

# Noninvasive Ultrasonic Drug Uncaging Maps Whole-Brain Functional Networks

## Highlights

- Ultrasound-sensitive nanoparticles enable localized drug delivery to the brain
- Ultrasonic drug uncaging allows noninvasive and precise control of brain activity
- Drug effects are limited to the ultrasound focus and by the kinetics of the drug
- Uncaging and neuroimaging together causatively maps whole-brain functional networks

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## In Brief

Wang, Aryal, et al. demonstrate that nanoparticle-mediated ultrasonic drug uncaging noninvasively modulates brain activity with precision determined by the ultrasound focus extent and the kinetics of the uncaged drug and establish that this technology can causatively map whole-brain functional networks.



# Noninvasive Ultrasonic Drug Uncaging Maps Whole-Brain Functional Networks

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## SUMMARY

Being able to noninvasively modulate brain activity, where and when an experimenter desires, with an immediate path toward human translation is a long-standing goal for neuroscience. To enable robust perturbation of brain activity while leveraging the ability of focused ultrasound to deliver energy to any point of the brain noninvasively, we have developed biocompatible and clinically translatable nanoparticles that allow ultrasound-induced uncaging of neuromodulatory drugs. Utilizing the anesthetic propofol, together with electrophysiological and imaging assays, we show that the neuromodulatory effect of ultrasonic drug uncaging is limited spatially and temporally by the size of the ultrasound focus, the sonication timing, and the pharmacokinetics of the uncaged drug. Moreover, we see secondary effects in brain regions anatomically distinct from and functionally connected to the sonicated region, indicating that ultrasonic drug uncaging could noninvasively map the changes in functional network connectivity associated with pharmacologic action at a particular brain target.

## INTRODUCTION

Realizing novel techniques for improved control of the human nervous system is a key goal of the neuroscience community (Jorgenson et al., 2015). The ideal neuromodulatory technique would allow noninvasive manipulation of any part of the brain with high spatial and temporal precision. It would flexibly allow control over any of the varied modes of communication in the brain, including excitatory, inhibitory, and modulatory inputs. Importantly, it would have a short and straightforward path to safe and effective use in wild-type large animals and humans.

We recently proposed accomplishing these objectives with ultrasonic drug uncaging, in which noninvasive neuromodulation is accomplished by using ultrasound to uncage neuromodulatory drugs from nanoparticle drug carriers (Airan, 2017). Focused ultrasound is a near-ideal modality to noninvasively transmit energy to the brain, given that ultrasound systems currently in

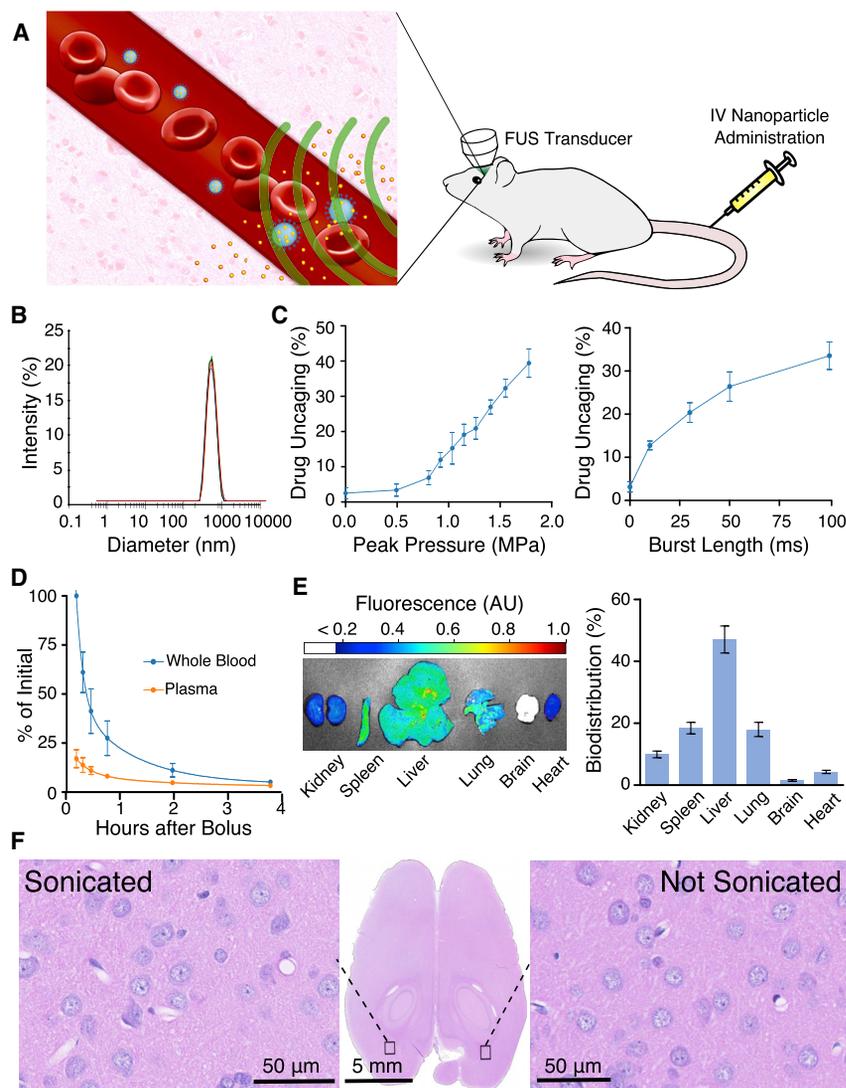
clinical use can sonicate the central brain transcranially with spatial and temporal precision on the order of millimeters and milliseconds (Ghanouni et al., 2015; Lipsman et al., 2014). Additionally, polymeric nanoscale emulsions of perfluorocarbons have been used as ultrasound-mediated delivery vehicles for chemotherapeutics (Fabiilli et al., 2010; Rapoport, 2016; Sheeran and Dayton, 2012; Sheeran et al., 2016). Previously, we adapted these nanoparticles to a biocompatible formulation that allows encapsulation and uncaging of the small molecule anesthetic propofol (Airan et al., 2017), a potent GABA(A) agonist, and designed these nanoparticles to be composed of ingredients that are each approved for human administration in different contexts (Makadia and Siegel, 2011; Robbin and Eisenfeld, 1998). However, until now, there has been no demonstration that ultrasonic drug uncaging could indeed capitalize on the principal attractive features of focused ultrasound for neuroscience: noninvasive localization in space and time without physical constraints on the depth of penetration. Here, we deliver on that promise by demonstrating—for the first time—that the neuromodulation induced by ultrasonic propofol uncaging is indeed limited by the spatial and temporal extent of sonication and the pharmacokinetics of the uncaged drug. We further demonstrate the power of this technique for neuroscientific inquiry by showing that ultrasonic drug uncaging drives secondary changes in brain regions that are functionally connected to the sonicated target, thereby mapping the network-level changes in functional connectivity induced by a localized pharmacologic stimulus.

## RESULTS

### Drug-Loaded Nanoparticles Uncage Propofol upon Sonication Efficaciously and Safely

In the envisioned application, drug-loaded ultrasound-sensitive nanoparticles would be intravenously administered and then uncaged with ultrasound while in the intravascular blood volume of the target brain region. The drug would then diffuse across the intact blood-brain barrier to act on the sonicated brain (Figure 1A). We optimized these nanoparticles for ultrasonic drug uncaging with sub-megahertz ultrasound frequencies to match the frequencies used for clinical transcranial sonication (Ghanouni et al., 2015; Hynynen and Jolesz, 1998; Lipsman et al., 2014, 2018). Importantly, compared to prior formulations (Airan et al., 2017; Rapoport, 2012), our current nanoparticles showed improved monodispersity, stability, drug loading, and free drug fraction





**Figure 1. Nanoparticles for Ultrasonic Drug Uncaging Are Effective and Well Tolerated**

(A) Schematic of nanoparticle use. Intravenously administered nanoparticles (blue) distribute in the blood volume. When focused ultrasound (FUS; green) is applied to a parenchymal target, drug (yellow) is uncaged from the nanoparticles into the blood and then diffuses into the brain across the blood-brain barrier.

(B) Typical nanoparticle dynamic light scattering results show a monodisperse peak of nanoscale material with Z-averaged diameter  $397.3 \pm 10.0$  nm, polydispersity index  $0.068 \pm 0.023$ , and zeta potential  $-26.7 \pm 0.6$  mV.

(C) Drug uncaging efficacy *in vitro* with 650 kHz focused sonication while varying *in situ* pressure (left; with  $60 \times 50$  ms burst length at 1 Hz burst frequency) or burst length (right; with 1.2 MPa peak *in situ* pressure) applied to an aqueous suspension of propofol-loaded nanoparticles quantified by the amount of uncaged drug that partitions to an organic solvent as a fraction of the total loaded drug ( $n = 3$ , mean  $\pm$  SEM).

