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Review

Freestanding nanomaterials for subcellular neuronal interfaces

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SUMMARY

Current technological advances in neural probing and modulation have enabled an extraordinary glimpse into the intricacies of the nervous system. Particularly, nanomaterials are proving to be an incredibly versatile platform for neurological applications owing to their biocompatibility, tunability, highly specific targeting and sensing, and long-term chemical stability. Among the most desirable nanomaterials for neuroengineering, freestanding nanomaterials are minimally invasive and remotely controlled. This review outlines the most recent developments of freestanding nanomaterials that operate on the neuronal interface. First, the different nanomaterials and their mechanisms for modulating neurons are explored to provide a basis for how freestanding nanomaterials operate. Then, the three main applications of subcellular neuronal engineering—modulating neuronal behavior, exploring fundamental neuronal mechanism, and recording neuronal signal—are highlighted with specific examples of current advancements. Finally, we conclude with our perspective on future nanomaterial designs and applications.

INTRODUCTION

One of the most pressing challenges modern medicine faces today is treating neurological disorders. To treat these disorders, the underlying mechanisms that govern the nervous system to target the disease must be comprehended. Traditionally, the signaling pathways of the brain's neuronal circuits were studied through attached electrodes that restrict our scope to a few isolated neurons rather than the millions of neurons involved in a typical neural circuit (Kumar et al., 2017). Without accounting for the great complexity of neural networks, efforts to significantly improve neural technology on the subcellular interface are hindered. Moreover, neural probes such as the Utah and Michigan arrays are limited by their mechanical invasiveness and imprecision in recording neuronal activity, making it desirable for new neural technologies to expand specific capabilities and minimize dimensions for a more seamless integration (Chen et al., 2017). Moreover, current disease therapies are nonspecific and have high mortality rates (Kumar et al., 2017), invigorating research efforts into developing new neurotherapeutic interventions that can target molecules with high specificity. Among the many new technologies proposed, one stands out for its versatility and specificity.

Freestanding nanomaterials are standalone nanostructures that operate close to the neuronal interface. These nanomaterials are among the most desirable neuromodulation technology because of their minimal invasiveness and ability to remotely control neuronal signaling. Freestanding nanomaterials are preferred over implantable electronic devices because they do not require complex assembly processes and are administered like a drug, making it easier to remotely adjust and control (Parameswaran et al., 2018). And by virtue of its nanoscale size, nanomaterials display a unique set of physical and biochemical properties that facilitates its powerful functionality (Shi et al., 2020). Its reduced mechanical rigidity promotes a seamless integration with the subcellular interface, enhancing biocompatibility and chemical stability. Additionally, it enables high resolution single-molecule detection (Li et al., 2020), allowing for high spatial specificity and administration in a drug-like fashion. Overall, freestanding nanomaterials hold much potential as a remotely controlled subcellular platform to spearhead innovative therapies, adding more precision to imaging, recording, and modulation of neurons.

This article will first highlight the mechanism of neuronal modulation for different types of freestanding nanomaterials. Modulation, or regulation, of neurons specifically stimulates action potentials and is one

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https://doi.org/10.1016/j.isci.2021.103534

of the key methods for treating neurological diseases. When a neuron receives enough signals from neighboring neurons to depolarize beyond threshold potential, the neuron then sends its electrical signal in the form of an action potential down its axon and to other neurons. Through this straightforward signaling mechanism, billions of neurons can propagate signals to each other within milliseconds to regulate the body's ability to grow, adapt, and heal. But when their functional integrity is compromised, neurons become diseased and are unable to properly regulate signals. Thus, modulating neuron signaling has been a crucial goal in neuroengineering to treat diseases and enhance neural functionality, with freestanding nanomaterials paving the way for improved modulation. Then, the article will introduce the three major applications of freestanding nanomaterials, namely the modulation of neuronal behavior, exploration of fundamental signaling mechanisms, and enhancement of intraneuronal recording.

MECHANISMS OF NEURONAL MODULATION

Out of the many approaches of freestanding nanomaterials to neuronal engineering, the most commonly used mechanisms to modulate neurons are defined below and grouped according to the type of nanomaterial.

Organic conductive nanomaterial

Organic conductive nanomaterials have sparked great interest in their ability to electrically modulate neurons. These polymeric hydrogel nanomaterials are often used as supportive scaffolds which satisfy the biochemical and biophysical microenvironment needs for optimal neural functioning while allowing for electrical stimulation to control the activities of the neuron (Farokhi et al., 2021). Organic semiconductors can also inhibit or stimulate action potentials in neurons through light excitation (Leccardi et al., 2020). For example, optical controlling of conjugated polymer films interfaced on neurons can promote neuronal silencing (Feyen et al., 2016).

For neural tissue engineering, conductive polymeric hydrogels are particularly useful owing to similarities in their mechanical properties and three-dimensional structure with the native extracellular matrix (Farkhondehnia et al., 2018) and are used to establish a bioelectronic interface with ions and electrons as charge carriers to enable direct signal transduction (Liang et al., 2021). Neuronal activity can be modulated electrochemically (Nam and Park, 2020) by first injecting a nanodevice that produces NO, a gaseous signaling molecule, through electrochemical nitrite reduction. By remotely applying an external voltage, a nitrite reduction at the cathode is induced and NO is produced to modulate neuronal signaling. Moreover, organic conductive nanomaterials derive their high electrical conductivity from alternating single and double bonds with overlapping π–π interactions (Song and George, 2017). Minimized mechanical mismatch with soft biological tissue have also extended their functional lifetimes, permitting accurate placement of the nanomaterial in specific regions of the curvilinear brain tissue (Ganji et al., 2018). Examples of widely used conductive polymers include polyaniline (PANI) and poly(3,4-ethylenedioxythiophene): polystyrene sulfonate (PEDOT: PSS). PANI is useful for its high availability, high conductivity, easy synthesis, and low cost, but has suboptimal biocompatibility. Thus, it is often combined with more biodegradable and biocompatible polymers to minimize immunogenic reactions (Figure 1A) (Boni et al., 2018). PEDOT: PSS is PEDOT doped with polystyrene sulfonate, which significantly increases its ability to retain high electrical conductivity under physiological conditions (Magaz et al., 2020). Living neurons have also been genetically targeted to chemically synthesize PEDOT or PANI on its plasma membrane through single-enzyme-facilitated polymerization. This method not only maintains neuronal viability but also changes the properties of the membrane to allow for modulation (Liu et al., 2020).

