of those nanobubbles resulted in nanomechanical stress that rapidly ejected the encapsulated compound within 0.1 ms.⁸ This ultrafast release speed crosses the critical biological threshold and enables studies on fast cell signaling processes, such as neurotransmission. Recently, ultra-light-sensitive nanotransducers were developed based on Au-coated mechanoresponsive nanovesicles for releasing molecules in the deep brain. NIR picosecond laser pulses illumination induced nanomechanical stress to trigger cargo release in sub-seconds. The laser energy threshold of Au-coated mechanosensitive nanovesicles is 40 times lower than that of Au-coated traditional liposomes. The technique enable release of calcein in the deep brain region (4 mm),⁴⁹ suggesting its potential for deep brain neuromodulation.

Secondly, magnetic fields can remotely heat nanotransducers to release packaged molecules (magneto-uncaging). Neuromodulatory compounds can either be conjugated to magnetic nanoparticles via a thermally labile linker³⁴ or loaded in magnetic nanoparticles containing hydrogel.⁵⁵ Rao et al.⁵⁶ applied an alternating magnetic field to heat magnetic nanoparticles and subsequently release encapsulated molecules from thermally sensitive lipid vesicles (20 s latency) to provide non-invasive molecular control of neural circuits. The released small molecules activated both genetically engineered and endogenously expressed receptors with high temporal and spatial precision.⁵⁶ When combined with polymeric scaffolds, magnetic transducers can also convert magnetic fields into protons in physiological environments. Upon magnetic field stimulation, the magnetothermal effect caused by magnetic nanoparticles triggered the hydrolytic degradation of surrounding polyanhydride or polyester to release protons into the extracellular space, open acid-sensing ion channels, and induce Ca^{2+} influx in neurons.⁵⁷

The third mechanism involves ultrasonic release of molecules (sono-uncaging). Several sono-transducers, including perfluorocarbons (PFCs) containing microbubbles, nanoemulsions, and nanodroplets, have been explored for chemical neuromodulation.^{36,63} Ultrasonic nanotransducer-enabled chemical neuromodulation is spatially and temporally controlled by the size of the ultrasound focus, the timing of sonication, and the pharmacokinetics of the neuromodulatory agents.⁴ Wang et al.⁴ showed that ultrasonic uncaging of propofol can lead to brain activity changes in brain regions that are anatomically distinct from and functionally connected to the stimulated region. This has allowed for non-invasive mapping of the network connectivity in the brain under pharmacological activation of specific targets.⁴ Airan et al.³⁵ reported that transcranial stimulation of propofol-loaded emulsions silenced seizures in an acute rat seizure model. No brain parenchymal damage or blood-brain barrier (BBB) opening associated with their use was observed. These studies suggest that further development of ultrasonic nanotransducers will offer new tools for non-invasive, spatiotemporally precise neuromodulation, which may find a variety of biological applications.

Engineered neuromodulator sensors

In parallel with the developments of nanotransducers for chemical neuromodulation, there has been significant interest in new sensors to report and image neuromodulator release locally in the brain. Two categories of sensors were reported. The first

category involves genetically encoded sensors for neuromodulators based on fluorescent proteins. Two dopamine sensors, dLight1 and GRAB_{DA}, were first reported by Tian's group¹²³ and Li's group,¹²⁴ respectively. These genetically encoded sensors show a large fluorescence increase in response to extracellular dopamine release and allow high-spatiotemporal-resolution imaging using advanced microscopies such as two-photon microscopy. These sensors exhibit nanomolar to sub-micromolar affinity and have been validated with various pharmacological, electrophysiological, and optogenetic stimulations.^{125,126} This type of sensor has proved to be a versatile platform and has been extended for other neuromodulators, such as acetylcholine (GRAB_{ACh}),¹²⁷ serotonin or 5-hydroxytryptamine (5-HT, GRAB_{5-HT}),¹²⁸ norepinephrine,^{123,129} and opioid neuropeptide.^{123,130} Other genetically encoded sensors for neuropeptides are also being developed, such as for oxytocin¹³¹ or neuropeptide release reporters.¹³²

The second category involves fluorescent sensors based on synthetic nanomaterials,^{133–135} such as carbon nanotube. Landry and co-workers reported a series of developments in using carbon nanotubes functionalized with single-stranded DNA (ssDNA) to generate a unique class of nanosensors. These nanosensors can detect dopamine and norepinephrine,^{133,134,136} and serotonin.¹³⁵ They exhibit fluorescent emission in the infrared range (1,000–1,300 nm), and have shown promise in monitoring the release of neuromodulators in acute brain slices upon electrically or optogenetically evoked release. The infrared emission provides advantages for imaging the brain tissue. Further discussions on brain imaging are available in other review articles and are not discussed further here.¹³⁷

NANO-BRAIN INTERFACE

Understanding the interaction between nanomaterials and the brain (nano-brain interface) is critical for the rational design and safe application of nanotransducers for neuromodulation. Nano-brain interface includes nanomaterials delivery to the brain, interaction of nanomaterials with different types of cells in the brain (i.e., cellular tropism), nanomaterials transport (extracellular, intracellular, and intercellular), the immune response following nanomaterials administration, and the brain clearance of nanomaterials (Figure 5 and Table 2). We will discuss and highlight recent advances in these areas.

Delivery of nanomaterials to the brain

To enable nanotransducer-mediated neuromodulation, nanotransducers must be delivered into targeted brain regions. There are four main routes for nanomaterials delivery to the brain, including intraparenchymal, systemic, intrathecal, and intranasal administration. The major drawback of systemic administration is the limited accessibility of the nanomaterials to the brain from the blood. This is largely due to the presence of the BBB, which blocks most nano/micro particles from entering into the brain.

Most studies so far directly inject nanomaterials into local brain regions (intraparenchymal injection). Intraparenchymal injection bypasses the BBB but requires stereotactic insertion of an invasive injection needle. Direct administration to the CNS via intraparenchymal injection can achieve high local concentration near the injection site with relatively low dose. While this works well for proof-of-concept studies and may be adapted for large animals and humans, the invasive procedure may pose a challenge, especially if it requires repeated injection over time. The approach is promising for selectively modulating specific brain regions, but it is less suitable

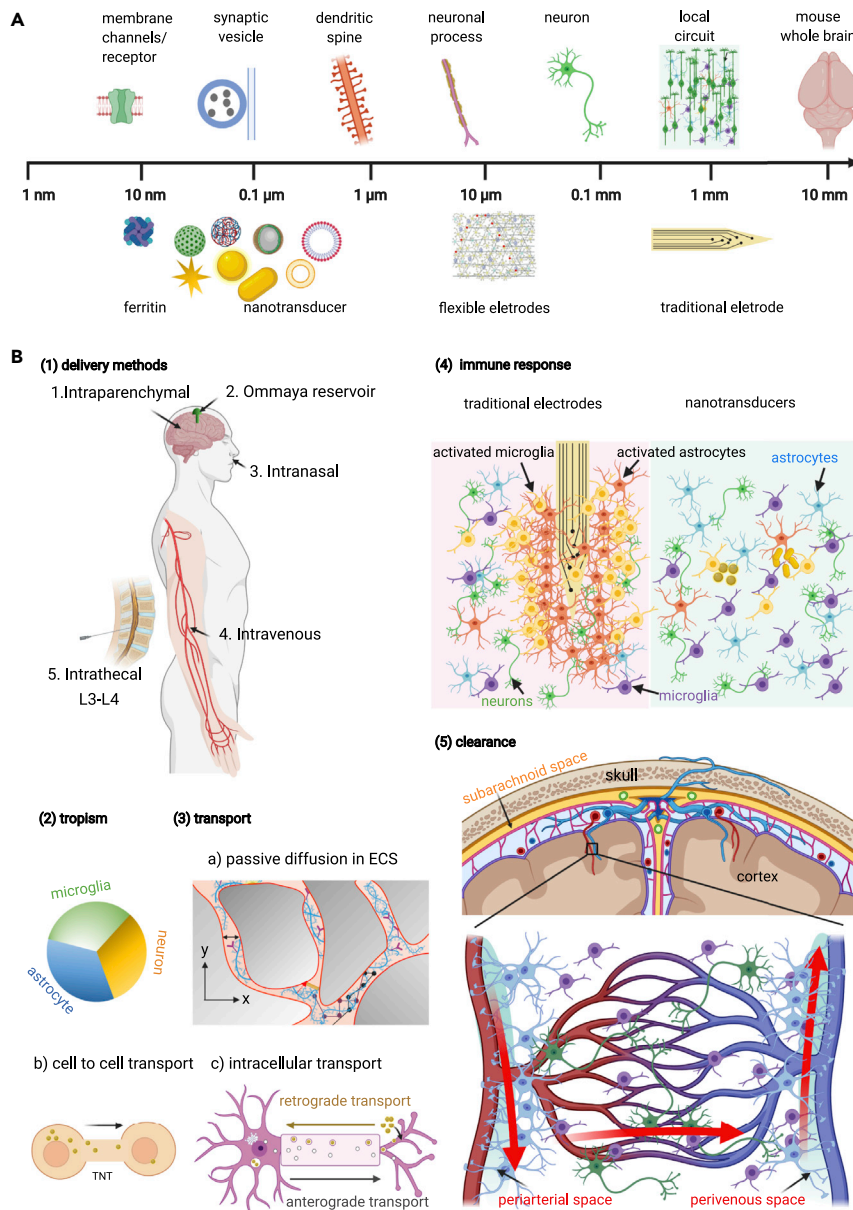


Figure 5. Nano-brain interface

(A) Multi-scale brain-materials interface including nanotransducers. Modified from Allegra Mascaro et al.¹³⁸ Schematic for flexible electrodes.¹