(D) Particle clearance kinetics in whole blood and plasma following intravenous bolus administration of 1 mg/kg of propofol encapsulated in nanoparticles doped with an infrared fluorescent dye. Curves represent a double-exponential model fit to the data ( $n = 3$ , mean  $\pm$  SEM).

(E) Nanoparticle biodistribution in a single animal (left) and quantified (right;  $n = 3$ , mean  $\pm$  SEM) in end organs 24 hr after intravenous bolus administration of propofol-loaded nanoparticles doped with an infrared fluorescent dye, presented as the percent of fluorescence seen across the six harvested organs.

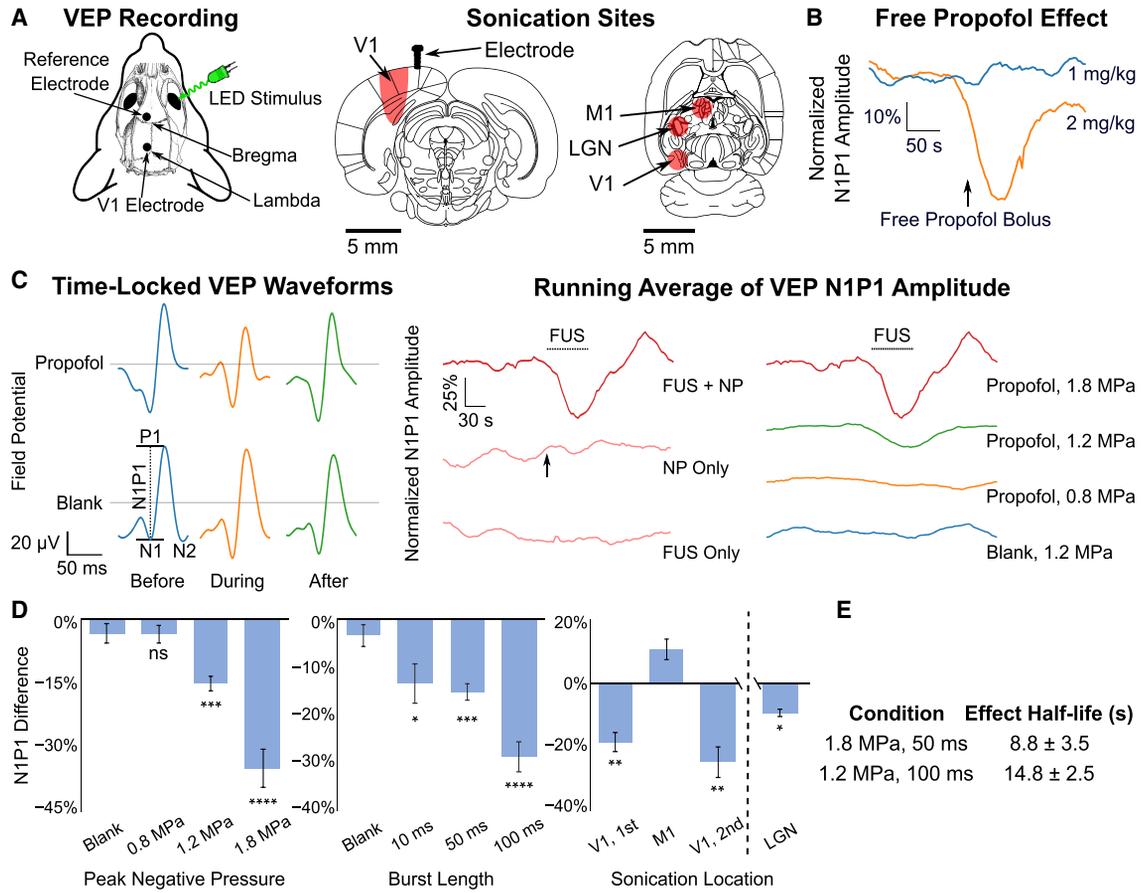
(F) Representative hematoxylin and eosin-stained transverse section of the brain (center) of a rat administered propofol-loaded nanoparticles intravenously and exposed to focused sonication ( $60 \times 100$  ms bursts with 1 Hz burst frequency at 1.8 MPa) directed to the occipital cortex, with 40 $\times$  views of the sonication target (left) and the contralateral non-sonicated brain (right).

(Figure 1B; typical dynamic light scattering results of Z-averaged diameter  $397.3 \pm 10.0$  nm, with polydispersity index  $0.068 \pm 0.023$ , zeta potential  $-26.7 \pm 0.6$  mV, and drug loading of 1.19% of nanoparticle weight). These nanoparticles uncaged propofol with an estimated *in vitro* pressure dose-response inflection at 0.8 MPa with 650 kHz sonication, with uncaging efficacy increasing with both sonication pressure and burst length (Figure 1C). In circulation, the nanoparticles cleared from the blood with a dual-exponential profile with estimated half-lives of 12.6 and 91.4 min for each exponential (Figure 1D), with no detectable nanoparticles in circulation 24 hr after administration. At 24 hr after administration, the nanoparticles were primarily sequestered in the liver, with less uptake in the spleen and lungs, minimal uptake in the kidney and heart, and importantly, no evidence of nonspecific binding to the brain (Figure 1E). Furthermore, the brains of animals that underwent focused sonication using maximal sonication pressure and maximal duty cycle (650 kHz sonication;  $60 \times 100$  ms bursts with 1 Hz burst repetition frequency, 1.8 MPa peak *in situ* pressure) following nanoparticle administra-

tion (10 min following bolus administration of 1 mg/kg of encapsulated propofol) showed no evidence of acute injury or blood-brain barrier disruption by either histology or contrast-enhanced MRI (Figure 1F; Figure S1A). Additionally, there was no evidence of parenchymal damage in animals receiving repeated nanoparticle dosing and sonication up to nine times over several weeks. Indeed, we have seen no evidence of nanoparticle or sonication-related toxicity in any of the  $>100$  rats that have undergone ultrasonic drug uncaging using these nanoparticle concentrations and with these sonication parameters. Together, these results show that this nanoparticle formulation allows safe and effective ultrasonic drug uncaging, with practical sonication dose-response relationships and *in vivo* particle kinetics.

#### Ultrasonic Propofol Uncaging Reversibly Silences Visual Evoked Potentials

To assess the spatial and temporal resolution of neuromodulation with this technique, we first noted the limited diffusion in brain tissue (Gredell et al., 2004) and rapid kinetics of action



**Figure 2. Dose-Response Relationship and Temporal Kinetics of Ultrasonic Propofol Uncaging, Revealed by VEPs**

(A) Schematic of recording electrode and light stimulus configuration (left) and sonication sites (right) represented by the expected full-width half-maximum (FWHM) of the sonication targets overlaid onto atlas slices (Paxinos and Watson, 2013) middle:  $-6.5$  mm caudal to bregma, right:  $-5.1$  mm ventral to bregma. V1, primary visual cortex; M1, primary motor cortex; LGN, lateral geniculate nucleus.

(B) Running average ( $n = 3$  animals each) of the visual evoked potential (VEP) N1P1 amplitude over time during a flashing light stimulus (10 ms monocular light stimulus at 1 Hz), normalized by the 60 s of stimulus presentation prior to bolus administration. Intravenous-free propofol administration shows no effect with a 1 mg/kg bolus (blue) of free propofol and a pronounced effect only with 2 mg/kg (orange). In all nanoparticle experiments, 1 mg/kg of encapsulated propofol was given in a bolus.

(C) Left: time-locked VEP waveforms (10 ms monocular light stimulus at 1 Hz), averaged over 60 s from an individual representative session before, during, or after sonication ( $60 \times 50$  ms bursts, 1 Hz burst frequency at 1.2 MPa est. peak *in situ* pressure) applied to V1 contralateral from the light stimulus, following intravenous administration of propofol-loaded (top) or blank (bottom) nanoparticles. Scheme of N1P1 amplitude measurement indicated on the bottom left waveform. Middle: running average ( $n = 5$  animals) of the VEP N1P1 amplitude over time for presentations of focused ultrasound (FUS) only (bottom;  $60 \times 50$  ms bursts, 1 Hz burst frequency at 1.8 MPa est. peak *in situ* pressure), followed by nanoparticle (NP) administration (middle; arrow indicates start of bolus administration), and then focused ultrasound with nanoparticles in circulation (FUS + NP, top) during the indicated time period (dashed bar). Time traces are normalized by the 60 s prior to intervention (i.e., FUS for the “FUS + NP” and “FUS Only” conditions or bolus administration for the NP Only condition) Right: running average ( $n = 5-9$  animals) of VEP N1P1 amplitude following sonication ( $60 \times 50$  ms bursts, 1 Hz burst frequency) applied to V1 with the indicated peak *in situ* pressure, normalized by the 60 s prior to FUS administration. 1.8 MPa trace repeated from the middle panel. Dashed bar, sonication time.