Optical nanomaterial

Within the field of minimally invasive neuromodulation techniques, the advent of non-genetic optomodulation, which uses light to modulate and stimulate the activity of neurons, has revolutionized the field of neuroscience. Optical nanomaterials transduce light signals, such as lasers, into an electrical/thermal/chemical signal to elicit specific neural responses. Current methods either tether the freestanding nanomaterial to the neuronal plasma membrane (Parameswaran et al., 2018) or to various components of the membrane (Carvalho-de-Souza et al., 2015; Efros et al., 2018). Photothermal stimulation is achieved by converting the energy from laser pulses into thermal energy, which diffuses to local neuronal membrane regions. The rate of membrane temperature change is proportional to the rate of membrane capacitance change and results in a capacitive current. Once the capacitive current exceeds the threshold, an action potential

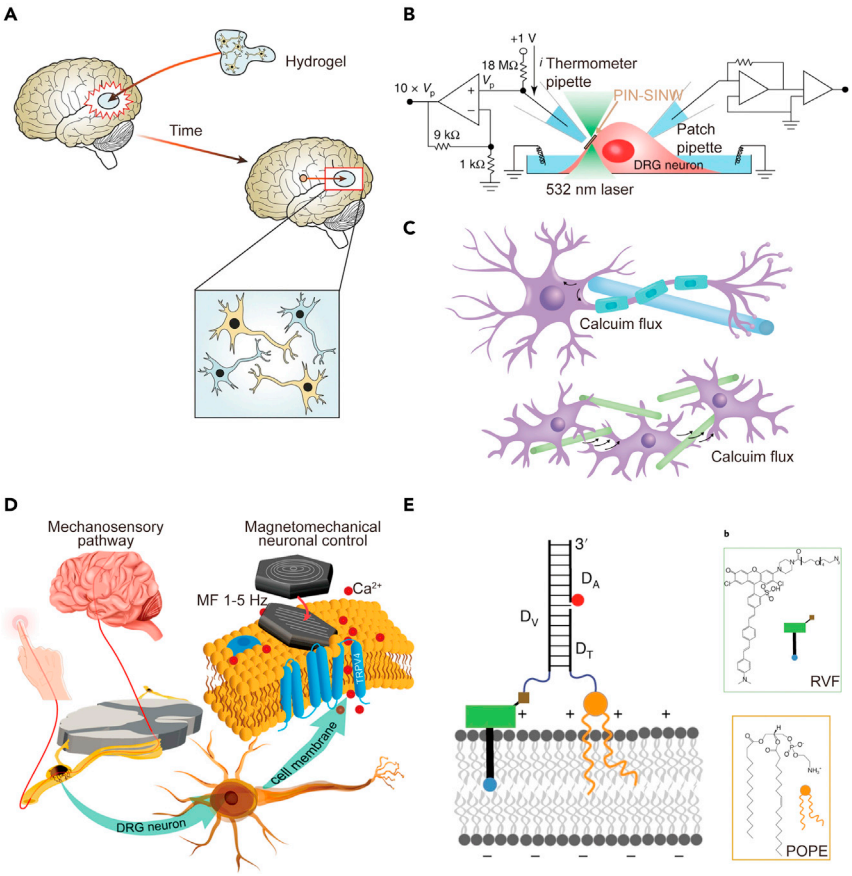

Figure 1. Different mechanisms of freestanding nanomaterials on neural interface

(A) Illustration of polymeric hydrogel seeded with neural cells implanted into brain cavity for neural regeneration. Reproduced with permission (Boni et al., 2018).Copyright 2018, Springer Nature.

(B) Example setup of photothermal stimulation with 532 nm laser pulses of primary dorsal root ganglion neurons by coaxial p-type/intrinsic/n-type Si nanowires (PIN SiNWs). Reproduced with permission (Parameswaran et al., 2018).Copyright 2018, Springer Nature.

(C) Photostimulation of SiNWs that underwent surface-level texturing shows significantly increased photothermal efficiency through stimulation of local calcium dynamics to propagate action potentials.

(D) Illustration of magnetic nanodiscs transducing torque forces to activate mechanosensitive ion channels on neuronal membrane. Reproduced with permission (Gregurec et al., 2020).Copyright 2020, American Chemical Society.

(E) Schematic diagram of a DNA-based nanodevice tethered to the plasma membrane to measure absolute membrane potential.

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will be invoked regardless of the duration of the laser pulse stimulation (Rastogi et al., 2020). On the other hand, photoelectrochemical stimulation is achieved by directly eliciting an action potential through laser pulse stimulation (Figure 2A) (Parameswaran et al., 2018). Optical nanomaterials come in various forms, such as nanowires (Fang et al., 2018), nanoparticles (Zhang et al., 2016), nanowire templates (Jiang et al., 2016), nanotubes (Barrejon et al., 2019), and even quantum dots (Efros et al., 2018). One example of a widely used optical nanomaterial in biological applications is silicon-based nanostructures, which are desired for their high tunability. To quantify the fundamental physicochemical processes of silicon's photoresponse, the following formula is derived from Ohm's law:

$$\Delta I_{light}(t) = \left(\frac{R_0}{R(t)} - 1\right) \times I_0 + \Delta I_{electric}(t)$$

where $\Delta I_{light}(t)$ is the light-generated current induced by laser pulses of a certain duration on the Si surface, R_0 is the dark-state pipette resistance, R(t) is the pipette resistance tip, I_0 is the dark-state current, and