(B) Aspects of nano-brain interface. (1) Delivery of nanotransducers to the brain. (2) Cellular tropism of nanomaterials in the brain. (3) Passive diffusion of nanomaterials in extracellular space and active transport of nanomaterials in the brain. (4) The immune response following traditional electrode implantation and nanomaterials. (5) Brain clearance of nanomaterials via the perivascular pathway. L3–L4, lumbar segment 3–4; ECS, extracellular space.

for a large brain region, since injected particles can be restricted to the area surrounding the injection site.¹³⁹ Systemic administration of nanomaterials has been used for cases that do not require nanomaterials entry into the brain or nanomaterials transport across the BBB. For example, mechanoluminescent nanoparticles for sono-optogenetics³⁷ can be infused into blood circulation to allow remote ultrasound activation. Moreover, blood circulation provides an endogenous mechanism

for recharging the mechanoluminescent nanoparticles in superficial blood vessels and transporting the recharged energy to the brain for ultrasound-triggered localized light emission. Furthermore, ultrasound-responsive nanodroplets/nanoemulsions were systemically delivered to release pentobarbital, which crosses the BBB for targeted neuromodulation.^{4,63} Nance et al.¹⁴⁰ reported that systemically delivered dendrimers (size ~3–12 nm) can cross the impaired BBB to diffuse efficiently within the brain parenchyma and target activated microglia and astrocytes in regions of injury.

Other routes of administration are less defined for neuromodulation by nanotransducers. Intrathecal injection relies on administering substances into the cerebrospinal fluid (CSF) that surrounds the brain and spinal cord. While intrathecal injection circumvents the BBB, there is limited evidence that nanoparticles can penetrate into the brain parenchyma via the perivascular space. Studies show that 100-nm PEGylated-polystyrene nanoparticles rapidly distributed through the subarachnoid space along the entire neuraxis following injection into cisterna magna, with nanoparticles showing some preference for ventral surfaces and minimal penetration into the parenchyma.⁶⁹ This is in contrast to studies that show widespread expression of injected antisense oligonucleotide (ASO) to treat amyotrophic lateral sclerosis and other neurological diseases.¹⁴¹ Intrathecal injection of adeno-associated virus (AAV) also led to broad distribution throughout the brain and spinal cord parenchyma.¹⁴² Further work is required to establish the feasibility of this route for neuromodulation. Ommaya reservoir, a subcutaneous device directly inserted in the lateral ventricles, also offers a route for CNS penetration. Drugs delivered via Ommaya reservoir directly reach the ventricular CSF, and then are homogeneously distributed in the subarachnoid space. This technology has been used in clinic for improving brain delivery of chemotherapeutic agents and adopted to enhance nanoparticles transport. However, Ommaya reservoirs may be ineffective for delivering nanoparticles to parenchyma since agents can only diffuse within millimeters from the ependymal surface.¹⁴³ Nasal to CNS drug delivery is another approach to bypass the BBB, which has demonstrated its potential in clinical trials for the treatment of pain¹⁴⁴ and recurrent glioblastoma. This pathway also has been explored to deliver nanomaterials. Studies show that solid lipid nanoparticles modified with brain-targeting peptide (mApoE) can be delivered to the brain via pulmonary administration.¹⁴⁵

Cellular tropism of nanomaterials

The interaction of nanomaterials with different cell types in the brain is an important and basic question to understand the nano-brain interface. Jenkins et al.¹⁴⁶ studied the interaction of nanoparticles with different cell types in cell culture, and showed that nanoparticles with polyethylene glycol (PEG) modification showed modest reduction in cell uptake for all cell types (microglia, astrocytes, oligodendrocyte progenitor cells, neural stem cells, neurons) compared with nanoparticles with bio-adhesive end groups (carboxymethyl dextran [CMX]). Song et al.¹⁴⁷ investigated the cellular tropism of poly(lactic acid) nanoparticles with different surface chemistries (bare, PEG coating, hyperbranched glycerol [HPG], and aldehyde [–CHO]-modified HPG). “Stealth” properties (PEG, HPG) mostly reduced internalization by all cell types, while bio-adhesive end groups (–CHO) enhanced cellular uptake. Furthermore, the measured rates of uptake *in vitro* correlate with uptake of nanoparticles in specific cell types *in vivo*. Dante et al.¹⁴⁸ demonstrated that surface charge of nanoparticles played a critical role in their neuronal interaction. Anionic nanoparticles interact with the membrane of neurons and locate at the synaptic cleft, while no neutral and cationic nanoparticles were observed on neurons. The anionic

particles selectively bound to excitable neurons but did not interact with non-excitable glial cells. However, these studies do not have sufficient resolution (e.g., by electron microscopy) to examine the nanoscale details of nanoparticle interactions with the brain parenchyma.

Extracellular, intracellular, and intercellular transport of nanomaterials

Nanoparticles can passively diffuse in the extracellular space upon injection into the brain. The diffusion of nanoparticles is significantly reduced in the brain compared with in free medium, due to the tortuosity and narrow gap of the extracellular space. Several seminal works in this area have investigated the diffusion of QD in the extracellular space and characterized the diffusion coefficient (Table 2). Recently, Cognet and co-workers have developed a nanoscale imaging technique to track the movement of individual nanotubes in the extracellular space, and they use this as a tool to locally map the geometry and rheology in local brain regions.¹⁴⁹ The results show that the extracellular space is highly heterogeneous in the dimension (down to 40 nm). Hyaluronan is the major diffusion barrier and local tissue organizer and undergoes significant changes in neurodegenerative conditions.¹⁵⁰ The group also reported photoswitchable single-walled carbon nanotubes for super-resolution imaging in the NIR wavelength ($>1\ \mu\text{m}$).¹⁵¹ A hybrid nanomaterial was created by covalently linking photoswitching molecules on the carbon nanotube. The photoswitching molecules control the intrinsic emission of the carbon nanotubes to generate a photoswitchable carbon nanotube with controllable blinking for localization and super-resolution imaging.

Active transport of nanoparticles in the brain involves retrograde axonal transport and anterograde transport. Retrograde axonal transport conveys materials from axon to cell body and travels long distances (millimeters) along neuronal projections. The nanomaterial surface charge and endosome uptake have an effect on the intracellular transport.¹⁵² In primary mouse cortical neurons, negatively charged polystyrene nanoparticles smaller than 100 nm undergo axonal retrograde transport upon uptake by the axons and accumulate in the soma. In cortical neurons, negatively charged polystyrene nanoparticles inside lysosomes and 40-nm positively charged polystyrene nanoparticles undergo slow axonal transport, while negatively charged free polystyrene nanoparticles outside lysosomes undergo fast axonal transport mediated by dynein. Anterograde transport of exogenous particles was first reported in the 1980s. Particles with size up to 500 nm (e.g., polystyrene nanoparticles) traveled rapidly along the axon in the anterograde direction after microinjection into crab axons. Studies in squid axons have shown that GFP-labeled herpes simplex virus underwent anterograde axonal transport at an average speed of $0.9\ \mu\text{m/s}$, four times faster than that of mitochondria and 10 times faster than negative fluorescence beads.¹⁵³ The motor protein kinesin mediates the anterograde axonal transport of vesicles, organelles, and particles (Figure 5B).¹⁵⁴ Nanoparticles can enter nerve terminals and undergo axonal transport to neuron cell bodies after intrapulmonary,¹⁵⁵ intramuscular, or intradermal administration.¹⁵⁶

In glial and neuronal cells, nanoparticle intercellular transport is mediated by membrane nanotubes; i.e., tunneling-nanotube (TNT)-like structures. TNTs mediate the intercellular transport of various cellular components by generating membrane continuity between cells, a process facilitated by forming membranous F-actin-rich structures between cells. Studies show QDs are actively transported via membrane nanotubes between cardiac myocytes with a mean velocity of $1.23\ \mu\text{m/s}$.¹⁵⁷ Surface modification of nanoparticles with neural cell adhesion molecule 1 (NCAM1) and