(D) Quantification of the difference in N1P1 amplitude seen between the beginning and end of sonication ( $60$  bursts at 1 Hz burst frequency) while varying the peak *in situ* pressure (left; 50 ms bursts) or burst length (middle; 1.2 MPa pressure) and for two separate experiments (right) first while moving the sonication (1.2 MPa,  $60 \times 50$  ms bursts, 1 Hz burst frequency) between V1 and M1 successively in the same animal (statistical comparison to M1 sonication) or separately sonicating LGN. Presented are mean  $\pm$  SEM for groups of  $n = 4-9$ . \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , by two-tailed t tests, comparing to blank nanoparticle sonication unless otherwise noted. Process for computing the difference in N1P1 amplitude is described in STAR Methods.

(E) Effect half-life indicated by a model (see Figure S2; STAR Methods) fitted to the N1P1 time traces for the indicated condition.

(Upton and Ludbrook, 1997) of the anesthetic propofol. We quantified the temporal kinetics of anesthetizing visual evoked potentials (VEPs) with ultrasonic propofol uncaging directed to the primary visual cortex (V1; Figure 2A). In all experiments of this current study, animals received a total dose of 1 mg/kg of

encapsulated propofol. At that dose, free propofol given intravenously as a bolus is insufficient to affect VEP amplitudes (Figure 2B). Nonetheless, ultrasonic propofol uncaging directed to V1 markedly attenuated this physiologic activity, with a decrease of the N1P1 amplitude commencing with sonication, and rapid

recovery of this amplitude seen in the immediate post-sonication period (Figure 2C, left). Furthermore, neither this sonication protocol alone nor nanoparticle administration itself significantly affected the N1P1 VEP amplitude (Figure 2C, middle). This effect was limited to the sonication period and showed a dose-response relationship with each of the applied sonication pressure and the burst length (Figures 2C, right, 2D, left and middle), consistent with the *in vitro* uncaging results (Figure 1C). Interestingly, at 1.8 MPa, at the end of sonication, we observed an increase in VEP amplitude relative to baseline in some animals, potentially due to post-inhibitory rebound spiking (Nortmann et al., 2015; Sanchez-Vives et al., 2000). Notably, this phenomenon has been observed previously with optogenetic halorhodopsin inhibition (Yang et al., 2018) and has also been hypothesized *in silico* to underlie the paradoxical excitatory action of subanesthetic doses of propofol (McCarthy and Kopell, 2012).

Recognizing recent findings that neuromodulation from focused ultrasound can arise primarily from an indirect auditory response (Guo et al., 2018; Sato et al., 2018), we conducted the same sonication protocol with non-drug-loaded blank nanoparticles. We found that blank nanoparticles yielded no effect, indicating that the observed neuromodulation is specific to propofol uncaging and not a result of the sonication itself or the other nanoparticle constituents.

To investigate whether this anesthesia was specific to sonication of V1, we sonicated the motor cortex (M1; Figure 2A, right) as a target that is both spatially and functionally separated from the visual system. Furthermore, as there is little arterial communication between the two hemispheres in the rat brain (Lee, 1995), we opted to sonicate the M1 cortex ipsilateral to the investigated V1 and contralateral to the light stimulus to assess for any nonspecific effects due to released propofol spreading by perfusion or diffusion. As predicted, sonication of this target showed no such attenuation of the N1P1 VEP amplitude (Figure 2D, right). We further found that sonication of a target encompassing part of the lateral geniculate nucleus (LGN; Figure 2A, right) was also able to significantly attenuate the VEP amplitude (Figure 2D, right), suggesting that ultrasonic propofol uncaging could yield secondary effects in regions functionally downstream of the sonicated target. Notably, this effect is smaller than direct propofol uncaging in V1 likely because our focus spot size only encompassed part of the LGN while also encompassing non-LGN regions. The temporal kinetics of these effects showed half-lives of 8–15 s (Figure 2E; Figure S2), correlating well with the known pharmacokinetics of propofol action (Upton and Ludbrook, 1997) and indicating that the temporal kinetics of this technique are determined by the timing of sonication and the pharmacokinetics of the uncaged drug. Together, these results show—for the first time—that ultrasonic drug uncaging may noninvasively and reversibly modulate physiologic brain activity, with dose-response relationships with the applied sonication parameters *in vivo* and with a temporal extent that is determined by the timing of sonication and the pharmacokinetics of the uncaged drug.

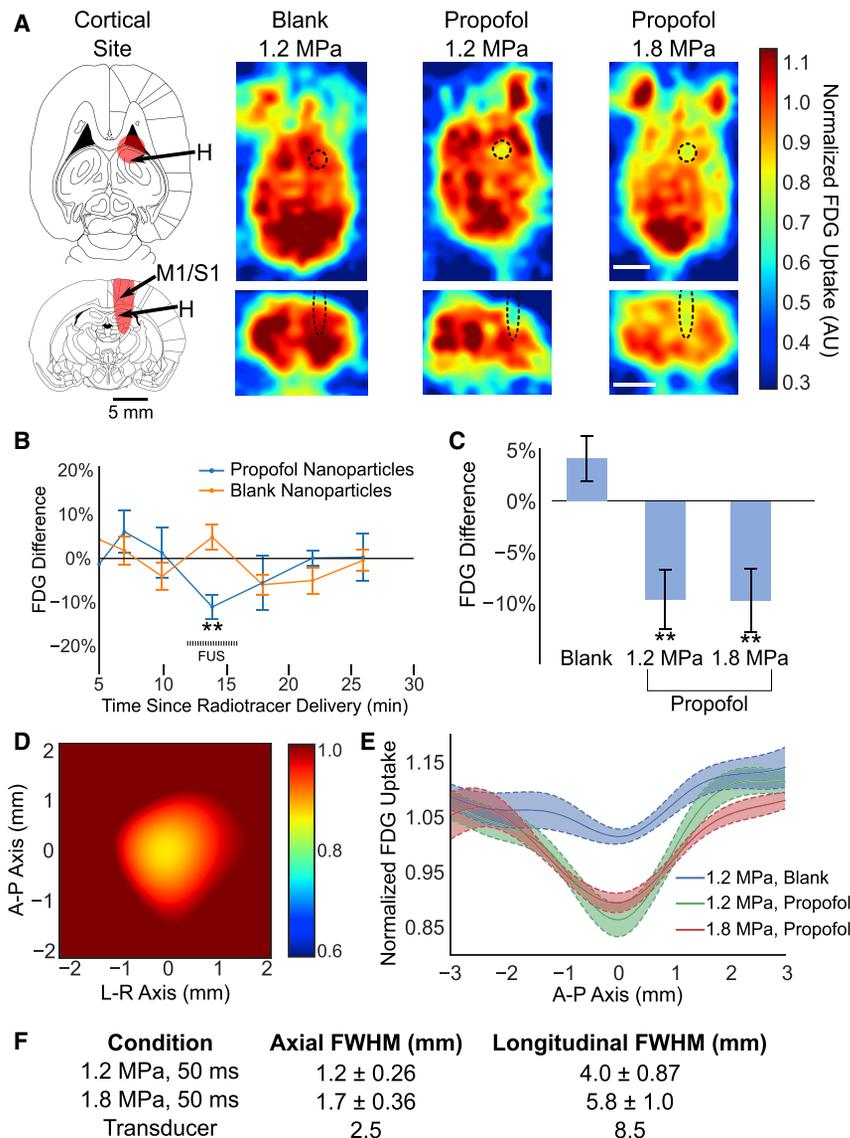
### Ultrasonic Propofol Uncaging Induces Spatially Localized Anesthesia

To more fully assess the spatial profile of the anesthesia induced with ultrasonic propofol uncaging, we turned to functional brain