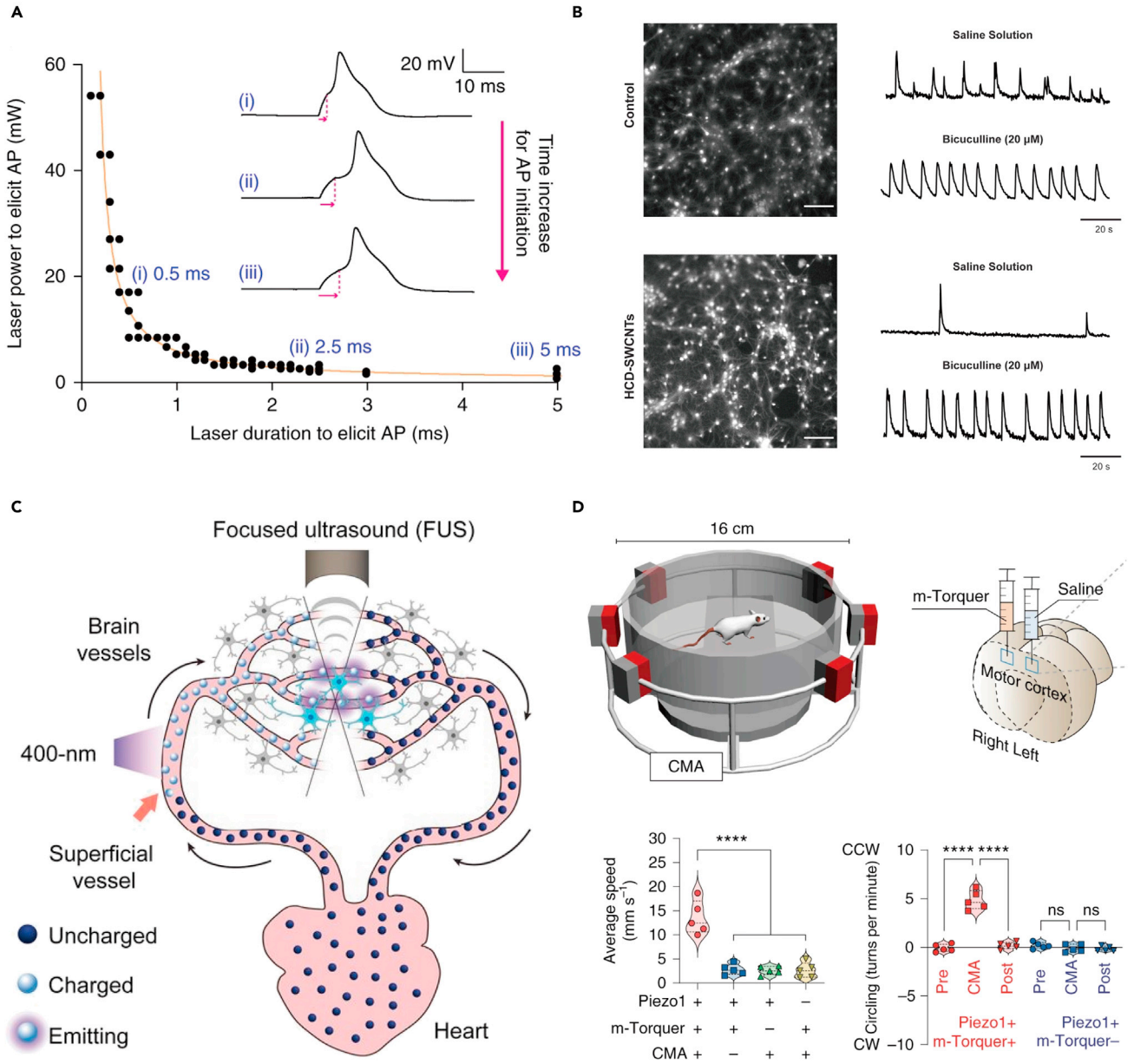

Figure 2. Remote modulation of neuronal signaling

(A) Excitability curves of neurons linked with single PIN SiNWs being laser-stimulated with 532 nm pulses at (I) 0.5 ms, (ii) 2.5 ms, and (iii) 5 ms. Displays laser power and duration required to induce action potential. Reproduced with permission (Parameswaran et al., 2018).Copyright 2018, Springer Nature.

(B) Control group of normal neuronal cultures (top left) and experimental group of neurons with higher degree of cross-linked single-walled carbon nanotubes (HCD-SWCNTs) (bottom left). Scale bar: 50 μm. Recorded repetitive Ca^{2+} events spontaneously (top) or bicuculline-induced (bottom). Reproduced with permission (Barrejon et al., 2019).Copyright 2019, American Chemical Society.

(C) Schematic of "sono-optogenetics" showing mechanoluminescent nanoparticles circulating in blood before applying 400 nm laser pulses to enable deep-brain stimulation. Reproduced with permission (Wu et al., 2019).Copyright 2019, United States National Academy of Sciences.

(D) Setup of m-Torquer system, composed of a magnetic torquer and rotating circular magnet array (CMA) (top left). Injection of m-Torquer nanostructures with saline into motor cortex of mouse (top right). Statistics of average mouse movement speed (bottom left). Analysis of movement direction, counterclockwise (CCW) and clockwise (CW) (bottom right).

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$\Delta I_{electric}(t)$ is the photovoltaically induced ionic current (Jiang et al., 2019). In addition to inorganic optical nanostructures, organic amphiphilic azobenzene derivatives can also respond to external light signals to modulate neurons. Through the isomerization process of the azobenzene photoswitch on the inner leaflet

of the membrane bilayer, the neuron membrane becomes thinner, leading to an increase of membrane capacitance at the millisecond scale (DiFrancesco et al., 2020).