CD44 antibodies was not found to change the cell-to-cell transport mechanism in neurons.¹⁵⁸

The immune response of nanomaterials

Mechanical insertion of bulky implants and subsequent micromotions within the skull lead to immune activation and glial scar formation that encapsulates the implants. This can displace neurons of interest, decrease the overall performance, and remodel the structure and function of the neural network surrounding the implant.⁵ Lessons learned from minimizing electrode implants suggest that implants smaller than individual cell bodies or nerve fibers may overcome the limitations of traditional electrodes¹⁵⁹ without being recognized by the immune system. Compared with bulk implants, nanotransducers can elicit less immune response due to their small size. McKenzie et al.¹⁶⁰ reported that nanoscale carbon fibers with diameter less than or equal to 100 nm inhibited the adhesion of astrocytes (glial scar tissue-forming cells) and decreased astrocyte proliferation compared with fibers larger than 100 nm. In addition, this study also showed that surface coating of carbon fibers with some polymers, such as polycarbonate urethane, can also effectively inhibit astrocyte adhesion and decrease astrocyte proliferation, thus leading to decreased glial scar tissue formation. In addition to formation of glia scar, exogenous nanomaterials may also induce transcriptomic changes in microglia. Yang et al.¹⁶¹ reported that single-walled carbon nanotubes upregulated genes specific to the immune response and induced morphological changes in SIM-A9 microglial cells *in vitro*. The transcriptomic and morphological changes in microglia were mitigated by surface coating with PEGylated phospholipids.

Nanomaterials clearance

Understanding the retention and clearance of nanomaterials in the brain is important for the design and application of nanotransducer-enabled neuromodulation. Firstly, the retention and clearance time varies significantly for different nanomaterials. While one study suggests a long retention of magnetic nanoparticles for weeks,⁷ another study showed that two organic nanoparticles, reconstituted high-density lipoprotein and poly(ethylene glycol)-b-poly(lactic acid) nanoparticles, were cleared rapidly from the brain following intraparenchymal administration, with half-life less than 5 h.¹⁶² The rapid clearance may facilitate applications for transient neuromodulation, while the long retention time is beneficial for chronic neuromodulation. Secondly, it was suggested that microglia-mediated transport mediates the nanoparticle clearance via the glymphatic pathway.¹⁶² Lastly, an impaired glymphatic system such as in Alzheimer disease can significantly slow down the clearance of interstitial solutes and nanoparticles.¹⁶² This suggests the need to take the brain disease into account when analyzing brain clearance of nanotransducers.

CONCLUSION AND OUTLOOK

Approaches for non-invasive and remote control of neuronal activity are important for interrogation of neural systems and treatment of many neurological diseases. There has been significant interest to develop wireless neuromodulation with nanotransducers and it has shown significant potential in terms of non-invasiveness, spatiotemporal precision, cell-type specificity, deep brain penetration, and translation to large animals and humans. The capability to transduce various energy modalities into local stimuli and interface with the neural system opens up new possibilities to modulate neural activity. New innovations in combining the energy modalities can overcome the limitations of a single energy modality and may continue to drive the field to the next phase of development. For example, sono-optogenetics³⁷ utilizes

mechanoluminescent nanoparticles and takes advantage of the deep penetration of ultrasound with the high spatiotemporal control of optogenetic modulation.

A better understanding of the nanomaterials-brain interface is critical for the success of nanotransducers for neuromodulation. However, limited work has been done in this area. Future research should develop minimally invasive approaches to deliver nanomaterials in local brain regions, and gain a better understanding of the immune response, cellular interaction, and retention and clearance of the nanomaterials in the brain. This is particularly important for applications requiring long-term utility of modulation.

One important consideration is the size and the complexity of the energy sources, which may limit broader dissemination and the clinical translation of nanotransducer-enabled neuromodulation techniques. For clinical use, the energy sources, such as light sources, magnetic system, and ultrasound system, have to be small and/or portable. Recently, biocompatible light sources that emit light with specific wavelengths have been implanted to overcome the limited tissue penetration of excitation lights for optogenetics.¹⁶³ A portable acoustic system for neuromodulation has been developed to potentially broaden the use of ultrasound systems.¹⁶⁴ Most of these studies are still in their very early stages. However, with advances in smaller and portable energy sources, we will be one step closer to broader laboratory use and clinical translation of nanotransducer-enabled neuromodulation techniques.

A promising direction for neuromodulation is genetically engineered functional materials. Currently, electrical modulation with electrodes or nanotransducers do not show cell-type specificity. Recently Liu et al.¹⁶⁵ genetically modified neurons to express peroxidase enzyme to synthesize electrically functional polymers that are conductive or insulating on the cell membrane, upon local infusion of precursor reagents. The *in situ* synthesized conductive polymers change neuron membrane electrical properties and allow cell-type-specific neuron and behavioral modulation in living animals. This work may inspire the creation of diverse and complex functional materials to seamlessly interface with the nervous system and allow next-generation neuromodulation techniques.

Another area of significant interest is to integrate sensing capabilities for bidirectional closed-loop neuromodulation and allow bidirectional communication with the local brain microenvironment. Nanotransducers with sensing ability will enable neuronal activity-guided neuromodulation, increasing the temporal precision and avoiding unwanted side effects due to overstimulation. Furthermore, distributed transducer systems that can communicate individually or with each other provide powerful tools to sense and modulate more brain activity. This could be useful, especially for mapping complex neuronal signaling in different brain regions. Advances in genetics, materials science, and engineering will provide us with next-generation nanotransducers with advanced functions. Ultimately, the emerging applications of new nanomaterials at the neural interfaces will lead to promising nanotransducers to modulate neural activity without physical wiring and transgenes. The capability to precisely modulate neural activity in deep brain regions with high spatiotemporal control enabled by those tools will strongly enhance the power to understand, modify, and control the human CNS.

In addition to enhancing understanding of signal transmission and processing in the CNS, nanotransducer-enabled wireless neuromodulation has shown therapeutic

potential for CNS disorders. Nanotransducer-enabled neural inhibition can restore normal firing of a hyperactive neuronal network and provide therapeutic effects for some brain disorders associated with neuronal hyperactivity, such as epilepsy and Parkinson disease.⁹⁶ Ye et al.⁴⁰ showed that Au nanorod-mediated neural inhibition of the left stellate ganglion can alleviate myocardial ischemia-induced ventricular arrhythmias. Stimulation of nanotransducers has the potential to restore ion channel equilibrium for diseases that exhibit off-balance ratio of excitatory to inhibitory ion channels/receptors, such as FXS.¹⁴ Another emerging application for neuromodulation is vision repair. Opto-stimulation of retinal neurons mediated by semiconducting polymer nanoparticles can rescue vision, suggesting its potential for retinitis pigmentosa treatment.¹¹ With more clinically translatable techniques being developed, nanotransducer-enabled neuromodulation will pave the way for therapies for many brain disorders.

ACKNOWLEDGMENTS

This work was partially supported by the National Science Foundation (1631910), and National Institute of Neurological Disorders and Stroke of the National Institutes of Health (RF1NS110499). Schematics were created with BioRender.com