imaging. Specifically, we used positron emission tomography (PET) imaging of the uptake of [<sup>18</sup>F]-fluorodeoxyglucose (FDG), a glucose analog, with a relative decrease in FDG avidity indicating effective anesthesia induced by the uncaged propofol (Tasbihgou et al., 2018). Given the low rate of diffusion for propofol within the brain (Gredell et al., 2004), the spatial extent of the anesthesia induced by ultrasonic propofol uncaging would be determined by the extent of parenchyma whose perfusion is supplied by the vessels in which the nanoparticles are uncaged. As rodent cortical parenchyma is supplied by penetrating arteries extending radially inward from the outer cortical surface (Xiong et al., 2017), sonication near the cortical surface would likely yield the broadest spatial extent of the effect and, therefore, the worst spatial resolution of the technique. To establish a worst-case bound of this spatial resolution, we therefore targeted the sonication focus (650 kHz, 240 × 50 ms bursts, with 1 Hz burst repetition frequency) to the cortical surface, with the majority of the focus in the rat dorsal primary motor cortex and with the far field of sonication entering the dorsal hippocampus (Figure 3A, left; *in vivo* mapping of the maximal sonication extent in Figure S1B). Indeed, following propofol-loaded nanoparticle administration, during sonication with 1.2 MPa peak *in situ* pressure, a robust decrease in FDG avidity was visualized precisely within the full-width at half-maximum (FWHM) profile of the sonication focus, exactly at the sonication target (Figure 3A, right). This neuromodulation was reliably visualized across this cohort (Figures 3D and 3E; Figure S3A). This decrease in avidity was not seen with blank nanoparticles, indicating that this effect is specific to propofol uncaging and not driven by sonication itself or the other nanoparticle constituents. As with VEP attenuation (Figure 2), this effect was limited to the time period of sonication (Figure 3B). With 1.8 MPa sonication, the magnitude of the decrease of FDG avidity was unchanged (Figure 3C), but the spatial extent of the affected region increased (Figure 3A, right, 3E, and 3F). This spatial extent at 1.8 MPa was still within the sonication FWHM, corresponding to the dose-response relationship of propofol uncaging and sonication pressure seen *in vitro* (Figure 1C) as well as *in vivo* with VEP attenuation (Figure 2D). Notably, increasing the sonication pressure by 50% (1.8 MPa versus 1.2 MPa) increased the effect FWHM by 44% (Figure 3F), as would be expected from the rectified linear pressure dose-response relationship that is implied by our *in vitro* results (Figure 1C). Comparing the spatial extent of the anesthetic decrease in FDG avidity with the expected sonication profile *in situ*, an effective *in vivo* threshold of approximately 1.0 MPa with 650 kHz sonication was estimated for the localized anesthesia induced by ultrasonic propofol uncaging.

### Ultrasonic Propofol Uncaging Induces Secondary Anesthesia of a Functionally Connected Network

Next, we noted qualitatively that with 1.8 MPa sonication, in addition to the primary decrease of FDG avidity seen in the sonicated target, there appeared to be other sites spatially distinct and separated from the sonication target showing apparent decrease in FDG avidity, albeit to a lesser extent (Figure 3A, right). To assess the consistency of this observation, the imaging volumes from animals receiving cortical sonication of 1.8 MPa peak negative pressure were coregistered to each other.



### Figure 3. Spatial Profile of Ultrasonic Drug Uncaging Quantified by PET Imaging

(A) Left: full-width half-maximum (FWHM) of the cortical sonication target (red) indicated on atlas sections (Paxinos and Watson, 2013) top:  $-6.9$  mm ventral to bregma, bottom:  $-2$  mm caudal to bregma. H, hippocampus; M1, primary motor cortex; S1, primary somatosensory cortex. Right: transverse (top) and axial (bottom) PET images obtained during sonication ( $60 \times 50$  ms bursts at 1 Hz burst frequency; black dashed ellipse: expected sonication FWHM) at the indicated peak *in situ* pressure following blank or propofol-loaded nanoparticle administration. Color bar represents FDG uptake, normalized against the average FDG uptake in the contralateral hemisphere. Note that the anterior paired structures outside the brain are the Harderian glands. Scale bar, 5 mm.

(B) Difference of the minimum FDG avidity across time for the voxels within the sonication FWHM averaged across animals receiving blank (orange) or propofol-loaded (blue) nanoparticles at 2 min following radiotracer administration. FDG avidity values are normalized against the contralateral field to account for different temporal features of FDG uptake in different animals due to different body weights, blood glucose level, and general anesthesia level (Figure S3B).

(C) Quantification of FDG uptake during sonication for voxels in the sonication field minus the contralateral non-sonicated field following blank or propofol-loaded nanoparticle administration.

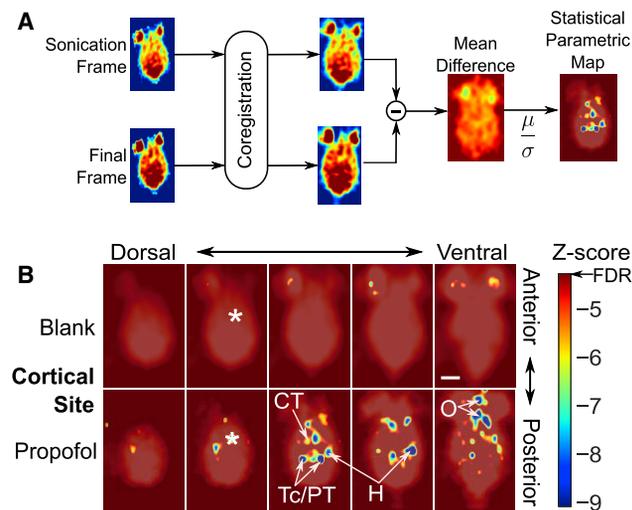
(D) Locally registered, interpolated, and averaged image of FDG uptake at the sonication site for 1.2 MPa sonication with propofol-loaded nanoparticles, normalized by the contralateral field. A-P, anterior-posterior; L-R, left-right.

(E) FDG uptake across a sagittal slice centered at the sonication site for each condition. Each individual animal was registered to each other by the minimal FDG uptake within the sonication site.

(F) Estimated FWHM of the sonication-induced changes in FDG avidity for each condition and for the transducer used in these experiments. Presented are mean  $\pm$  SEM for groups of  $n = 5-7$ . \*\* $p < 0.01$  by two-tailed t tests.

Statistical parametric maps were calculated across the whole brain for the Z-scores of the difference in FDG avidity of a given voxel between the sonication time period and a delayed time period at the end of the PET acquisition (Figure 4A). These maps were then thresholded at the false discovery rate (FDR) to account for multiple statistical comparisons in this whole-brain analysis. While no brain voxels showed a significant difference of FDG avidity during cortical target sonication of blank nanoparticle treated animals (Figure 4B, top), a substantial number of brain voxels for propofol nanoparticle-treated animals showed such a difference with sonication at 1.8 MPa (Figure 4B, bottom), but notably not at 1.2 MPa or with sham sonication (Figure S4A). These sites were spatially distinct and separated from the sonication target, with some even in the contralateral brain. These foci localized primarily to the ipsilateral more than contralateral thalamus (Bosch-Bouju et al., 2013) and hippocampus (Hess et al., 1995), with additional sites of functional decrease

localizing to the tectum (Rubelowski et al., 2013), and the ipsilateral olfactory bulb and cortex (Cinelli et al., 1987; van Groen and Wyss, 1990). Interestingly, we found that all voxels that showed significance beyond the FDR had less metabolic activity associated with the cortical uncaging of propofol. This is in line with previous studies identifying that the majority of projection neurons of the cortex are excitatory (Spruston, 2008). The lack of significant voxels at the primary sonication target at 1.2 MPa is likely due to the effect spatial size being on the order of a single voxel, the inevitable small differences in experimental targeting, and the whole-brain coregistration process translating these small deviances to yield no substantial overlap of the sonicated areas in these whole-brain coregistered and averaged images (Figure S5A). In contrast, at 1.8 MPa, the spatial size of the effect was large enough for the anesthetized areas to overlap, resulting in statistically significant voxels at the sonication target (Figure S5B).



**Figure 4. Ultrasonic Propofol Uncaging Maps Functional Connectivity of a Cortical Target**

(A) Analysis scheme for statistical parametric mapping of the whole-brain effects of targeted ultrasonic propofol uncaging. Following whole-brain coregistration, FDG PET frames during sonication and at the end of the acquisition were subtracted. This difference was averaged across animals, divided by the standard error across each group on a voxel-by-voxel basis, and thresholded at the false discovery rate (FDR), producing a Z-score statistical parametric map.

(B) Functional connectivity maps for cortical target sonication of animals receiving blank (top) or propofol-loaded (bottom) nanoparticles. Unannotated maps are provided in Figures S4A and S4B. CT, central thalamus; Tc/PT, tectum/posterior thalamus; H, hippocampus; O, olfactory bulbs and cortex.