Mechanostimulative nanomaterials

Another type of freestanding neuromodulation device utilizes mechanoresponsive nanomaterials. This class includes nanomaterials activated by ultrasound, piezoelectric nanomaterials, and magnetic nanomaterials. Ultrasound has been found to activate neurons by way of mechanical, rather than thermal, stimulation (Kubanek et al., 2018). The neuron's mechanosensitive ion channels, such as the transient receptor potential channel V4 (TRPV4), undergo conformational changes under mechanical force, increasing its permeability to select ions and thereby inducing electrical signaling (Gu and Gu, 2014). Next, piezoelectric materials describe the crystal's ability to convert between mechanical and electrical energy (Hinchet et al., 2018). Nanoparticles such as tetragonal barium titanate can be harnessed as nanotransducers for their piezoelectric properties. They mainly associate with the plasma membrane and generate electricity in response to mechanical ultrasound perturbations. The electrical current can then mediate membrane depolarization, giving rise to signaling mechanisms such as the flux in calcium ion concentrations (Marino et al., 2015). On the other hand, magnetic nanoparticles can transduce torque at localized areas of the cell membrane under alternating magnetic fields (Gregurec et al., 2020). The delivery of localized torque forces can activate mechanosensitive ion channels and have been shown to stimulate mice neurons (Lee et al., 2021).

Organic nanocomposites

Organic nanocomposites are normally assembled from biological macromolecules such as DNA (Qi et al., 2018). Nanocomposites include DNA-based nanodevices (Saha et al., 2015) and polymer nanoparticles (Edelbrock et al., 2018). DNA-based nanodevices present an exciting new opportunity to improve resolution at the subcellular level. Instead of being tethered to the leaflets of the neuronal plasma membrane, these nanodevices are internalized into the neuron and designed to localize in organelles along the endo-lysosomal pathway (Saha et al., 2015). Once there, they can measure and probe various biological signals, such as recording the neuron's absolute membrane potential (Saminathan et al., 2021). On the other hand, polymer nanoparticles that incorporate a mimetic sequence can activate specific tyrosine kinases to stimulate neuronal differentiation and maturation (Edelbrock et al., 2018).

APPLICATIONS IN SUBCELLULAR NEURONAL ENGINEERING

Among the many promising applications of freestanding nanomaterials in neuroengineering, three prominent areas of research can undergo significant advancements through nanomaterials—modulation of neuron behavior by activating and controlling signal transduction pathways, exploration of the fundamental mechanisms that underlie signaling pathways in neurons, and enhanced intraneuronal resolution.

Modulation of neuron signaling

One class of neuromodulation devices utilizes non-genetic optomodulation. An example of such a device is the coaxial p-type/intrinsic/n-type (PIN) silicon nanowires (SiNWs) with atomic Au on the surface. PIN SiNWs have shown high tunability and specificity in wireless, free-standing photoelectrochemical modulation of primary rat dorsal root ganglion neurons. The atomic Au acts as the catalyst by reducing the kinetic energy barrier required for generating the photoelectrochemical current. In an in-vitro experiment, the PIN SiNWs were drop-casted onto the cultured rat neurons and induced action potentials identical to those induced by classical external electrodes upon laser stimulation. Furthermore, scanning electron microscopy (SEM) confirmed that the SiNWs form close interactions with the neural membranes (Figure 1B) (Parameswaran et al., 2018). With their small radial dimensions, SiNWs can also offer highly localized neural modulation. However, the single-crystalline form of SiNWs causes poor photothermal efficiency due to their rapid heat dissipation and limited light absorption. Instead, Fang et al. showed that the surface modification of SiNWs to form textured porous SiNWs enhances its photothermal efficiency by photostimulation of extracellular junctions to promote local calcium dynamics and therefore action potential signaling (Figure 1C). They synthesized the desired porous texture through periodic pressure perturbations to allow Au to deposit and diffuse at determined locations (Fang et al., 2018). Jiang et al. further investigated the viability of heterogeneous, biomimetic structural and chemical forms of silicon in expanding exciting new avenues for biomaterials research. Referred to as amorphous silicon, it is formed by a chemical vapor deposition process using mesoporous silica as its template (Jiang et al., 2016). It should be noted that while

silicon-based materials are widely used for biological applications, there is still an overall limited understanding of the interactions between freestanding silicon devices like PIN SiNWs and biological interfaces. To overcome this, Jiang et al. have introduced a rational design principle that shows silicon's light-induced capacitive and Faradaic effects, quantifying its usefulness for neural modulation in humans and other large non-primate animals (Jiang et al., 2018).

Si-based nanostructures are not the only optical neuromodulation devices under development — carbon-based structures have also attracted much attention (Figure 2B) (Barrejon et al., 2019). Freestanding carbon nanotube (CNT) films are desired for their large surface area, low density, thermal and electrical conductivity, and strength that can control adhesion, survival, and growth of neurons through axonal and synaptic signaling modulation. Despite its difficult translation to practical applications due its poor processability at the macroscopic scale, CNT films have been improved upon by cross-linkage. Additionally, Rastogi et al. explored the use of nanowire-templated 3D fuzzy graphene for advancing therapeutic applications as well as our understanding of basic neural signaling mechanisms. Particularly, the addition of graphene enables easy surface customization that endows it with excellent optical and photothermal properties to electrically stimulate neurons with lower laser energies than those required for Si-based nanodevices (Rastogi et al., 2020).

