NEUROMODULATION

Neuromodulation with nanoparticles
Ultrasonic drug uncaging shows potential for noninvasive manipulation of the brain

By Raag Airan

Current strategies for clinical neuromodulation are limited by either high invasiveness, low precision, or poor depth of penetration. Deep brain stimulation (DBS) and other electrical strategies for deep brain neuromodulation necessitate the use of invasive device placement. Similarly, optogenetic interventions generally require the placement of a fiber-optic cable into the tissue for light delivery and would necessitate gene therapy. Noninvasive techniques for electrical neuromodulation, such as transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (tDCS), on the other hand, have an overly broad spatial extent and limited depth of penetration.

The National Institutes of Health BRAIN Initiative has therefore identified a critical need for technologies capable of noninvasively modulating the activity of the living brain, at any depth, with high spatial and temporal precision and with high clinical translation potential (1). Our recent work on focused ultrasound and nanotechnology is exactly such a technology (2). I have been interested in new techniques for neuromodulation since completing my graduate studies with Karl Deisseroth at Stanford. For my thesis, I developed the optoXRs, a family of opsin-receptor chimeras that control G protein–coupled signaling pathways using light (3). Using optoXRs, we were able to control not just neuronal firing and intracellular calcium fluxes in the mouse brain but also conditioned place preference, a behavioral model of reward and addiction.

My graduate work, which sought to establish optogenetics as a highly precise neuromodulatory technique, convinced me of the need for better tools to dissect brain circuits. My experiences as a medical student, and now as a practicing physician, have further compelled me to focus on techniques with high clinical translation potential.

ULTRASOUND AS A TOOL FOR NEUROMODULATION

Focused ultrasound (FUS) is a near-ideal modality for clinical noninvasive neuromodulation, because it can efficiently deliver energy at significant depth throughout the body. Clinicians worldwide, including myself, routinely use focused ultrasound to noninvasively ablate deep brain regions (4). FUS can create millimeter-sized sonication foci in magnetic resonance imaging (MRI)-defined brain regions, across intact skin and skull, while the patient is awake and participating in a neuropsychiatric exam.

Several groups have explored using FUS for neuromodulation of the brain and peripheral nerves. This literature extends from the work of the Fry brothers in the 1950s (5) to recent works across a range of preparations, including salamander retinae (6), mice (7, 8), nonhuman primates (9), and even humans (10, 11).

In these experiments, the intensity and duty cycle of the applied ultrasound were low enough that no appreciable tissue temperature rise or damage to the brain parenchyma was likely. However, the mechanism by which FUS yields a neuromodulatory effect is hotly debated and, to some, a matter of controversy (12). Certain investigators go so far as to describe this effect as a probabilistic rather than a deterministic one (13). For clinical applications, a robust and deterministic neuromodulatory mechanism of action is needed.

Prior to the work described here, I had verified through simulation studies that a clinical FUS system can develop a sonication focus in most brain regions that would be targeted for neuromodulation, including both cortical and subcortical locations, and in locations near the skull base (14). I had also experimentally confirmed that blood-brain barrier opening—an application demanding similar sonication pressures and duty cycles as those needed for neuromodulation—can be completed by sonicating across a human skull with the focused ultrasound transducer that I incorporated into the prior simulation (15). The last piece of the puzzle was to identify a robust neuromodulatory mechanism to couple with the efficient energy delivery of FUS.

JUST ADD NANOPARTICLES

In 2015, I turned to FUS-mediated drug delivery, because it is known that pharmacology can provide a robust neuromodulatory effect. I mainly considered methods that did not rely on a tissue temperature rise, because heating alone can bias neural activity and lead to tissue damage and because obtaining a reliable increase in tissue temperature near the skull base is uniquely challenging (4).

Phase-change nanoparticles, on the other hand, provide an ideal drug-delivery approach for neuromodulation. In these particles, a perfluorocarbon core undergoes a liquid-to-gas phase transition when sonicated at a sufficient peak negative pressure (16). That phase change, with its associated volume of expansion, induces the release of drug cargo.

This mechanism does not rely on a particular temperature rise but instead relies on the mechanical action of ultrasound. Additionally, the vaporization event can be induced with millisecond- or even microsecond-long ultrasound pulses (17), which is more suitable for the interrogation of neural circuits. It is also more straightforward to achieve a particular peak negative

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www.sciencemag.org/content/357/6350/465-A
pressure than a specific temperature increase in peripheral brain regions.

The chemistry of phase-change nanoparticles allows for stable encapsulation of almost any hydrophobic small-molecule drug (16). Given that hydrophobic small molecules may generally cross the blood-brain barrier (18), phase-change nanoparticles could, in principle, encapsulate nearly any drug of neuropsychiatric interest.