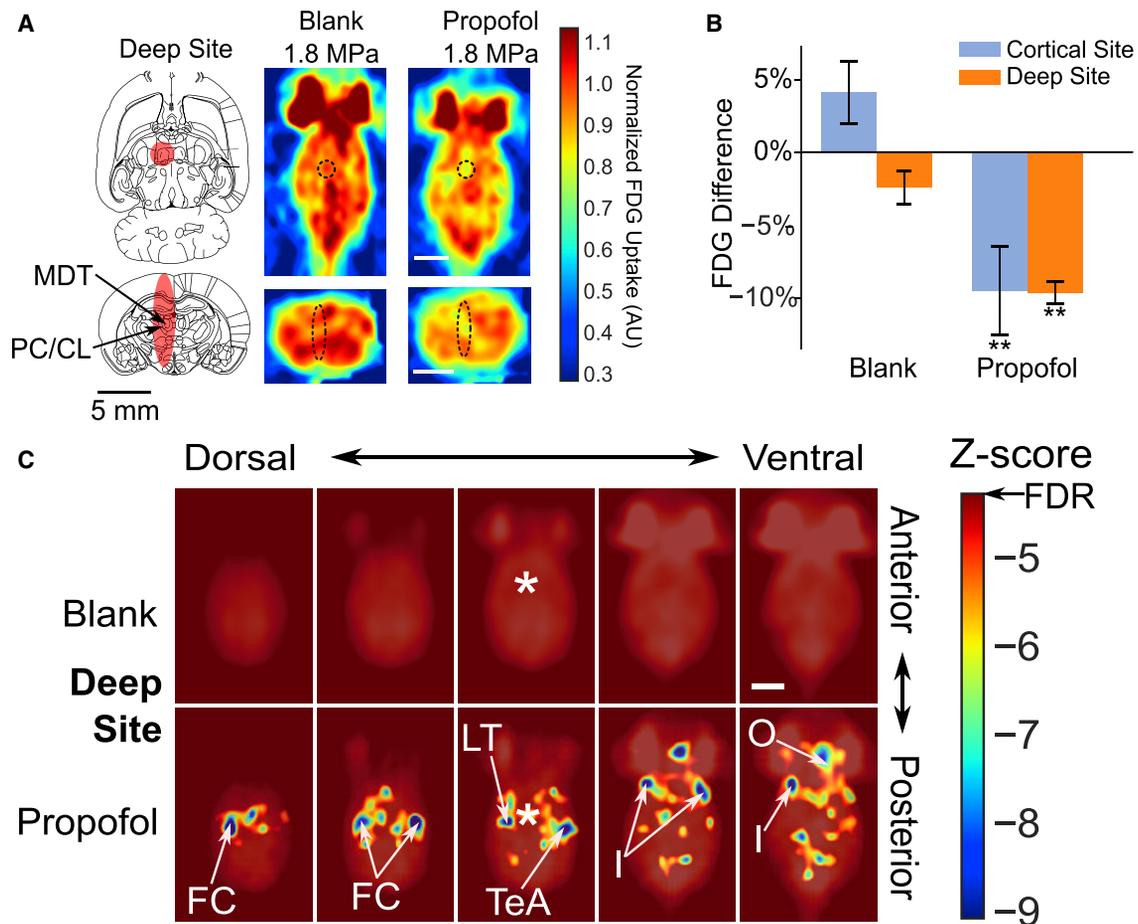
To validate that this analysis was indeed assessing changes in functional connectivity, we noted the significant bidirectional interconnectedness of the central thalamus and frontal cortex (Brown et al., 2011) and the secondary decrease in central thalamic metabolism with frontal cortical propofol uncaging (Figure 4B). We decided to assess propofol uncaging in the central thalamus under the hypothesis that this intervention should yield a corresponding decrease of activity in the frontal cortex, as an inverse of what has been seen with optogenetic activation of this region (Liu et al., 2015) and to test the efficacy of ultrasonic propofol uncaging to noninvasively modulate this deep brain region. Overall, we expected that deep subcortical structures, such as the thalamus, would likely show an even greater extent of functional connectivity across the brain (Brown et al., 2011) compared to cortical sonication. A sonication target centered on the paracentral and mediodorsal nuclei of the thalamus was chosen (Figure 5A, left; *in vivo* mapping of the maximal sonication extent in Figure S1B), with the transducer sonication profile extending dorsally into the dorsal hippocampus and ventrally into the hypothalamus. As with the cortical target, with ultrasonic propofol uncaging in this deep target, a robust decrease in FDG avidity was seen within the sonication target, which was spatially restricted to the sonication focus, with no such effect in animals receiving blank nanoparticles (Figures 5A and 5B). The same functional connectivity analysis that was used for the cortical sonication data was then applied to the deep sonication data. Indeed, while blank nanoparticle-treated animals showed no

such change in functional connectivity during deep sonication (Figure 5C, top), a whole-brain network of activity changes was seen with ultrasonic propofol uncaging in the deep target, with these changes principally localized to the ipsilateral more than contralateral frontal, prefrontal, and insular cortices (Brown et al., 2011; Liu et al., 2015), the olfactory bulbs and cortex (Courtillot and Wilson, 2014), and the midbrain (Brown et al., 2011; Heckers et al., 1992) (Figure 5C, bottom). All statistically significant voxels were inhibited during propofol uncaging in this deep target, agreeing with previous studies establishing that central thalamic projection neurons are primarily excitatory (Ellender et al., 2013).

## DISCUSSION

Overall, we have seen that these ultrasound-sensitive nanoparticles have desirable physicochemical properties and drug loading, show suitable ultrasonic uncaging efficacy *in vitro*, and are well tolerated by rats (Figure 1; Figure S1). We observed that ultrasonic propofol uncaging can selectively attenuate VEPs when targeting regions in the visual pathway, with a temporal resolution of approximately 10 s with respect to the sonication timing (Figure 2; Figure S2), consistent with the known pharmacokinetics of propofol action (Upton and Ludbrook, 1997). Using FDG PET, we observed that ultrasonic propofol uncaging induced silencing of the brain that was limited to the sonication field (Figure 3; Figure S3) and that, with higher ultrasound intensities, secondary changes were induced in regions functionally connected to the sonication target (Figures 4 and 5; Figures S4 and S5), creating functional connectivity maps of the network effects of localized propofol action. Together, these results demonstrate that ultrasonic drug uncaging can be used not only for spatially and temporally localized drug delivery but also for mapping the brain network-level physiological responses to localized pharmacologic action.

We have also validated that these nanoparticles may encapsulate and uncage a wide variety of drugs, so long as they are lipophilic, including ketamine, dexmedetomidine, and nicardipine (Q. Zhong et al., 2018, Controlled Release Soc., abstract). As lipophilicity is a hallmark of drugs that naturally may cross the blood-brain barrier (Chen and Liu, 2012), ultrasonic drug uncaging from these nanoparticles is particularly suited for the delivery of neuromodulatory drugs. Given its limited diffusion in the brain (Gredell et al., 2004) and short time of action (Upton and Ludbrook, 1997), propofol may be taken as a model drug to define the spatial and temporal resolution of ultrasonic drug uncaging more generally. Certainly, given the estimated diffusion coefficient of propofol of  $0.02 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  (Gredell et al., 2004) and an estimated capillary transit time of 1.4 s (Jespersen and Østergaard, 2012), we estimate the extent of diffusion beyond the capillary bed during this transit time to be only 1.7  $\mu\text{m}$ , suggesting that perfusion-related effects dominate in the distribution of propofol following uncaging. Indeed, the results presented here (Figures 2 and 3) suggest that the resolution of ultrasonic drug uncaging is defined principally by the spatio-temporal extent of sonication and the action of the particular drug that is uncaged. Even with a more volatile agent, such as halothane, which has an order of magnitude faster diffusion



**Figure 5. Ultrasonic Propofol Uncaging in Deep Structures Yields a Unique Functional Connectivity Signature**

(A) Left: deep sonication target FWHM (red) overlaid onto transverse (top:  $-5.6$  mm ventral to bregma) and axial (bottom:  $-3$  mm caudal to bregma) rat atlas sections (Paxinos and Watson, 2013). MDT, mediodorsal thalamus; PC/CL, paracentral/centrolateral nuclei of the thalamus. Right: individual animal transverse (top) and axial (bottom) PET images obtained during sonication ( $60 \times 50$  ms bursts at 1 Hz burst frequency, 1.8 MPa peak *in situ* pressure; black dashed ellipse: expected sonication FWHM) following blank or propofol-loaded nanoparticle administration. Color bar represents FDG uptake, normalized against the average FDG uptake in the contralateral hemisphere. Scale bars, 5 mm.

(B) Quantification of FDG avidity difference during sonication in the sonication field minus the contralateral non-sonicated field following blank or propofol-loaded nanoparticle administration for either cortical (repeated from Figure 3 for ease of comparison) or deep target sonication. Presented are mean  $\pm$  SEM for groups of  $n = 6-7$ . \*\* $p < 0.01$  by two-tailed t tests.

(C) Functional connectivity maps for deep target sonication of animals receiving blank or propofol-loaded nanoparticles. Unannotated maps are provided in Figure S4C. FC, frontal cortex; LT, lateral thalamus; TeA, tegmental association area; I, insula; O, olfactory bulbs and cortex.

coefficient (Chesney et al., 2003), we estimate the extent of diffusion to be only up to  $11 \mu\text{m}$  during capillary transit, indicating that this result and model should hold generally for a wide range of drugs with different rates of transport within the brain.

The relatively restricted spatial extent of these effects is an important surprising and welcome feature, given the potential for vascular perfusion to carry the uncaged drug beyond the sonication focus. Regarding this point, we first note that uncaged drug will enter the brain parenchymal compartment primarily at the capillary level, the typical site for blood-parenchyma solute exchange (Upton, 2007). Also, the typical linear extent of brain parenchymal transit of an agent within the tortuous capillary bed is only  $150-500 \mu\text{m}$  (Hudetz, 1997). Additionally, even if there is significant diffusion of the drug within the brain parenchyma, dilution of the drug into the volume of diffusion would lower the

effective drug concentration and limit the diffusion-related spread of drug action. We would therefore expect that the maximal spatial extent of the action of ultrasonically uncaged drug would be less than  $\sim 0.5$  mm beyond the brain region that is sonicated with a pressure that is above the effective uncaging threshold (1.0 MPa for propofol uncaging with 650 kHz sonication; Figures 1, 2, and 3).