Another optical neuromodulation device of interest is gold nanoparticles (AuNP). Keeping AuNPs close to the plasma membrane of neurons can stimulate action potential generation using only millisecond-long pulses of laser light. Carvalho-de-Souza et al. hypothesized that this optocapacitance mechanism is enabled photothermally, and successfully defended this hypothesis by showing that reducing laser pulses would decrease the minimum laser energy required to generate an action potential (Carvalho-de-Souza et al., 2018). For more specific, highly localized stimulation of neurons, AuNPs were conjugated to three membrane proteins of dorsal root ganglion neurons. The AuNPs were found to only heat their immediate environment, with neuron depolarization achieved dependent on the rate of change of temperature rather than the actual temperature itself. This allows for neuron stimulation without reaching damaging temperature levels and also keeps the heating duration short (Carvalho-de-Souza et al., 2015). Current methods tether AuNPs to the membrane ion channels on the extracellular leaflet of the plasma membrane, but putting a cholesterol conjugate on the AuNP may provide a more generalized and facile way to attach AuNP to both sides of the membrane (Carvalho-de-Souza et al., 2019).

A common issue with most neurological diseases is oxidative stress. To counteract this, lipid-coated polydopamine nanoparticles, an example of a near-infrared light-responsive nanomaterial, can act as neuroprotective agents and antioxidants along with being a photothermal conversion platform to stimulate neuronal activity. Their ability to convert photothermally can transform the energy from NIR (near-infrared) laser stimulation to increase the intracellular temperature of neuron-like cells, stimulating their activity and growth (Battaglini et al., 2020). Moreover, Chen et al. proposed using upconversion nanoparticles that absorb tissue penetrating NIR light and emit visible light to optogenetically stimulate dopamine release in neurons located in the ventral tegmental area. Among various effects, this release triggered memory recall and silenced seizure in hippocampal excitatory cells, underscoring its capacity for remote neural therapy (Chen et al., 2018). On top of sensing NIR light patterns, near-infrared-light-responsive nanomaterials can also enable remote and noninvasive modulation of signal transduction pathways. From upconversion nanoparticles to microcapsules containing plasmonic gold nanoparticles, NIR-sensitive nanomaterials offer new avenues in wireless optogenetic control (Zhang et al., 2016).

However, to reach the deep recesses of the brain, non-genetic optomodulation often requires resorting to invasive methods such as fiber optics or craniotomy due to poor photon tissue penetration. To overcome this, Wu et al. devised a new method called "sono-optogenetics", which uses mechanoluminescent nanoparticles that act as local light sources in the brain when triggered by brain-penetrant focused ultrasound (FUS) (Figure 2C). Instead of the conventional "outside-in" approach utilized by the previously mentioned research in which the light source is from the outside, they employ an "inside-out" approach in which the light source, the nanoparticles, are delivered through the circulatory system, expanding the functionality of non-genetic optomodulation to the deepest regions of the brain (Wu et al., 2019).

Magnetic nanomaterials are another class of remotely controlled neuromodulation devices that use magnetic fields rather than light. In contrast to optical nanomaterials, which directly induce electrical signals on

the cell membrane, magnetic nanomaterials regulate the neuron by mechanically activating ion channels that generates cell depolarization. Gregurec et al. revealed that magnetic nanodisc-induced torque activated mechanotransduction ion channels, driving neural signaling. Adjusting the vortex parameters of magnetic nanodiscs may be exploited in future studies involving nanoscale force transduction of nervous systems (Figure 1D) (Gregurec et al., 2020). Another magnetic neurostimulation toolkit named m-Torquer delivers localized torque forces in the piconewton scale. Lee et al. introduced m-Torquer which combines the advantages of magnetism —deep penetration and freestanding interventions— with long working distance and neural targeting to create a versatile neuromodulation nanodevice that has been shown to stimulate mice neurons expressing the mechanosensitive ion channel Piezo1 and can potentially be applied to larger animals such as primates (Figure 2D) (Lee et al., 2021). On the other hand, when magnetic nanoparticles heat up under alternating magnetic fields, thermally sensitive liposomes would release small molecules. Along with the chemogenetic activation of engineered receptors, this remote chemomagnetic modulation of neurons allows for temporal precision and spatial targeting of neurons (Rao et al., 2019). Additionally, Kozielski et al. introduced injectable, magnetoelectric nanoelectrodes that remotely transmit electrical signals to the deep brain of mice by applying an external magnetic field. This modulation has been shown to shape mouse behavior and regulate regions of the corticobasal ganglia-thalamocortical circuit (Kozielski et al., 2021).

Neuromodulation techniques are not restricted to the positive regulation of neurons through stimulating action potentials and can also silence neural activity. For example, biocompatible gold nanostars (AuNS) can silence neuronal activity (Lee et al., 2018a). Moreover, both plasmonic gold nanofilms and plasmonic nanoparticles have been shown to effectively inhibit neuronal activity photothermally (Lee et al., 2018b). In fact, the methods of silencing neuronal activity have drastically expanded besides the optogenetically inhabitation approach (Wiegert et al., 2017).

Exploration of underlying fundamental mechanisms

Nanostructures offer an unprecedented platform to explore the fundamental mechanisms of signal transduction. For example, they can be used to explore the biophysical mechanisms behind ultrasound's ability to stimulate neurons and elicit behavior. To determine whether the neuron is activated by thermal or mechanical stimulation, Kubanek et al. applied pulsed ultrasound to a behavioral-genetic assay of the Caenorhabditis elegans's neurons. They showed that thermal-insensitive mutants responded similarly to wild-type, whereas mechanoinsensitive mutants exhibited no response. Their findings underscore the mechanical, rather than thermal, basis for neuron activation by ultrasound (Kubanek et al., 2018). Additionally, our ability to deliver nanotherapeutics to injured neurons is restrained by our poor understanding of the effects of endosomal trafficking on axon growth. Steketee et al. suggested engineering superparamagnetic particles (MNPs) as TrkB agonist antibodies that are endocytosed into signaling endosomes. Once inside, the MNP signaling endosomes can be controlled by a magnetic field to alter motility and neuronal growth. MNPs provide a promising platform for nanotherapeutic delivery as well as subcellular localization studies (Steketee et al., 2011). Furthermore, brain-derived neurotrophic factor (BDNF) is a neurotrophin that specifically binds to the tyrosine kinase B receptor to stimulate neuronal differentiation and maturation, helping the central nervous system (CNS) recover from an injury. However, native BDNF-based therapies have shown little success in clinical trials. Instead, Edelbrock et al. found that incorporating a BDNF mimetic sequence into a supramolecular peptide amphiphile filamentous nanostructure can similarly activate the tyrosine kinase B receptor to create a highly functional bioactive matrix for injured areas in the CNS (Edelbrock et al., 2018).