AUTHOR CONTRIBUTIONS

Writing – original draft, X.L. and Z.Q.; writing – review & editing, X.L., X.H., N.J.R., X.X., J. H., P.A.S., G.H., and Z.Q.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Hong, G., and Lieber, C.M. (2019). Novel electrode technologies for neural recordings. *Nat. Rev. Neurosci.* 20, 330–345.
- Woods, G.A., Rommelfanger, N.J., and Hong, G. (2020). Bioinspired materials for in vivo bioelectronic neural interfaces. *Matter* 3, 1087–1113.
- Shemesh, O.A., Tanese, D., Zampini, V., Linghu, C., Piatkevich, K., Ronzitti, E., Papagiakoumou, E., Boyden, E.S., and Emiliani, V. (2017). Temporally precise single-cell-resolution optogenetics. *Nat. Neurosci.* 20, 1796–1806.
- Wang, J.B., Aryal, M., Zhong, Q., Vyas, D.B., and Airan, R.D. (2018). Noninvasive ultrasonic drug uncaging maps whole-brain functional networks. *Neuron* 100, 728–738 e727.
- Salatino, J.W., Ludwig, K.A., Kozai, T.D.Y., and Purcell, E.K. (2017). Glial responses to implanted electrodes in the brain. *Nat. Biomed. Eng.* 1, 862–877.
- Li, J., Duan, H., and Pu, K. (2019). Nanotransducers for near-infrared photoregulation in biomedicine. *Adv. Mater.* 31, e1901607.
- Chen, R., Romero, G., Christiansen, M.G., Mohr, A., and Anikeeva, P. (2015). Wireless magnetothermal deep brain stimulation. *Science* 347, 1477–1480.
- Li, X., Che, Z., Mazhar, K., Price, T.J., and Qin, Z. (2017). Ultrafast near-infrared light-triggered intracellular uncaging to probe cell signaling. *Adv. Funct. Mater.* 27, 1605778.
- Nakatsuji, H., Numata, T., Morone, N., Kaneko, S., Mori, Y., Imahori, H., and Murakami, T. (2015). Thermosensitive ion channel activation in single neuronal cells by using surface-engineered plasmonic nanoparticles. *Angew. Chem. Int. Ed.* 54, 11725–11729.
- DiFrancesco, M.L., Lodola, F., Colombo, E., Maragliano, L., Bramini, M., Paterno, G.M., Baldelli, P., Serra, M.D., Lunelli, L., Marchioretto, M., et al. (2020). Neuronal firing modulation by a membrane-targeted photoswitch. *Nat. Nanotechnol.* 15, 296–306.
- Maya-Vetencourt, J.F., Manfredi, G., Mete, M., Colombo, E., Bramini, M., Di Marco, S., Shmal, D., Mantero, G., Dipalo, M., Rocchi, A., et al. (2020). Subretinally injected semiconducting polymer nanoparticles rescue vision in a rat model of retinal dystrophy. *Nat. Nanotechnol.* 15, 698–708.
- Guduru, R., Liang, P., Hong, J., Rodzinski, A., Hadjikhani, A., Horstmyer, J., Levister, E., and Khizroev, S. (2015). Magnetolectric ‘spin’ on stimulating the brain. *Nanomedicine (Lond)*. 10, 2051–2061.
- Munshi, R., Qadri, S.M., Zhang, Q., Castellanos Rubio, I., Del Pino, P., and Pralle, A. (2017). Magnetothermal genetic deep brain stimulation of motor behaviors in awake, freely moving mice. *eLife* 6, e27069.
- Tay, A., and Di Carlo, D. (2017). Magnetic nanoparticle-based mechanical stimulation for restoration of mechano-sensitive ion channel equilibrium in neural networks. *Nano Lett.* 17, 886–892.
- Engels, J., and Schlaefer, E.J. (1972). Synthesis, structure, and reactivity of adenosine cyclic 3',5'-phosphate benzyl triesters. *J. Med. Chem.* 20, 907–911.
- Bechtereva, N.P., Bondartchuk, A.N., and Smirnov, V.M. (1972). Therapeutic electrostimulations of the deep brain structures. *Vopr. Neurokhir.* 36, 7–12.
- Barker, A.T., Jalinous, R., and Freeston, I.L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet* 1, 1106–1107.
- Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268.
- Nagel, G., Ollig, D., Fuhrmann, M., Kateriya, S., Musti, A.M., Bamberg, E., and Hegemann, P. (2002). Channelrhodopsin-1: a light-gated proton channel in green algae. *Science* 296, 2395–2398.
- Wells, J., Kao, C., Jansen, E.D., Konrad, P., and Mahadevan-Jansen, A. (2005). Application of infrared light for in vivo neural stimulation. *J. Biomed. Opt.* 10, 064003.