It is unlikely for the results of the functional connectivity mapping analysis (Figures 4 and 5) to be confounded by effects of diffusion or perfusion given the considerations noted above. Furthermore, given the contiguous nature of arterial blood supply and the lack of arterial communication across hemispheres (Lee, 1995), diffusion or perfusion confounds should result in statistically significant modulations of voxels that are interconnected and do not cross into the contralateral brain. In contrast, we

observe that the secondary sites of uncaged propofol-induced changes are multiple, discrete, unconnected, and even in the contralateral brain (Figures 4 and 5), highlighting that the observed changes are unlikely to represent perfusion or diffusion confounds. Also, temperature changes are unlikely to be responsible for this neuromodulation, given that with these sonication protocols (i.e., 50 ms bursts at 1.8 MPa or 100 ms bursts at 1.2 MPa), each sonication burst would result in an estimated temperature increase of  $\sim 0.1^\circ\text{C}$  (calculations detailed in STAR Methods) (Azhari, 2010; Haar and Coussios, 2007; Hasgall et al., 2015; Nyborg, 1988). This temperature change would rapidly dissipate during the 900–950 ms of time for which the transducer is not active in each period, indicating that there should be no accumulated thermal dose.

Simulation studies have suggested that there may be minimal off-target sonication of substantial intensity due to scatter during focused ultrasound transmission in small rodent skulls (Younan et al., 2013), serving as a potential confound. However, the *in vivo* spatial profile of sonication in our work is restricted to the sonication trajectory and does not directly overlap with these functional maps (Figures 4 and 5; Figure S1B), in agreement with other *in vivo* characterizations of similar sonication fields in rat skulls (McDannold et al., 2013; O'Reilly et al., 2010). It is therefore unlikely that scatter of the ultrasound would yield the variety of distant discrete foci that we observe in our functional connectivity maps, especially given that: there was a lack of similar effects seen with 1.2 MPa sonication (Figure S4A); the scattered ultrasound would likely be of lower intensity than that in the primary sonication target; and targeting different sites a few millimeters apart yielded wholly different functional signatures (Figures 4B and 5C). We do note that our spatial analysis may be limited by the inevitable likelihood of standing wave generation (O'Reilly et al., 2010) with sub-megahertz sonication in small rodent skulls. A more complete analysis will likely necessitate the use of larger animal models whose cranial vaults are significantly larger than the spatial length of the applied ultrasound pulses in tissue. Indeed, the main limitation of this work is that current small-animal focused ultrasound systems generally have foci that are broad longitudinally to the point of spanning the entire dorsal-ventral axis of the brain (Figure S1B). Future work with this system in small animals will depend on the development of ultrasound transducers with smaller focal spot sizes and that are ideally wearable to allow awake, behaving analyses (Lee et al., 2018; Sieu et al., 2015; Tiran et al., 2017; Urban et al., 2015). Furthermore, the use of multi-element transducers that could surround the cranium would allow for tighter focal lengths in the longitudinal direction (Ghanouni et al., 2015; Lipsman et al., 2014).

Given its temporal resolution defined by the ultrasound timing and drug pharmacokinetics (Figure 2), spatial resolution defined by the ultrasound focus (Figure 3), and versatility for delivering a wide range of lipophilic compounds (Q. Zhong et al., 2018, Controlled Release Soc., abstract), noninvasive neuromodulation using ultrasonic drug uncaging offers complementary features to other techniques for neuromodulation. With localized drug application, ultrasonic drug uncaging could add a pharmacological adjunct to either invasive (Bichot et al., 2011; Leuchter et al., 2012; Mayberg et al., 2005) or noninvasive (Allan et al.,

2012; Farzan et al., 2016; Grossman et al., 2017; Vöröslakos et al., 2018) electromagnetic neurostimulation or direct ultrasound neuromodulation techniques (Casella et al., 2017; Chaplin et al., 2018; Deffieux et al., 2013; Guo et al., 2018; King et al., 2013; Lee et al., 2015; Legon et al., 2014; McDannold et al., 2015; Sato et al., 2018; Tyler et al., 2018; Ye et al., 2016). To enable clinical translation, these nanoparticles are by design composed of ingredients that are each approved for human administration in different contexts (Makadia and Siegel, 2011; Robbin and Eisenfeld, 1998), and their application does not appear to disrupt the blood-brain barrier (Figure S1A) or otherwise adversely impact the brain (Figure 1). Ultrasonic drug uncaging could therefore be readily translated and applied in larger wild-type animals, and potentially in humans, to test hypotheses generated by other neuromodulatory techniques that, for example, genetically modify the brain (Chen et al., 2015; Ibsen et al., 2015; Lee et al., 2014; Szablowski et al., 2018; Zhang et al., 2010). Future studies will work toward this large animal and clinical translation, will map changes in the functional connectome induced by focal pharmacologic perturbation (Figures 4 and 5), and will also determine the potential behavioral changes associated with ultrasonic drug uncaging within different brain regions and with a variety of neuromodulatory drugs.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Nanoparticle Synthesis and Characterization
  - Visual Evoked Potential (VEP) Recordings
  - PET Functional Imaging
  - Calculation of Heating Induced by Focused Ultrasound Protocols
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - EEG Recordings
  - PET Imaging
- DATA AND SOFTWARE AVAILABILITY

## SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and two tables and can be found with this article online at <https://doi.org/10.1016/j.neuron.2018.10.042>.

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#### AUTHOR CONTRIBUTIONS

J.B.W., M.A., Q.Z., and R.D.A. designed the experiments. J.B.W. implemented the EEG hardware, wrote the EEG acquisition and analysis software, wrote the PET analysis software, and completed the EEG and PET data analysis. M.A. completed the EEG experiments, including parameter optimization for the *in vivo* uncaging experiments, and completed the safety studies with MRI and histological endpoints. M.A. and J.B.W. implemented and calibrated the ultrasound systems, including characterizing the ultrasound fields *in vivo*. Q.Z. produced the nanoparticles and completed their physicochemical characterization and *in vivo* particle kinetics and biodistribution analysis. J.B.W. and D.B.V. completed the PET experiments. J.B.W., M.A., and R.D.A. wrote the paper and generated the figures, with input from all the authors.

Conceptualization, R.D.A., M.A., J.B.W., and Q.Z.; Methodology, J.B.W., M.A., Q.Z., and R.D.A.; Software, J.B.W.; Validation, M.A., J.B.W., Q.Z., and R.D.A.; Formal Analysis, J.B.W. and Q.Z.; Investigation, J.B.W., M.A., Q.Z., and D.B.V.; Resources, Q.Z., M.A., J.B.W., and R.D.A.; Data Curation, J.B.W.; Writing – Original Draft, J.B.W., M.A., and R.D.A.; Writing – Review & Editing, J.B.W., M.A., Q.Z., D.B.V., and R.D.A.; Visualization, J.B.W., M.A., Q.Z., and R.D.A.; Supervision, R.D.A.; Project Administration, R.D.A.; Funding Acquisition, R.D.A.

#### DECLARATION OF INTERESTS

Patent applications have been filed on the nanoparticles described in this manuscript (17-163 – Provisional application with Stanford University; Q.Z. and R.D.A.) and on related technology (PCT/US2017/033226 with Johns Hopkins University; R.D.A.).