Furthermore, nanostructures can delve deeper into basic biochemical processes in neurons. First, our understanding of nitric oxide's (NO) role in the nervous system is curtailed by the lack of tools to see it function real-time in live cells. Park et al. have proposed locally generating NO through an electrochemical denitrification reaction catalyzed by iron sulfide nanoclusters. NO release can then be controlled by tuning the applied voltage, allowing for an optimizable tool that can aid NO research studies (Figure 3A) (Park et al., 2020a). Second, there have been extensive studies revealing the active role that clathrin-mediated endocytosis (CME) proteins play in curving the membrane into a vesicle, such as when neurons take in neurotransmitters, but much less have focused on the effect of the membrane curvature on the surrounding membrane proteins. This is mainly attributed to the lack of tools that offer real-time membrane curvature control in live cells. Zhao et al. used vertically aligned nanostructures to modulate local membrane curvature. Utilizing this novel device revealed that the proteins involved in mediating CME spatially preferred

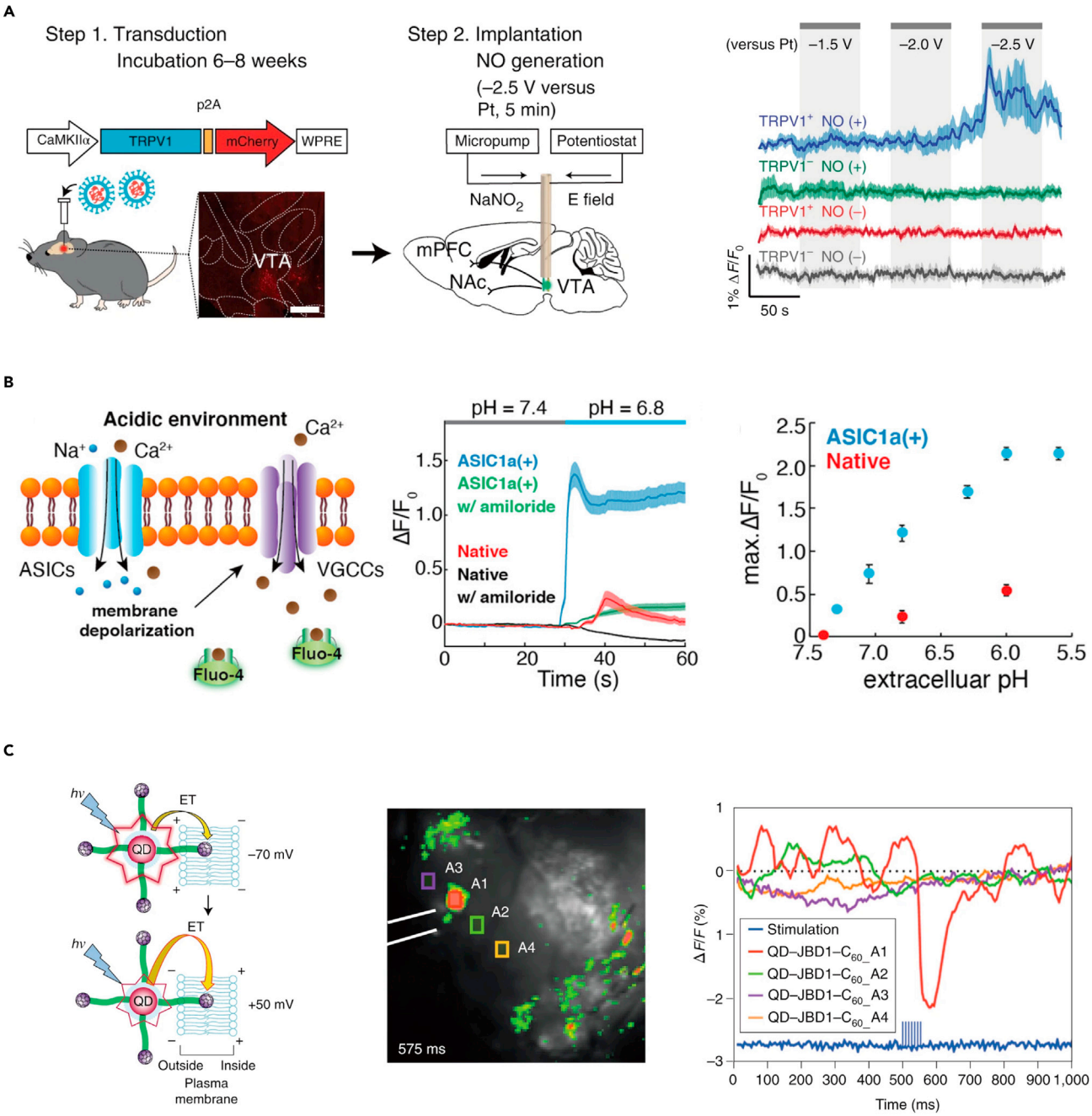

Figure 3. Nanodevices to advance knowledge of underlying mechanisms of the nervous system

(A) Schematic of set up for virus-assisted gene therapy (Step 1) and nanofiber implantation (Step 2) to control local generation of nitric oxide (NO) for studies. Transient receptor potential vanilloid family member 1 (TRPV1) and NO are indicated by fluorescent fluo-4 Ca^{2+} indicators GCaMP6s to record behavior of neuron. Reproduced with permission (Park et al., 2020a).Copyright 2020, Springer Nature.