21. Lugo, K., Miao, X., Rieke, F., and Lin, L.Y. (2012). Remote switching of cellular activity and cell signaling using light in conjunction with quantum dots. *J. Biomed. Opt.* 3, 447–454.
22. Huang, H., Delikanli, S., Zeng, H., Ferkey, D.M., and Pralle, A. (2010). Remote control of ion channels and neurons through magnetic-field heating of nanoparticles. *Nat. Nanotechnol.* 5, 602–606.
23. Tufail, Y., Matyushov, A., Baldwin, N., Tauchmann, M.L., Georges, J., Yoshihiro, A., Tillery, S.I., and Tyler, W.J. (2010). Transcranial pulsed ultrasound stimulates intact brain circuits. *Neuron* 66, 681–694.
24. K.A. Deisseroth, P.A. Upconversion of light for use in optogenetic methods. United States Patent 2011, PCT/US2011/059287.
25. Ghezzi, D., Antognazza, M.R., Dal Maschio, M., Lanzarini, E., Benfenati, F., and Lanzani, G. (2011). A hybrid bioorganic interface for neuronal photoactivation. *Nat. Commun.* 2, 166.
26. Yong, J., Needham, K., Brown, W.G., Nayagam, B.A., McArthur, S.L., Yu, A., and Stoddart, P.R. (2014). Gold-nanorod-assisted near-infrared stimulation of primary auditory neurons. *Adv. Healthc. Mater.* 3, 1862–1868.
27. Tay, A., Sohrabi, A., Poole, K., Seidlits, S., and Di Carlo, D. (2018). A 3D magnetic hyaluronic acid hydrogel for magnetomechanical neuromodulation of primary dorsal root ganglion neurons. *Adv. Mater.* e1800927.
28. Li, W., Luo, R., Lin, X., Jadhav, A.D., Zhang, Z., Yan, L., et al. (2015). Remote modulation of neural activities via near-infrared triggered release of biomolecules. *Biomaterials*. 65, 76–85.
29. Ibsen, S., Tong, A., Schutt, C., Esener, S., and Chalasani, S.H. (2015). Sonogenetics is a non-invasive approach to activating neurons in *Caenorhabditis elegans*. *Nat. Commun.* 6, 8264.
30. Marino, A., Arai, S., Hou, Y., Sinibaldi, E., Pellegrino, M., Chang, Y.T., Mazzolai, B., Mattoli, V., Suzuki, M., and Ciofani, G. (2015). Piezoelectric nanoparticle-assisted wireless neuronal stimulation. *ACS. Nano* 9, 7678–7689.
31. Wheeler, M.A., Smith, C.J., Ottolini, M., Barker, B.S., Purohit, A.M., Grippo, R.M., Gaykema, R.P., Spano, A.J., Beenhakker, M.P., Kucenas, S., et al. (2016). Genetically targeted magnetic control of the nervous system. *Nat. Neurosci.* 19, 756–761.
32. Stanley, S.A., Kelly, L., Latcha, K.N., Schmidt, S.F., Yu, X., Nectow, A.R., Sauer, J., Dyke, J.P., Dordick, J.S., and Friedman, J.M. (2016). Bidirectional electromagnetic control of the hypothalamus regulates feeding and metabolism. *Nature* 531, 647–650.
33. Wang, H.Q., Zhou, X.Q., Cui, D., Liu, R.X., Tan, R.X., Wang, X., Liu, Z.P., and Yin, T. (2019). Comparative study of transcranial magneto-acoustic stimulation and transcranial ultrasound stimulation of motor cortex. *Front. Behav. Neurosci.* 13, 214.
34. Romero, G., Christiansen, M.G., Barbosa, L.S., Garcia, F., and Anikeeva, P. (2016). Localized excitation of neural activity via rapid magnetothermal drug release. *Adv. Funct. Mater.* 26, 6471–6478.
35. Airan, R.D., Meyer, R.A., Ellens, N.P., Rhodes, K.R., Farahani, K., Pomper, M.G., Kadam, S.D., and Green, J.J. (2017). Noninvasive targeted transcranial neuromodulation via focused ultrasound gated drug release from nanoemulsions. *Nano Lett.* 17, 652–659.
36. Szablowski, J.O., Lee-Gosselin, A., Lue, B., Malounda, D., and Shapiro, M.G. (2018). Acoustically targeted chemogenetics for the non-invasive control of neural circuits. *Nat. Biomed. Eng.* 2, 475–484.
37. Wu, X., Zhu, X., Chong, P., Liu, J., Andre, L.N., Ong, K.S., Brinson, K., Jr., Mahdi, A.I., Li, J., Fenno, L.E., et al. (2019). Sono-optogenetics facilitated by a circulation-delivered rechargeable light source for minimally invasive optogenetics. *Proc. Natl. Acad. Sci. U S A*. 116, 26332–26342.
38. Chen, S., Weitemier, A.Z., Zeng, X., He, L., Wang, X., Tao, Y., Huang, A.J.Y., Hashimoto, Y., Kano, M., Iwasaki, H., et al. (2018). Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics. *Science* 359, 679–684.
39. Ao, Y., Zeng, K., Yu, B., Miao, Y., Hung, W., Yu, Z., Xue, Y., Tan, T.T.Y., Xu, T., Zhen, M., et al. (2019). An upconversion nanoparticle enables near infrared-optogenetic manipulation of the *Caenorhabditis elegans* motor circuit. *ACS Nano* 13, 3373–3386.
40. Ye, T.Y., Lai, Y.Q., Wang, Z.Y., Zhang, X.G., Meng, G.N., Zhou, L.P., Zhang, Y.F., Zhou, Z., Deng, J.L., Wang, M., et al. (2019). Precise modulation of gold nanorods for protecting against malignant ventricular arrhythmias via near-infrared neuromodulation. *Adv. Funct. Mater.* 29, 1902128.
41. Rastogi, S.K., Garg, R., Scopelliti, M.G., Pinto, B.I., Hartung, J.E., Kim, S., Murphey, C.G.E., Johnson, N., San Roman, D., Bezanilla, F., et al. (2020). Remote nongenetic optical modulation of neuronal activity using fuzzy graphene. *Proc. Natl. Acad. Sci. U S A*. 117, 13339–13349.
42. Lyu, Y., Xie, C., Checheta, S.A., Miyako, E., and Pu, K. (2016). Semiconducting polymer nanobioconjugates for targeted photothermal activation of neurons. *J. Am. Chem. Soc.* 138, 9049–9052.
43. Nelidova, D., Morikawa, R.K., Cowan, C.S., Raics, Z., Goldblum, D., Scholl, H.P.N., Szikra, T., Szabo, A., Hillier, D., and Roska, B. (2020). Restoring light sensitivity using tunable near-infrared sensors. *Science* 368, 1108–1113.
44. Kang, P.Y., Li, X.Q., Liu, Y.N., Shiers, S.I., Xiong, H.J., Giannotta, M., Dejana, E., Price, T.J., Randrianalisoa, J., Nielsen, S.O., and Qin, Z.P. (2019). Transient photoinactivation of cell membrane protein activity without genetic modification by molecular hyperthermia. *ACS Nano* 13, 12487–12499.
45. Jalali, H.B., Aria, M.M., Dikbas, U.M., Sadeghi, S., Kumar, B.G., Sahin, M., Kavakli, I.H., Ouyang, C.W., and Nizamoglu, S. (2018). Effective neural photostimulation using indium-based type-II quantum dots. *ACS Nano* 12, 8104–8114.
46. Rand, D., Jakesova, M., Lubin, G., Vebrat, I., David-Pur, M., Derek, V., Cramer, T., Sariciftci, N.S., Hanein, Y., and Glowacki, E.D. (2018). Direct electrical neurostimulation with organic pigment photocapacitors. *Adv. Mater.* 30, e1707292.
47. Tang, J., Qin, N., Chong, Y., Diao, Y., Yiliguma, Wang, Z., Xue, T., Jiang, M., Zhang, J., and Zheng, G. (2018). Nanowire arrays restore vision in blind mice. *Nat. Commun.* 9, 786.
48. Cabre, G., Garrido-Charles, A., Moreno, M., Bosch, M., Porta-de-la-Riva, M., Krieg, M., Gascon-Moya, M., Camarero, N., Gelabert, R., Lluch, J.M., et al. (2019). Rationally designed azobenzene photoswitches for efficient two-photon neuronal excitation. *Nat. Commun.* 10, 907.
49. Xiong, H., Li, X., Kang, P., Perish, J., Neuhaus, F., Ploski, J.E., Kroener, S., Ogunyakin, M.O., Shin, J.E., Zasadzinski, J.A., et al. (2020). Near-infrared light triggered-release in deep brain regions using ultra-photosensitive nanovesicles. *Angew. Chem. Int. Ed.* 59, 8608–8615.
50. Duret, G., Polali, S., Anderson, E.D., Bell, A.M., Tzouanas, C.N., Avants, B.W., and Robinson, J.T. (2019). Magnetic entropy as a proposed gating mechanism for magnetogenetic ion channels. *Biophys. J.* 116, 454–468.
51. Munshi, R., Qadri, S.M., and Pralle, A. (2018). Transient magnetothermal neuronal silencing using the chloride channel anoctamin 1 (TMEM16A). *Front. Neurosci.* 12, 560.
52. Gregurec, D., Senko, A.W., Chuvilin, A., Reddy, P.D., Sankaranarayanan, A., Rosenfeld, D., Chiang, P.H., Garcia, F., Tafel, I., Varnavides, G., et al. (2020). Magnetic vortex nanodiscs enable remote magnetomechanical neural stimulation. *ACS Nano* 14, 8036–8045.
53. Wang, G., Zhang, P., Mendu, S.K., Wang, Y., Zhang, Y., Kang, X., Desai, B.N., and Zhu, J.J. (2019). Reevaluation of magnetic properties of Magneto. *Nat. Neurosci.* 23, 1047–1050.
54. Kole, K., Zhang, Y., Jansen, E.J.R., Brouns, T., Bijlsma, A., Calcini, N., Yan, X., Lantyer, A.D.S., and Celikel, T. (2019). Assessing the utility of Magneto to control neuronal excitability in the somatosensory cortex. *Nat. Neurosci.* 23, 1044–1046.
55. Kondaveeti, S., Semeano, A.T.S., Cornejo, D.R., Ulrich, H., and Petri, D.F.S. (2018). Magnetic hydrogels for levodopa release and cell stimulation triggered by external magnetic field. *Colloids Surf. B. Biointerfaces*. 167, 415–424.
56. Rao, S., Chen, R., LaRocca, A.A., Christiansen, M.G., Senko, A.W., Shi, C.H., Chiang, P.H., Varnavides, G., Xue, J., Zhou, Y., et al. (2019). Remotely controlled chemomagnetic modulation of targeted neural circuits. *Nat. Nanotechnol.* 14, 967–973.
57. Park, J., Tabet, A., Moon, J., Chiang, P.H., Koehler, F., Sahasrabudhe, A., and Anikeeva, P. (2020). Remotely controlled proton generation for neuromodulation. *Nano Lett.* 20, 6535–6541.
58. Huang, Y.S., Fan, C.H., Hsu, N., Chiu, N.H., Wu, C.Y., Chang, C.Y., Wu, B.H., Hong, S.R.,