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE  | SOURCE  | IDENTIFIER  |
|--|---|---|
| Chemicals, Peptides, and Recombinant Proteins  |   |   |
| mPEG-PLGA (Mw: 2,000:5,000 Da)   | Akina   | AK090   |
| Propofol   | Alfa-Aesar  | L06841; CAS: 2078-54-8  |
| Ketamine Hydrochloride   | Stanford University Environmental Health & Safety | CAS: 1867-66-9  |
| Tetrahydrofuran (THF)  | Sigma-Aldrich                                     | 401757; CAS: 109-99-9   |
| Hexane   | Sigma-Aldrich                                     | 296090; CAS: 110-54-3   |
| n-Perfluoropentane (PFP)   | FluoroMed   | APF-30M; CAS: 678-26-2  |
| MultiHance Gadobenate Dimeglumine  | Bracco Diagnostics                                | 0270-5164; CAS: 127000-20-8   |
| [18F]-Fluorodeoxyglucose (FDG)   | Stanford Cyclotron                                | CAS: 105851-17-0  |
| Microbubbles   | Lantheus  | Definity (DE4)  |
| IRDye 800RS  | LI-COR Biotechnology                              | IR800   |
| Experimental Models: Organisms/Strains   |   |   |
| Rats; Long-Evans Wild-Type   | Charles River                                     | Strain ID: 006  |
| Software and Algorithms  |   |   |
| Python v.3.6   | Anaconda  | <a href="https://www.anaconda.com/download/">https://www.anaconda.com/download/</a>                   |
| ImageJ (Fiji)  | <a href="#">Schindelin et al., 2012</a>           | <a href="https://imagej.net/Downloads">https://imagej.net/Downloads</a>                               |
| Simple ITK Python Package v.4.12.12  | ITK   | <a href="https://itk.org/ITK/resources/software.html">https://itk.org/ITK/resources/software.html</a> |
| Data Acquisition and Analysis code   | This paper  | Available upon request  |
| Other  |   |   |
| Focused Ultrasound System: Integrated Generator, RF Amplifier, Circulation System, and Transducer (2×) | Image Guided Therapy                              | Custom model  |
| Stereotactic Frame   | Image Guided Therapy                              | Custom model  |
| Bath Sonicator   | Thermo Scientific                                 | Bransonic M1800H  |
| Membrane Extruder  | Avestin   | Liposofast LF-50  |
| Zetasizer  | Malvern Panalytical                               | Zetasizer Nano ZS90   |
| Microtiter Plate Reader  | Tecan   | Infinite M1000 PRO  |
| Fluorescent Imaging System   | Spectral Instruments Imaging                      | Lago Part #: A1666  |
| 3T MRI   | MR Solutions                                      | MRS 3017  |
| EEG Recording Board  | OpenBCI   | Cyton   |
| USB Breakout Board   | Adafruit  | FT232H  |
| USB Isolator   | Adafruit  | 2107  |
| Green LED  | Linrose   | B4304H5-10  |
| Raspberry Pi   | RS Components Ltd                                 | RPi 2 Model B   |
| PET Scanner  | Concorde Microsystems                             | MicroPET R4   |

### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Raag D. Airan ([rairan@stanford.edu](mailto:rairan@stanford.edu)).

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

All experiments described in this work involving vertebrate animals were approved under the Stanford Administrative Panel on Laboratory Animal Care (APLAC), protocols 22100 and 32874, conforming to institutional and national guidelines and regulations. In this

work, healthy wild-type male Long-Evans rats (6–15 weeks old) were used. Subjects were not previously used for any other procedures. For EEG recordings and PET experiments, animals were used for multiple recording or imaging sessions, with at least 48 hr between each session. For the blood-barrier opening experiments used to visualize the sonication field, each animal was used once. Unless stated otherwise, rats were housed in cages with 2–3 inhabitants, with a day-night cycle enforced from 7 AM–7 PM. Animal husbandry was provided by veterinarians and technicians at the Stanford Veterinary Services Center (VSC). Food and water were freely available, unless otherwise stated.

## METHOD DETAILS

### Nanoparticle Synthesis and Characterization

#### Chemicals

Di-block copolymers are made up of a hydrophilic block of polyethylene glycol (PEG; mol. wt. 2 kDa) and a hydrophobic block of poly(lactic-co-glycolic acid) (PLGA). Two molecular weights of hydrophobic block chains were used: 2 kDa and 5 kDa. The example of nomenclatures for di-block copolymer is polyethylene glycol 2 kDa - poly(lactic-co-glycolic acid) = PEG (2 kDa)-PLGA (5 kDa). All di-block copolymers were purchased from Akina (West Lafayette, IN, USA). Propofol was purchased from Alfa Aesar (Haverhill, MA, USA). Ketamine hydrochloride injectable solution is a controlled substance and was purchased via Stanford University Environmental Health & Safety. Tetrahydrofuran (THF) and hexane were obtained from Sigma-Aldrich (St Louis, MO, USA). n-Perfluoropentane (PFP) was purchased from FluoroMed (Round Rock, TX, USA). A hydrophobic IRDye 800RS infrared dye was purchased from LI-COR Biotechnology (Lincoln, NE, USA).

#### Production of Nanoparticles

150 mg of di-block copolymer and 15 mg of propofol were combined in a 20 mL glass beaker and 10 mL THF was added to dissolve the polymer and drug. Then, 10 mL of phosphate buffer saline (PBS) was added dropwise to the organic solution over 5 min. The THF was fully evaporated by placing the mixture overnight in atmosphere and then in vacuum for 1 h. This produced drug-loaded polymeric micelles in saline suspension. Then, 300  $\mu$ l cold PFP was added to the suspension, followed by 5 min sonication in a 40 kHz Bransonic M1800H bath sonicator (Thermo Scientific; Waltham, MA, USA) which was pre-filled with iced water. The solution was centrifuged at 4°C at 2000 g for 10 min. The supernatant was decanted and the resulting pellet was resuspended in cold PBS. Centrifugation-resuspension was repeated two more times to remove and dilute residual free drug, polymer, and PFP-free micelles. Finally, the nanoemulsion suspension was extruded twice using an Avestin Liposofast LF-50 extruder (Ottawa, ON, Canada) equipped with compressed nitrogen (40 psi) and loaded with a polycarbonate membrane of 0.6  $\mu$ m pores. The extruded nanoemulsion suspension was either used fresh or mixed with glycerin (2.25%, w/v) as a cryoprotectant, frozen immediately, and stored at –80°C until it was thawed for use.

#### Characterization of Nanoparticles

The Z-average diameter, polydispersity index (PDI) and zeta potential of the drug-loaded phase-change nanoemulsions were measured via dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS90 (Malvern, United Kingdom). A 10  $\mu$ l nanoemulsion solution was thoroughly mixed with 990  $\mu$ l cold PBS. DLS parameters were: materials = perfluoropentane; Refractive Index (RI) = 1.330; absorption = 0.1; dispersant = ICN PBS tablets; viscosity = 0.8882 cP at 25°C; Mark-Houwink parameters; equilibration time = 60 s; disposable cuvettes = ZEN0118; measurement angle = 90 degree; measurement duration = automatic; number of measurements = 5; positioning method = seek optimum position; analysis model = general purpose (normal resolution). To measure the zeta potential, 10  $\mu$ l nanoemulsion solution was mixed with 990  $\mu$ l deionized water. Then 900  $\mu$ l of this solution was transferred to a disposable capillary cell. Measurement parameters were: cell type = DTS1070, dispersant = water; viscosity = 0.8872 cP at 25°C; dielectric constant = 78.54; F( $\kappa$ a) selection model = Smoluchowski; F( $\kappa$ a) value = 1.5; measurement duration = automatic; measurement runs between 10 and 100; number of measures = 3 with no delay between measurements.

#### Quantification of Nanoparticle Drug Loading

A 100  $\mu$ l nanoemulsion solution was thoroughly mixed with 900  $\mu$ l methanol. The fluorescence of propofol was quantified with a Tecan Infinite M1000 microtiter plate reader (San Jose, CA, USA) at excitation/emission = 276/302 nm. The propofol content was calculated with respect to a standard curve of propofol prepared in varying concentrations in the same solvent.

#### In Vitro Assay of Ultrasonic Drug Uncaging

A 50  $\mu$ l nanoemulsion suspension (1 mg/ml drug equivalent) was added to a Fisherbrand 0.2 mL PCR tube (Fisher Scientific). 150  $\mu$ l of hexane was added atop the nanoemulsion suspension. The PCR tube was placed in a custom holder and coupled using degassed water to a focused ultrasound (FUS) transducer (Image Guided Therapy, Pessac, France) at room temperature, such that the FUS focus was contained within the nanoemulsion suspension layer. The nanoemulsions were sonicated with FUS for 60 s total, with varying peak negative pressure and burst length, with a burst repetition frequency of 1 Hz. While varying pressure, the burst length was 50 ms; while varying burst length, the peak *in situ* pressure was 1.2 MPa. The center frequency of the transducer was 650 kHz. Following FUS, 100  $\mu$ l of the organic phase was collected without disturbing the aqueous layer. The amount of the uncaged drug was quantified by measuring its UV fluorescence and comparing to a standard curve of the drug prepared in varying concentrations in hexane.

### Pharmacokinetics of Nanoparticles

All animal experiments were carried out in accordance with the Stanford IACUC. Long-Evans rats with body weight 180-200 g (Charles River Laboratories, Wilmington, MA, USA) were used in all *in vivo* studies. Propofol-loaded PFP/PEG (2 kDa)-PLGA (5 kDa) nanoemulsions were doped with a hydrophobic near-infrared fluorescent dye, IR800 (LI-COR, Lincoln, NE), during nanoemulsion production and then used to test *in vivo* blood-pool particle kinetics and systemic biodistribution.