A ROAD MAP TO CLINICAL USE

In eventual clinical use, nanoparticles that are loaded with the desired drug would be infused intravenously. The nanoparticles would distribute throughout the blood pool in their inert form. FUS would then be applied only to the desired part of the brain. Sonication would induce drug release from the nanoparticles into the intravascular space. The drug would cross the blood-brain barrier and modulate activity only in the sonicated brain region. Drug redistribution and metabolism would serve to limit the temporal extent of drug activity. The remainder of the drug in the nonactivated particles would remain inert until the particles were sequestered and metabolized by the body. Throughout this process, the patient would be awake and participating in neuropsychiatric assessment, functional brain imaging, and/or cognitive behavioral therapy. The uncaging sonication could be repeated or applied to an additional brain region while the nanoparticles reside in the blood.

PROOF OF PRINCIPLE

During my clinical residency at Johns Hopkins, I approached Jordan Green, a world expert in polymeric nanoparticle drug-delivery technology, about collaborating on the development of a protocol for neuromodulation using ultrasound-mediated drug uncaging from nanoparticles. With the help of Randall Meyer, a talented senior graduate student, we were able to successfully modify published protocols for phase-change nanoparticle production to encapsulate and release propofol, a small-molecule anesthetic. Notably, we constructed the particles with individual components that are each approved in different contexts for human application, in anticipation of clinical translation.

Propofol is an ideal compound to serve as a first test case for this type of application because it is small, highly lipophilic, and can rapidly diffuse into the brain during a first-pass of perfusion. Indeed, we were able to stably encapsulate propofol and see a dose-response relationship between the sonication dose and the induced propofol release (2). Moreover, we were able to confirm that the particle size was reliably <500 nm, indicating that there is no significant risk of vessel embolization during vaporization.

After intravenous administration, the particles cleared from the blood pool with an overall half-life of ~30 minutes, which is suitable for a clinical protocol. Twenty-four hours later, no particles were detected in blood samples. The particles were sequestered by the expected organs for nanoparticle uptake, namely liver and spleen, without nonspecific binding to the brain.

STOPPING SEIZURES WITH SONICATION

To assess the efficacy of achieving a neuromodulatory effect, we sought the help of Shilpa Kadam, director of the preclinical in vivo electrophysiology laboratory at the Kennedy Krieger Institute in Baltimore. We used a chemoconvulsant seizure model to increase the neural activity of rats.

After nanoparticle administration alone, the seizures persisted (see the figure) (2). However, after sonication directed to the hippocampus and thalamus, the seizures in rats that had received propofol-loaded particles were reliably and immediately silenced. In contrast, seizures persisted well after sonication was completed in rats that received particles that did not contain the drug.

There was no evidence of brain parenchymal damage on high-field MRI or histology, nor was there evidence of blood-brain barrier opening. These experiments validated that this technique could be used for robust

Ultrasonic release of propofol from nanoparticles stops seizures

Focused ultrasound (FUS) sonication targets ([A], red) and timeline ([B]. Raw EEG traces ([C] and population averages ([D] and [E]) before and after seizure induction and FUS show immediate decreases of EEG power with FUS in rats receiving propofol-loaded but not blank particles ([F] There was no nonspecific, systemic release of propofol. Adapted from (2).
and safe noninvasive focal neuromodulation.

THE FUTURE SOUNDS PROMISING

In my newly established laboratory at Stanford, we plan to use a combination of electroencephalography (EEG), positron emission tomography, and functional MRI to assess the spatial and temporal resolution of our technique for noninvasive neuromodulation with ultrasonic drug uncaging and to determine the relationship between the applied sonication dose and the neural silencing induced by propofol release.

After these initial experiments in rats, we plan to implement this protocol in large animals, in preparation for seeking regulatory approval to translate this technique to the clinic. Additionally, we are currently identifying the range of drugs that can be encapsulated in these nanoparticles, based on their chemical features. Finally, we are developing aseptic methods to produce these particles at scales suitable for large animals and humans.

We plan to use ultrasonic drug uncaging as a platform for clinical brain-mapping studies of specific biochemical pathways. This technique would enable pseudolesion studies, which could be used to locate functional brain regions and to validate targets before surgical intervention. This technique could also be used as a focal pharmacological adjunct to psychiatric talk or exposure therapy—for example, by noninvasively modulating brain regions such as the amygdala in patients with anxiety disorders.

With the development of focused ultrasonic drug uncaging from phase-change nanoparticles, the era of versatile, robust, and precise noninvasive neuromodulation is here.

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10.1126/science.aao1200
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Science 357 (6350), 465.
DOI: 10.1126/science.aao1200