- Chang, Y.C., Yan-Tang Wu, A., et al. (2020). Sonogenetic modulation of cellular activities using an engineered auditory-sensing protein. *Nano Lett.* 20, 1089–1100.
59. Ye, J., Tang, S.Y., Meng, L., Li, X., Wen, X.X., Chen, S.H., Niu, L.L., Li, X.Y., Qiu, W.B., Hu, H.L., et al. (2018). Ultrasonic control of neural activity through activation of the mechanosensitive channel MscL. *Nano Lett.* 18, 4148–4155.
60. Wu, C.Y., Fan, C.H., Chiu, N.H., Ho, Y.J., Lin, Y.C., and Yeh, C.K. (2020). Targeted delivery of engineered auditory sensing protein for ultrasound neuromodulation in the brain. *Theranostics* 10, 3546–3561.
61. Huang, Y., Jiang, Y., Luo, X., Wu, J., Zong, H., Shi, L., Heng, R., Jiang, S., Jia, X., Mei, J., et al. (2021). Neural stimulation in vitro and in vivo by photoacoustic nanotransducers. *Matter* 4, 675–687.
62. Rojas, C., Tedesco, M., Massobrio, P., Marino, A., Ciofani, G., Martinoia, S., and Raiteri, R. (2018). Acoustic stimulation can induce a selective neural network response mediated by piezoelectric nanoparticles. *J. Neural Eng.* 15, 036016.
63. Lea-Banks, H., O'Reilly, M.A., Hamani, C., and Hynynen, K. (2020). Localized anesthesia of a specific brain region using ultrasound-responsive barbiturate nanodroplets. *Theranostics* 10, 2849–2858.
64. Lee, J.W., Jung, H., Cho, H.H., Lee, J.H., and Nam, Y. (2018). Gold nanostar-mediated neural activity control using plasmonic photothermal effects. *Biomaterials* 153, 59–69.
65. Yoo, S., Hong, S., Choi, Y., Park, J.H., and Nam, Y. (2014). Photothermal inhibition of neural activity with near-infrared-sensitive nanotransducers. *ACS Nano* 8, 8040–8049.
66. Wang, Y.X., Feng, L.H., and Wang, S. (2019). Conjugated polymer nanoparticles for imaging, cell activity regulation, and therapy. *Adv. Funct. Mater.* 29, 1806818.
67. Roet, M., Heschem, S.A., Jahanshahi, A., Rutten, B.P.F., Anikeeva, P.O., and Temel, Y. (2019). Progress in neuromodulation of the brain: a role for magnetic nanoparticles? *Prog. Neurobiol.* 177, 1–14.
68. Thorne, R.G., and Nicholson, C. (2006). In vivo diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space. *Proc. Natl. Acad. Sci. U S A.* 103, 5567–5572.
69. Householder, K.T., Dharmaraj, S., Sandberg, D.I., Wechsler-Reya, R.J., and Sirianni, R.W. (2019). Fate of nanoparticles in the central nervous system after intrathecal injection in healthy mice. *Sci. Rep.* 9, 12587.
70. Wen, S., Zhou, J., Schuck, P.J., Suh, Y.D., Schmidt, T.W., and Jin, D. (2019). Future and challenges for hybrid upconversion nanosystems. *Nat. Photon.* 13, 828–838.
71. Svaasand, L.O., and Ellingsen, R. (1983). Optical properties of human brain. *Photochem. Photobiol.* 38, 293–299.
72. Holtzheimer, P.E., Kelley, M.E., Gross, R.E., Filkowski, M.M., Garlow, S.J., Barocas, A., Wint, D., Craighead, M.C., Kozarsky, J., Chismar, R., et al. (2012). Subcallosal cingulate deep brain stimulation for treatment-resistant unipolar and bipolar depression. *Arch. Gen. Psychiatry* 69, 150–158.
73. Mace, E., Montaldo, G., Cohen, I., Baulac, M., Fink, M., and Tanter, M. (2011). Functional ultrasound imaging of the brain. *Nat. Methods* 8, 662–664.
74. Ron, M., and Rob, P. (2015). *Cell Biology by the Numbers*, 1st Edition (Garland Science).
75. Ahmari, S.E., and Smith, S.J. (2002). Knowing a nascent synapse when you see it. *Neuron* 34, 333–336.
76. Wright, B.L., Lai, J.T., and Sinclair, A.J. (2012). Cerebrospinal fluid and lumbar puncture: a practical review. *J. Neurol.* 259, 1530–1545.
77. Pardridge, W.M. (2016). CSF, blood-brain barrier, and brain drug delivery. *Expert Opin. Drug Deliv.* 13, 963–975.
78. Tonnesen, J., Inavalli, V., and Nagerl, U.V. (2018). Super-resolution imaging of the extracellular space in living brain tissue. *Cell* 172, 1108–1121. e15.
79. Kitagawa, T. (2012). Newborn screening for inborn errors of metabolism in Japan. A history of the development of newborn screening. *Pediatr. Endocrinol. Rev.* 10 (Suppl 1), 8–25.
80. Ferenczi, E.A., Tan, X., and Huang, C.L. (2019). Principles of optogenetic methods and their application to cardiac experimental systems. *Front. Physiol.* 10, 1096.
81. Berry, M.H., Holt, A., Salari, A., Veit, J., Visel, M., Levitz, J., Agbi, K., Gaub, B.M., Sivy, B., Flannery, J.G., and Isacoff, E.Y. (2019). Restoration of high-sensitivity and adapting vision with a cone opsin. *Nat. Commun.* 10, 1221.
82. Mackenzie, L.E., Goode, J.A., Vakurov, A., Nampi, P.P., Saha, S., Jose, G., and Millner, P.A. (2018). The theoretical molecular weight of NaYF₄:RE upconversion nanoparticles. *Sci. Rep.* 8, 1106.
83. Yao, J., Liu, B., and Qin, F. (2010). Kinetic and energetic analysis of thermally activated TRPV1 channels. *Biophys. J.* 99, 1743–1753.
84. Schreiber, R., Ousingawatt, J., Wanitchakool, P., Sirianant, L., Benedetto, R., Reiss, K., and Kunzelmann, K. (2018). Regulation of TMEM16A/ANO1 and TMEM16F/ANO6 ion currents and phospholipid scrambling by Ca²⁺ and plasma membrane lipid. *J. Physiol.* 596, 217–229.
85. Cruz-Rangel, S., De Jesus-Perez, J.J., Contreras-Vite, J.A., Perez-Cornejo, P., Hartzell, H.C., and Arreola, J. (2015). Gating modes of calcium-activated chloride channels TMEM16A and TMEM16B. *J. Physiol.* 593, 5283–5298.
86. Shin, K.C., Park, H.J., Kim, J.G., Lee, I.H., Cho, H., Park, C., Sung, T.S., Koh, S.D., Park, S.W., and Bae, Y.M. (2019). The Piezo2 ion channel is mechanically activated by low-threshold positive pressure. *Sci. Rep.* 9, 6446.
87. Wang, L., Zhou, H., Zhang, M., Liu, W., Deng, T., Zhao, Q., Li, Y., Lei, J., Li, X., and Xiao, B. (2019). Structure and mechanogating of the mammalian tactile channel PIEZO2. *Nature* 573, 225–229.
88. Jakesova, M., Ejnemy, M.S., Derek, V., Schmidt, T., Gryszel, M., Brask, J., Schindl, R., Simon, D.T., Berggren, M., Elinder, F., and Glowacki, E.D. (2019). Optoelectronic control of single cells using organic photocapacitors. *Sci. Adv.* 5, eaav5265.
89. Lin, J.Y., Knutsen, P.M., Muller, A., Kleinfeld, D., and Tsien, R.Y. (2013). ReaChR, a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nat. Neurosci.* 16, 1499–1508.
90. Lin, J.Y. (2011). A user's guide to channelrhodopsin variants: features, limitations and future developments. *Exp. Physiol.* 96, 19–25.
91. Owen, S.F., Liu, M.H., and Kreitzer, A.C. (2019). Thermal constraints on in vivo optogenetic manipulations. *Nat. Neurosci.* 22, 1061–1065.
92. Jiang, Y., Lee, H.J., Lan, L., Tseng, H.A., Yang, C., Man, H.Y., Han, X., and Cheng, J.X. (2020). Optoacoustic brain stimulation at submillimeter spatial precision. *Nat. Commun.* 11, 881.
93. Wheeler, M.A., Deppmann, C.D., Patel, M.K., and Guler, A.D. (2019). Reply to, Magneto is ineffective in controlling electrical properties of cerebellar Purkinje cells, Assessing the utility of Magneto to control neuronal excitability in the somatosensory cortex and revaluation of magnetic properties of Magneto. *Nat. Neurosci.* 23, 1051–1054.
94. Gao, X., Jiang, Y., Lin, Y., Kim, K.H., Fang, Y., Yi, J., Meng, L., Lee, H.C., Lu, Z., Leddy, O., et al. (2020). Structured silicon for revealing transient and integrated signal transductions in microbial systems. *Sci. Adv.* 6, eaay2760.
95. Jiang, Y., Li, X., Liu, B., Yi, J., Fang, Y., Shi, F., Gao, X., Sudzilovsky, E., Parameswaran, R., Koehler, K., et al. (2018). Rational design of silicon structures for optically controlled multiscale biointerfaces. *Nat. Biomed. Eng.* 2, 508–521.
96. Yue, K., Guduru, R., Hong, J., Liang, P., Nair, M., and Khizroev, S. (2012). Magneto-electric nano-particles for non-invasive brain stimulation. *PLoS One* 7, e44040.
97. Feng, H.J., Kao, C., Gallagher, M.J., Jansen, E.D., Mahadevan-Jansen, A., Konrad, P.E., and Macdonald, R.L. (2010). Alteration of GABAergic neurotransmission by pulsed infrared laser stimulation. *J. Neurosci. Methods* 192, 110–114.
98. Miao, Q., Xie, C., Zhen, X., Lyu, Y., Duan, H., Liu, X., Jorke, J.V., and Pu, K. (2017). Molecular afterglow imaging with bright, biodegradable polymer nanoparticles. *Nat. Biotechnol.* 35, 1102–1110.
99. Li, J., and Pu, K. (2020). Semiconducting polymer nanomaterials as near-infrared photoactivatable protherapeutics for cancer. *Acc. Chem. Res.* 53, 752–762.
100. Wu, X.Y.J., Rommelfanger, N.J., Yin, R., Liu, J., Cai, S., Ren, W., Shin, A., Ong, K.S., Pu, K., and Hong, G. (2020). Through-scalp deep-brain stimulation in tether-free, naturally-behaving