(B) Acidic extracellular environment activates cation-permeable acid-sensing ion channels (ASICs) before triggering voltage-gated Ca^{2+} channels (VGCCs), leading to depolarization of cell membrane. Averaged fluo-4 fluorescence showing that proton-mediated Ca^{2+} influxes were significantly reduced with the presence of the acid-sensing ion channels (ASICs) blocker amiloride in both the 100 native neurons (black) and 100 ASIC1a+ neurons (green) in comparison to the 100 genetically normal neurons (red) and 100 ASIC1a-overexpressing ($ASIC1a^{+}$) neurons (blue). Reproduced with permission (Park et al., 2020b).Copyright 2020, American Chemical Society.

(C) Illustration of the mechanism of quantum dot (QD)-peptide fullerene (C60) electron transfer (ET)-based nanobioconjugate to record membrane potential *in vivo*. Fluorescence response of four regions of interest (A1, A2, A3, and A4) at a frame of 575 ms. QD-JBD1-C60 is a QD linked to the peptidyl sequence JBD1, with graph showing the average fluorescence response in the four regions of interest.

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high positive curvature over flat and negative curvature, suggesting that membrane curvature may also affect the activities of other proteins and opening new avenues for research (Zhao et al., 2017). Third, studying proton-mediated biochemical processes in neurons have been largely impacted by our lack of tool sets that can control local pH. Park et al. designed a wireless nanotransducer that comprises magnetic nanoparticles embedded in a polyanhydride or polyester scaffold. Tuning noninvasive alternating magnetic fields will increase the temperature within the nanotransducer, accelerating the hydrolytic degradation of polyanhydride and polyester polymers and thereby releasing protons that will decrease local pH. The remote magnetic modulation of local pH activated acid-sensing ion channels and induced intracellular calcium ion influx into the neurons, highlighting its potential for advancing mechanistic understanding of the role of protons in neurons (Figure 3B) (Park et al., 2020b). Moreover, current technology is insensitive to torques induced by motor activities in axonal transport studies. Kaplan et al. experimented with gold nanorod-labeled endosomes and visualized them with multichannel, polarized dark-field microscopy to track rotational and translational motion of the gold nanorods in dorsal root ganglion axons. They observed that translational and rotational dynamics are correlated with each other, and that the gold nanorods tend to align with the orientation of the microtubules (Kaplan et al., 2018). Some nanodevices are internalized into the neuron to modulate and study its intracellular environment, bypassing the challenges surrounding the creation of a stable enough biological-nanomaterial interface present in external nanodevices. Lee et al. demonstrated the viability of completely internalizing nanowires into neurons through TAT cell-penetrating peptides (TAT CPPs). TAT CPP typically delivers cargo composed of molecules as small as nucleic acids to larger molecules such as proteins inside the neuron. Linking TAT CPP to the surface of nanowires has been shown to enable spontaneous internalization of nanowires into primary neurons (Lee et al., 2016). Other devices utilize molecular machines that drill holes through the lipid bilayer to introduce drugs into the cell, release certain molecular compounds, or induce cell death (Garcia-Lopez et al., 2017).

Synapses are the junction in which information is passed from one neuron to the next. Much of our knowledge of synapses comes from voltage-clamp analysis. However, there are glaring errors with voltage-clamping dendritic spines, which contain the vast majority of excitatory synapses. It is crucial to maintain a constant membrane potential for accurate recordings from voltage clamps, which is not possible in most excitatory synapses owing to spine electrical compartmentalization. In other words, the compartmentalization would isolate electrical signals and prevent voltage control of the entire spiny synapse, distorting measurements and raising the question of how accurate our current understanding of synapses is. Instead, Beaulieu-Laroche et al. introduced a new approach that places importance on the concept of synaptic integration compartmentalization when designing and interpreting electrophysiological experiments (Beaulieu-Laroche and Harnett, 2018). However, the extent to which spines compartmentalize voltage remains a contentious idea because of a dearth of devices that can directly measure the voltage of spines. Jayant et al. tested this idea by utilizing quantum-dot-coated nanopipette electrodes, demonstrating that the spine head potentials are significantly larger than those downstream. This suggests that there is both a spine neck resistance that can contribute to voltage compartmentalization and that nanopipettes can act as direct electrical interfaces to aid future research (Jayant et al., 2017).

Recording of intraneural signaling

DNA-based nanodevices present an exciting new opportunity to improve resolution at the subcellular level. To increase our understanding about the role that membrane potential plays in organelle regulation, Saminathan et al. designed Voltair, a fluorescent DNA nanodevice that targets organelles *in situ* in live cells and measures their absolute membrane potential (Figure 1E). Voltair was endocytosed by human embryonic kidney cells and is then tethered to the lumenal leaflet of the organelle of interest. Currently, Voltair can only probe endocytic organelles and the *trans*-Golgi network (Saminathan et al., 2021). Moreover, current small-molecule calcium ion indicators are generally restricted to usage in the cytoplasm or the ER owing to their pH sensitivity. Naryanaswamy et al. injected a fluorescent, DNA-based nanodevice reporter named CalipHluor into the coelomocytes of C. elegans to expand the functionality of calcium ion indicators. CalipHluor is a pH-correctable indicator that can simultaneously and specifically report the pH and calcium ion concentration, bestowing new insights into the role of calcium ions at the organelle level (Narayanaswamy et al., 2019). Additionally, to study the activity and location of subcellular chloride in a pH-independent manner, the DNA nanodevice Clensor was developed. Clensor was created to localize in organelles along the endolysosomal pathway, and quantified the resting chloride concentration of organelles in Drosophila melanogaster, showing that the lumenal lysosomal chloride is regulated by intracellular chloride transporter DmClC-b (Saha et al., 2015). Finally, the pathophysiological link between defective

lysosomes and common neurological disorders such as Parkinson's and Alzheimer's is not yet clear. Based on the idea that different subpopulations of lysosomes have their own distinct functions owing to their differing pH and chloride ion concentration, Leung et al. proposed a DNA-based combination reporter called ChloropHore that can measure the two ions to chemically differentiate the lysosomes. ChloropHore utilized 2-IM (two-ion measurement) technology and was targeted to the lysosomes on human dermal fibroblasts, holding the potential to uncover the underlying principles of lysosome-related diseases (Leung et al., 2019).