- mice with widefield NIR-II illumination. *BioRxiv*. <https://doi.org/10.1101/2020.05.29.123075>.
101. Honore, E. (2007). The neuronal background K2P channels: focus on TREK1. *Nat. Rev. Neurosci.* 8, 251–261.
102. Shapiro, M.G., Homma, K., Villarreal, S., Richter, C.P., and Bezanilla, F. (2012). Infrared light excites cells by changing their electrical capacitance. *Nat. Commun.* 3, 736.
103. Tan, X., Jahan, I., Xu, Y., Stock, S., Kwan, C.C., Soriano, C., Xiao, X., Garcia-Anoveros, J., Fritsch, B., and Richter, C.P. (2018). Auditory neural activity in congenitally deaf mice induced by infrared neural stimulation. *Sci. Rep.* 8, 388.
104. Cayce, J.M., Friedman, R.M., Chen, G., Jansen, E.D., Mahadevan-Jansen, A., and Roe, A.W. (2014). Infrared neural stimulation of primary visual cortex in non-human primates. *NeuroImage* 84, 181–190.
105. Izzo, A.D., Walsh, J.T., Jr., Ralph, H., Webb, J., Bendett, M., Wells, J., and Richter, C.P. (2008). Laser stimulation of auditory neurons: effect of shorter pulse duration and penetration depth. *Biophys. J.* 94, 3159–3166.
106. Davis, H.C., Kang, S., Lee, J.H., Shin, T.H., Putterman, H., Cheon, J., and Shapiro, M.G. (2020). Nanoscale heat transfer from magnetic nanoparticles and ferritin in an alternating magnetic field. *Biophys. J.* 118, 1502–1510.
107. Wu, J., Goyal, R., and Grandl, J. (2016). Localized force application reveals mechanically sensitive domains of Piezo1. *Nat. Commun.* 7, 12939.
108. Mosabbir, A.A., and Truong, K. (2018). Genetically encoded circuit for remote regulation of cell migration by magnetic fields. *ACS Synth. Biol.* 7, 718–726.
109. Hutson, M.R., Keyte, A.L., Hernandez-Morales, M., Gibbs, E., Kupchinsky, Z.A., Argyridis, I., Erwin, K.N., Pegram, K., Kneifel, M., Rosenberg, P.B., et al. (2017). Temperature-activated ion channels in neural crest cells confer maternal fever-associated birth defects. *Sci. Signal.* 10, eaal4055.
110. Xu, F.X., Zhou, L., Wang, X.T., Jia, F., Ma, K.Y., Wang, N., Lin, L., Xu, F.Q., and Shen, Y. (2019). Magneto is ineffective in controlling electrical properties of cerebellar Purkinje cells. *Nat. Neurosci.* 23, 1041–1043.
111. Meister, M. (2016). Physical limits to magnetogenetics. *eLife* 5, e17210.
112. Brier, M.I., Mundell, J.W., Yu, X., Su, L., Holmann, A., Squeri, J., Zhang, B., Stanley, S.A., Friedman, J.M., and Dordick, J.S. (2020). Uncovering a possible role of reactive oxygen species in magnetogenetics. *Sci. Rep.* 10, 13096.
113. Hernandez-Morales, M., Shang, T., Chen, J., Han, V., and Liu, C. (2020). Lipid oxidation induced by RF waves and mediated by ferritin iron causes activation of ferritin-tagged ion channels. *Cell. Rep.* 30, 3250–3260. e7.
114. Barbic, M. (2019). Possible magneto-mechanical and magneto-thermal mechanisms of ion channel activation in magnetogenetics. *eLife* 8, e45807.
115. Legon, W., Ai, L., Bansal, P., and Mueller, J.K. (2018). Neuromodulation with single-element transcranial focused ultrasound in human thalamus. *Hum. Brain Mapp.* 39, 1995–2006.
116. Qiu, Z., Guo, J., Kala, S., Zhu, J., Xian, Q., Qiu, W., Li, G., Zhu, T., Meng, L., Zhang, R., et al. (2019). The mechanosensitive ion channel Piezo1 significantly mediates in vitro ultrasonic stimulation of neurons. *iScience* 21, 448–457.
117. Oh, S.J., Lee, J.M., Kim, H.B., Lee, J., Han, S., Bae, J.Y., Hong, G.S., Koh, W., Kwon, J., Hwang, E.S., et al. (2020). Ultrasonic neuromodulation via astrocytic TRPA1. *Curr. Biol.* 30, 948.
118. Qiu, Z., Kala, S., Guo, J., Xian, Q., Zhu, J., Zhu, T., Hou, X., Wong, K.F., Yang, M., Wang, H., and Sun, L. (2020). Targeted neurostimulation in mouse brains with non-invasive ultrasound. *Cell. Rep.* 32, 108033.
119. Towne, C.L. and Andersen, D.E. (2019). Minimally-invasive, high resolution neuromodulation of deep brain and cortical structures using circuit-specific promoters and focused ultrasound array (US20190321473A1, United States).
120. Sato, T., Shapiro, M.G., and Tsao, D.Y. (2018). Ultrasonic neuromodulation causes widespread cortical activation via an indirect auditory mechanism. *Neuron* 98, 1031–1041. e5.
121. Guo, H., Hamilton, M., 2nd, Offutt, S.J., Gloeckner, C.D., Li, T., Kim, Y., Legon, W., Alford, J.K., and Lim, H.H. (2018). Ultrasound produces extensive brain activation via a cochlear pathway. *Neuron* 98, 1020–1030. e4.
122. Parameswaran, R., Carvalho-de-Souza, J.L., Jiang, Y., Burke, M.J., Zimmerman, J.F., Koehler, K., Phillips, A.W., Yi, J., Adams, E.J., Bezanilla, F., and Tian, B. (2018). Photoelectrochemical modulation of neuronal activity with free-standing coaxial silicon nanowires. *Nat. Nanotechnol.* 13, 260–266.
123. Patriarchi, T., Cho, J.R., Merten, K., Howe, M.W., Marley, A., Xiong, W.H., Folk, R.W., Broussard, G.J., Liang, R., Jang, M.J., et al. (2018). Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. *Science* 360, eaat4422.
124. Sun, F., Zeng, J., Jing, M., Zhou, J., Feng, J., Owen, S.F., Luo, Y., Li, F., Wang, H., Yamaguchi, T., et al. (2018). A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. *Cell* 174, 481–496. e19.
125. Sun, F., Zhou, J., Dai, B., Qian, T., Zeng, J., Li, X., Zhuo, Y., Zhang, Y., Wang, Y., Qian, C., et al. (2020). Next-generation GRAB sensors for monitoring dopaminergic activity in vivo. *Nat. Methods* 17, 1156–1166.
126. Patriarchi, T., Mohebi, A., Sun, J., Marley, A., Liang, R., Dong, C., Puhger, K., Mizuno, G.O., Davis, C.M., Wiltgen, B., et al. (2020). An expanded palette of dopamine sensors for multiplex imaging in vivo. *Nat. Methods* 17, 1147–1155.
127. Jing, M., Li, Y., Zeng, J., Huang, P., Skrzewski, M., Kljakic, O., Peng, W., Qian, T., Tan, K., Zou, J., et al. (2020). An optimized acetylcholine sensor for monitoring in vivo cholinergic activity. *Nat. Methods* 17, 1139–1146.
128. Wan, J., Peng, W., Li, X., Qian, T., Song, K., Zeng, J., Deng, F., Hao, S., Feng, J., Zhang, P., et al. (2020). A genetically encoded GRAB sensor for measuring serotonin dynamics in vivo. *BioRxiv*. <https://doi.org/10.1101/2020.02.24.962282>.
129. Feng, J., Zhang, C., Lischinsky, J.E., Jing, M., Zhou, J., Wang, H., Zhang, Y., Dong, A., Wu, Z., Wu, H., et al. (2019). A genetically encoded fluorescent sensor for rapid and specific in vivo detection of norepinephrine. *Neuron* 102, 745–761. e8.
130. Dong, A., He, K., Dudok, B., Farrell, J.S., Guan, W., Liput, D.J., Puhl, H.L., Cai, R., Duan, J., Albarran, E., et al. (2020). A fluorescent sensor for spatiotemporally resolved endocannabinoid dynamics. *BioRxiv*. <https://doi.org/10.1101/2020.10.08.329169>.
131. Mignocchi, N., Krussel, S., Jung, K., Lee, D., and Kwon, H. (2020). Development of a genetically-encoded oxytocin sensor. *BioRxiv*. <https://doi.org/10.1101/2020.07.14.202598>.
132. Ding, K., Han, Y., Seid, T.W., Buser, C., Karigo, T., Zhang, S., Dickman, D.K., and Anderson, D.J. (2019). Imaging neuropeptide release at synapses with a genetically engineered reporter. *eLife* 8, e46421.
133. Beyene, A.G., Alizadehmojarad, A.A., Dorlhiac, G., Goh, N., Streets, A.M., Kral, P., Vukovic, L., and Landry, M.P. (2018). Ultralarge modulation of fluorescence by neuromodulators in carbon nanotubes functionalized with self-assembled oligonucleotide rings. *Nano Lett.* 18, 6995–7003.
134. Beyene, A.G., Delevich, K., Del Bonis-O'Donnell, J.T., Piekarski, D.J., Lin, W.C., Thomas, A.W., Yang, S.J., Kosillo, P., Yang, D., Prounis, G.S., et al. (2019). Imaging striatal dopamine release using a nongenetically encoded near infrared fluorescent catecholamine nanosensor. *Sci. Adv.* 5, eaaw3108.
135. Jeong, S., Yang, D., Beyene, A.G., Del Bonis-O'Donnell, J.T., Gest, A.M.M., Navarro, N., Sun, X., and Landry, M.P. (2019). High-throughput evolution of near-infrared serotonin nanosensors. *Sci. Adv.* 5, eaay3771.
136. Bonis-O'Donnell, J.T.D., Page, R.H., Beyene, A.G., Tindall, E.G., McFarlane, I.R., and Landry, M.P. (2017). Dual near-infrared two-photon microscopy for deep-tissue dopamine nanosensor imaging. *Adv. Funct. Mater.* 27, 1702112.
137. Yang, W., and Yuste, R. (2017). In vivo imaging of neural activity. *Nat. Methods* 14, 349–359.
138. Allegra Mascaró, A.L., Silvestri, L., Sacconi, L., and Pavone, F.S. (2015). Towards a comprehensive understanding of brain machinery by correlative microscopy. *J. Biomed. Opt.* 20, 61105.
139. Vite, C.H., McGowan, J.C., Niogi, S.N., Passini, M.A., Drobatz, K.J., Haskins, M.E., and Wolfe, J.H. (2005). Effective gene therapy for an inherited CNS disease in a large animal model. *Ann. Neurol.* 57, 355–364.

140. Nance, E., Zhang, F., Mishra, M.K., Zhang, Z., Kambhampati, S.P., Kannan, R.M., and Kannan, S. (2016). Nanoscale effects in dendrimer-mediated targeting of neuroinflammation. *Biomaterials* 101, 96–107.
141. Schoch, K.M., and Miller, T.M. (2017). Antisense oligonucleotides: translation from mouse models to human neurodegenerative diseases. *Neuron* 94, 1056–1070.
142. Hinderer, C., Bell, P., Katz, N., Vite, C.H., Louboutin, J.P., Bote, E., Yu, H., Zhu, Y., Casal, M.L., Bagel, J., et al. (2018). Evaluation of intrathecal routes of administration for adeno-associated viral vectors in large animals. *Hum. Gene Ther.* 29, 15–24.
143. Blasberg, R.G., Patlak, C., and Fenstermacher, J.D. (1975). Intrathecal chemotherapy: brain tissue profiles after ventriculocisternal perfusion. *J. Pharmacol. Exp. Ther.* 195, 73–83.
144. Landis, M.S., Boyden, T., and Pegg, S. (2012). Nasal-to-CNS drug delivery: where are we now and where are we heading? An industrial perspective. *Ther. Deliv.* 3, 195–208.
145. Dal Magro, R., Ornaghi, F., Cambianica, I., Beretta, S., Re, F., Musicanti, C., Rigolio, R., Donzelli, E., Canta, A., Ballarini, E., et al. (2017). ApoE-modified solid lipid nanoparticles: a feasible strategy to cross the blood-brain barrier. *J. Control Release.* 249, 103–110.
146. Jenkins, S.I., Weinberg, D., Al-Shakli, A.F., Fernandes, A.R., Yiu, H.H.P., Telling, N.D., Roach, P., and Chari, D.M. (2016). Stealth nanoparticles evade neural immune cells but also evade major brain cell populations: implications for PEG-based neurotherapeutics. *J. Control Release.* 224, 136–145.
147. Song, E., Gaudin, A., King, A.R., Seo, Y.E., Suh, H.W., Deng, Y., Cui, J., Tietjen, G.T., Huttner, A., and Saltzman, W.M. (2017). Surface chemistry governs cellular tropism of nanoparticles in the brain. *Nat. Commun.* 8, 15322.
148. Dante, S., Petrelli, A., Petrini, E.M., Marotta, R., Maccione, A., Alabastri, A., Quarta, A., De Donato, F., Ravasenga, T., Sathya, A., et al. (2017). Selective targeting of neurons with inorganic nanoparticles: revealing the crucial role of nanoparticle surface charge. *ACS Nano* 11, 6630–6640.
149. Godin, A.G., Varela, J.A., Gao, Z., Danne, N., Dupuis, J.P., Lounis, B., Groc, L., and Cognet, L. (2017). Single-nanotube tracking reveals the nanoscale organization of the extracellular space in the live brain. *Nat. Nanotechnol.* 12, 238–243.
150. Soria, F.N., Paviolo, C., Doudnikoff, E., Arotcarena, M.L., Lee, A., Danne, N., Mandal, A.K., Gosset, P., Dehay, B., Groc, L., et al. (2020). Synucleinopathy alters nanoscale organization and diffusion in the brain extracellular space through hyaluronan remodeling. *Nat. Commun.* 11, 3440.
151. Godin, A.G., Setaro, A., Gandil, M., Haag, R., Adeli, M., Reich, S., and Cognet, L. (2019). Photoswitchable single-walled carbon nanotubes for super-resolution microscopy in the near-infrared. *Sci. Adv.* 5, eaax1166.
152. Lesniak, A., Kilinc, D., Blasiak, A., Galea, G., Simpson, J.C., and Lee, G.U. (2019). Rapid growth cone uptake and dynein-mediated axonal retrograde transport of negatively charged nanoparticles in neurons is dependent on size and cell type. *Small* 15, e1803758.
153. Satpute-Krishnan, P., DeGiorgis, J.A., and Bearer, E.L. (2003). Fast anterograde transport of herpes simplex virus: role for the amyloid precursor protein of Alzheimer's disease. *Aging Cell.* 2, 305–318.
154. Kramer, T., Greco, T.M., Taylor, M.P., Ambrosini, A.E., Cristea, I.M., and Enquist, L.W. (2012). Kinesin-3 mediates axonal sorting and directional transport of alphaherpesvirus particles in neurons. *Cell. Host. Microbe* 12, 806–814.
155. Hopkins, L.E., Patchin, E.S., Chiu, P.L., Brandenberger, C., Smiley-Jewell, S., and Pinkerton, K.E. (2014). Nose-to-brain transport of aerosolised quantum dots following acute exposure. *Nanotoxicology* 8, 885–893.
156. Filler, A.G., Whiteside, G.T., Bacon, M., Frederickson, M., Howe, F.A., Rabinowitz, M.D., Sokoloff, A.J., Deacon, T.W., Abell, C., Munglani, R., et al. (2010). Tri-partite complex for axonal transport drug delivery achieves pharmacological effect. *BMC Neurosci.* 11, 8.
157. He, K., Luo, W., Zhang, Y., Liu, F., Liu, D., Xu, L., Qin, L., Xiong, C., Lu, Z., Fang, X., and Zhang, Y. (2010). Intercellular transportation of quantum dots mediated by membrane nanotubes. *ACS Nano* 4, 3015–3022.
158. Tosi, G., Vilella, A., Chhabra, R., Schmeisser, M.J., Boeckers, T.M., Ruozi, B., Vandelli, M.A., Forni, F., Zoli, M., and Grubbs, A.M. (2014). Insight on the fate of CNS-targeted nanoparticles. Part II: intercellular neuronal cell-to-cell transport. *J. Control Release.* 177, 96–107.
159. Zhou, T., Hong, G., Fu, T.M., Yang, X., Schuhmann, T.G., Viveros, R.D., and Lieber, C.M. (2017). Syringe-injectable mesh electronics integrate seamlessly with minimal chronic immune response in the brain. *Proc. Natl. Acad. Sci. U S A.* 114, 5894–5899.
160. McKenzie, J.L., Waid, M.C., Shi, R., and Webster, T.J. (2004). Decreased functions of astrocytes on carbon nanofiber materials. *Biomaterials* 25, 1309–1317.
161. Yang, D., Yang, S.J., Del Bonis-O'Donnell, J.T., Pinals, R.L., and Landry, M.P. (2020). Mitigation of carbon nanotube neurosensor induced transcriptomic and morphological changes in mouse microglia with surface passivation. *ACS Nano* 14, 13794–13805.
162. Gu, X., Song, Q., Zhang, Q., Huang, M., Zheng, M., Chen, J., Wei, D., Chen, J., Wei, X., Chen, H., et al. (2020). Clearance of two organic nanoparticles from the brain via the paravascular pathway. *J. Control Release.* 322, 31–41.
163. Matarese, B.F.E., Feyen, P.L.C., de Mello, J.C., and Benfenati, F. (2019). Sub-millisecond control of neuronal firing by organic light-emitting diodes. *Front. Bioeng. Biotechnol.* 7, 278.
164. Qiu, W., Zhou, J., Chen, Y., Su, M., Li, G., Zhao, H., Gu, X., Meng, W., Wang, C., Xiao, Y., et al. (2017). A portable ultrasound system for non-invasive ultrasonic neuro-stimulation. *IEEE Trans. Neural Syst. Rehabil. Eng.* 25, 2509–2515.
165. Liu, J., Kim, Y.S., Richardson, C.E., Tom, A., Ramakrishnan, C., Birey, F., Katsumata, T., Chen, S., Wang, C., Wang, X., et al. (2020). Genetically targeted chemical assembly of functional materials in living cells, tissues, and animals. *Science* 367, 1372–1376.