In addition to DNA-based nanodevices, quantum dots and nanodiamonds are promising candidates for tracking changes in neurons. Current technology to study brain circuits cannot produce functional 3D neural images that track instantaneous voltage changes within individual neurons. To produce such recordings requires a spatial resolution of 10 nm, on the same scale with the neuronal membrane, and a temporal resolution in the milliseconds, on the same timescale of neural electrical signals. A candidate for solving this persistent gap in neuroscience technology, luminescent nanocrystalline semiconductor quantum dots (QDs) have shown suitability for sensing real-time voltage changes on the neuronal membrane. In addition to meeting the size and timescale requirements for recording brain circuits, it can be conjugated onto peptides for targeted delivery to specific regions of the neuron and exhibits excellent optical stability with low levels of photobleaching and degradation (Figure 3C) (Nag et al., 2017). Moreover, abnormalities in intraneural transport have been linked to higher genetic risk factors in patients, which are implicated in brain diseases like autism and Alzheimer's. As current devices are not specific enough to detect minor genetic abnormalities, Haziza et al. devised a tracking method that uses fluorescent nanodiamonds (FNDs) to measure changes in intraneuronal transport caused by brain-disease-related genetic risk factors. FNDs are endocytosed into mouse hippocampal neurons and are sensitive enough to identify modifications to intraneuronal transport in transgenic neurons (Haziza et al., 2017).

OUTLOOK

Nanomaterials hold much potential to be the basis of a new class of neural devices, opening new avenues in countless areas of neuroscience research. Utilizing nanostructures is only the start toward appreciating the full capacity of the nervous system at the cellular and subcellular level. The nanomaterials and nanodevices described previously are new approaches to reduce mechanical mismatch with neural tissue, avoid complications during clinical translation, and target delivery into specific neurons. Nonetheless, these three areas remain challenging. Future rational design of neural nanodevices needs to be grounded in the physical properties of the neural interface it operates on to minimize mechanical mismatch and increase biocompatibility (Acaron Ledesma et al., 2019). Highly stiff devices tend to activate the body's foreign-body response and cause chronic inflammation in neural tissues, highlighting the importance of using nanomaterials as pliant as biological tissues (Won et al., 2021). Moreover, to reach the deep recesses of the brain, non-genetic optomodulation often requires resorting to invasive methods like fiber optics or craniotomy due to poor photon tissue penetration, highlighting the need for more minimally invasive technology capable of stimulating the deep brain. Next, successful clinical translation requires more research into not only the biocompatibility and functionality of the nanotechnology but also of its long-term fate and potential effects on the surrounding tissue (Benfenati and Lanzani, 2021), concerns surrounding the biosafety of nanomaterials, such as the physiological impact of optically stimulating neurons through heat. Testing gold nanoparticle-mediated laser stimulation on mouse neurons revealed that the cells exhibited a severe and complex stress response, underscoring the need to thoroughly examine the safety and efficacy of laser stimulation techniques (Johannsmeier et al., 2018). Additionally, scaling to clinical translation demands consideration of more practical aspects, such as whether the nanomaterial in question is affordable and easy to process and produce on the macroscopic scale. Finally, targeted delivery of nanomaterials to specific sites and neurons in the brain could greatly advance therapies for neurological diseases. Current technology has a long road ahead to achieve specific multisite drug delivery at the single neuron level (Johannsmeier et al., 2018).

Future nanomaterials design can include the incorporation of the nanomaterials as part of a complex living system, serving as a platform for neuromodulation. This requires that the nanomaterials have a high affinity for the cell, which can be achieved by a constant expression of the relevant protein complexes from the integrated transgenes. Particularly, as glial cells are increasingly recognized as integral to ensure normal nervous system functioning, glial cells can also be implanted with nanodevices to modulate simultaneous signaling. Moreover, even though this article primarily focused on neuronal modulation in regards to

promoting action potentials, there can be more freestanding materials research done on inhibiting neural activity, which are critical to applications such as improving therapies for epilepsy and protecting the brain from excitotoxicity. Other wireless, remote, and battery-free nanodevices can create a more dynamic system to achieve multiscale neural interfaces. In other words, the nanodevices can detect signals on both the nanoscopic level of the subcellular neuronal components as well as the macroscopic level of the organ itself. Moreover, instead of freestanding or permanent implantable devices, transient bioelectronics is another class of technology that can expand applications on the nervous system. After a certain period of time, the bioelectronics biodegrade. In particular, polymeric neural probes have been created that can monitor brain activity for several months rather than several days (Ferlauto et al., 2021). Furthermore, even though the focus of this review was on the various nanostructures used to restore the normal functioning of the nervous system, nanomaterials design can also complement normal functioning by enhancing the nervous system's native abilities. Indeed, new research has explored expanding mammalian vision beyond the 700 nm wavelength using ocular injectable photoreceptor-binding upconversion nanoparticles to perceive even the most sophisticated NIR light patterns. This new vision existed in tandem with native daylight vision, with minimal side effects in mice so far (Ma et al., 2019). Although freestanding nanostructures are still a relatively new field, it already holds much promise for making great strides in exploiting the robust functionality of the mammalian nervous system.

ACKNOWLEDGMENTS

This work was supported by the US Air Force Office of Scientific Research (FA9550-20-1-0387) and the National Science Foundation (NSF CMMI-1848613, NSF DMR-2011854).

AUTHOR CONTRIBUTIONS

J.S. conceived the theme and designed the manuscript structure. E.L. wrote the manuscript. J.S. and B.T. supervised this work and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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