To produce dye-doped nanoemulsions, 1 mg IR800 dye was added to the drug and polymer THF solution, and the rest of the nanoemulsion production protocol was unchanged. For the *in vivo* experiments, a nanoemulsion bolus (equivalent to 1 mg/kg of drug) was administered intravenously via a 24 g × 3/4" catheter paced in the rat tail vein in a total volume of ~0.4-0.5 mL (n = 3). Blood samples were collected via either left or right submandibular vein at 2 min 10 min, 20 min, 40 min, 2 h and 4 h, alternating sides for each sampling. The blood was split into two volumes. Whole blood sample fluorescence was assessed using a Lago (Spectral Instruments Imaging; Tucson, AZ, USA) imaging system (excitation/emission = 770/810 nm) and quantification was completed using regions of interest (ROIs) of the same size across samples, drawn within the capillary tube. The second volume of each sample was centrifuged in a microcentrifuge for a total of 10 min at 10,000 g at 4°C. The plasma fraction from these samples was then collected and their fluorescence was quantified in a similar fashion to that of whole blood samples. The nanoemulsion concentration in the whole blood and plasma were fitted with a two-compartment kinetic model, described in the next section.

For systemic biodistribution quantification, the same dye-doped propofol-loaded nanoemulsions were administered intravenously as a bolus to Long-Evans rats (n = 3). The rats were sacrificed at 24 h post administration to harvest major organs: heart, liver, lungs, kidneys, spleen, and brain. These organs were imaged for IR800 fluorescence (excitation/emission = 770/800 nm) using a Lago imaging system (Tucson, AZ) and quantified using regions of interest (ROI) of the same size, drawn to be within the image of each organ. The distribution of the nanoemulsion among the organs was calculated by dividing the ROI fluorescence of each tissue by the sum of ROI fluorescence values of all organs.

### Modeling Pharmacokinetic Data

For modeling, we use the two-compartment model

$$\dot{x}_c(t) = k_{21}x_p(t) - (k_{el} + k_{12})x_c(t)$$

$$\dot{x}_p(t) = k_{12}x_c(t) - k_{21}x_p(t),$$

where  $x_c(t)$  and  $x_p(t)$  are the nanoparticle concentration in the central blood pool and periphery, respectively,  $k_{12}$  and  $k_{21}$  are the rate constants for drug transfer from the blood pool to the periphery and vice-versa, and  $k_{el}$  is the elimination rate constant. Solving this equation by ansatz gives us a solution consisting of the double exponential of the form

$$x_c(t) = A_c e^{-r_d t} + B_c e^{-r_e t}$$

$$x_p(t) = A_p e^{-r_d t} + B_p e^{-r_e t},$$

where  $r_d$  is the rate of distribution and  $r_e$  is the rate of elimination (not to be confused with the elimination rate constant), and  $A_c/A_p$  and  $B_c/B_p$  are the coefficients for the distribution and elimination exponential phases, respectively. Note that our pharmacokinetics experiments primarily measure  $x_c(t)$ . Whole blood data contain contributions from both nanoparticles and micelles, whereas plasma contains contributions from micelles (and likely minimal free dye).

### Histology

A total of ten animals were used to evaluate the histological effects of ultrasonic drug uncaging. Following nanoparticle administration and sonication, the animals were deeply anesthetized with ketamine/xylazine, and sacrificed and fixed via transcardial perfusion (0.9% NaCl, 100ml; 4% paraformaldehyde, 250ml). The brains were then removed, embedded in paraffin, and serially sectioned at 4 μm in the transverse plane (perpendicular to the direction of ultrasound beam propagation). Every 50<sup>th</sup> section (200 μm apart) was stained with hematoxylin and eosin (H&E) and inspected using NanoZoomer-XR (Hamamatsu Photonics, Bridgewater, NJ), in consultation with a veterinary pathologist of the Stanford Veterinary Service Center.

### Magnetic Resonance Imaging for Safety of Nanoparticles

For evaluating safety, a transmit/receive surface coil (diameter/radius of curvature: 4/3ccm) was placed above the rat head, and the system was placed in an animal 3T MRI (preclinical cryogenic-free, MR Solutions, Guildford, UK). Before and after ultrasonic propofol uncaging (1.8 MPa *in situ* pressure, 60 × 100 ms bursts delivered with 1 Hz burst frequency after intravenous administration of 1 mg/kg encapsulated propofol) in visual cortex, axial T2-weighted fast spin-echo (FSE) images (parameters: repetition time (TR): 5475 ms; echo time (TE): 68 ms; matrix size: 256 × 256; slice thickness/spacing: 0.5 mm/interleaved), axial T2\*-weighted gradient echo images (TR/TE: 718/6 ms; matrix size: 256 × 256; slice thickness: 2 mm), and axial T1-weighted post-contrast FSE images (TR/TE: 720/11 ms; matrix size: 256 × 256; slice thickness/spacing: 1 mm/interleaved) were acquired. Post-contrast images were taken before and after the intravenous administration of an MRI contrast agent (MultiHance, gadobenate dimeglumine; Bracco Diagnostics Inc, NJ USA; 0.21 ml/kg).

## Visual Evoked Potential (VEP) Recordings

### EEG Electrode Implantation

A 9 mm rostrocaudal incision was made of the rat dorsal scalp (body weight 180–200 g) with surgical scissors and a scalpel. Then, 1-mm burr holes were drilled into the skull for two-electrode implantation. A stainless-steel skull screw (J.I. Morris, Southbridge, MA, USA) was implanted through the skull close to the visual cortex (6 mm posterior to bregma and 1 mm lateral to midline) as the signal electrode. A reference screw electrode was placed 2 mm anterior to bregma and 2 mm lateral to the midline. Dental cement (BASi, West Lafayette, IN, USA) was used to fix the screws. The skin incision was closed and 10 days were allowed for the animals to recover from the surgery before electroencephalography (EEG) recording.

### EEG Recording and LED Stimulus Setup

EEG recording was performed with an 8 Channel Cyton Biosensing Board (OpenBCI, Brooklyn, NY, USA) with a custom firmware that allowed for a sampling rate of 500 Hz and for recording the stimulus timings. To prevent aliasing, samples were recorded at 16 kHz with digital filtering before resampling at 500 Hz, according to the specifications of the OpenBCI ADS1299. The OpenBCI board was also modified to interface with a laptop via a USB breakout board (Adafruit, NY, USA) and USB isolator (Adafruit, NY, USA). For EMI shielding, the box was placed in a Faraday Cage consisting of a cardboard box with aluminum foil and copper tape. The stimulus was provided by a Mini-Ganzfeld Stimulator consisting of a 3D-printed cone with three green LEDs (Linrose B4304H5-10, Plainview, NY, USA) embedded, and shielded with black electrical tape and copper mesh. A Raspberry Pi 2 Model B (RS Components, Corby, Northants, UK) was used to coordinate stimulus delivery. To gate the LED stimulus, the Raspberry Pi was connected to a breadboard (Twin Industries, San Ramon, CA) and a MOSFET (NTE, Bloomfield, NJ).

### Combined FUS-EEG Setup

At least 10 days after electrode implantation, animals were anesthetized with ketamine/xylazine and were placed in a plastic stereotactic frame (Image Guided Therapy, Pessac, France) coupled to the FUS system, and immobilized with ear bars and a bite bar. Any remaining dorsal scalp fur in the sonication trajectory was removed by clipping and applying a chemical depilatory (Nair, [amazon.com](https://www.amazon.com)). A hair dryer was used for 20–30 s to remove moisture from around the electrodes. The signal and reference electrodes were coupled to the corresponding skull screw electrodes and the custom-made EEG system. A needle was inserted under the skin of the neck as the ground electrode. A digital multimeter was used to ensure that the electrode impedances were below 5 $\Omega$ . A monocular visual stimulus (Linrose B4304H5-10; 10ms flashes presented at 1 Hz) was applied contralateral to the sonicated hemisphere and the ipsilateral eye was covered with a plastic cone. A thin (< 1mm) ultrasound pad (Aquaflex, Parker Laboratories, Fairfield, NJ, USA) and minimal ultrasound gel were used to couple the FUS transducer membrane and the skin of the head. Stereotactic coordinates for sonication are given in [Table S2](#). The depth of sonication was controlled by adjusting the height of the coupling bag between the transducer face and the rat head. To account for skull attenuation, a 30% pressure insertion loss was assumed for this size and age of rats (O'Reilly et al., 2011). Prior to recording, animals were kept in a darkened room and allowed to adapt to darkness for at least 5 min.

### Visual Evoked Potential (VEP) Recording

To ensure an adequate anesthesia plane was achieved to yield the appropriate signal-to-noise ratio for the experiment, VEP N1P1 amplitudes were monitored until the amplitude measured at least 60  $\mu$ V over a 60 s epoch. Once this condition was achieved, 6 min VEP traces were acquired, with either focused ultrasound or nanoemulsion intravenous administration commencing at 3 min after the VEP recording started. At least 10 min passed between nanoemulsion administration and the next sonication.

## PET Functional Imaging

### Functional Imaging with [18F]-FDG

Dynamic PET Imaging was conducted on a Concorde Microsystems R4 MicroPET Scanner (Knoxville, TN). 5–7 rats (body weight 200–220 g) were randomly assigned per condition. Rats were fasted overnight for 15–18 hr. Shortly before each scan, tail vein access was established via cannulation while the animal was anesthetized with 3% isoflurane. Dorsal scalp fur in the sonication trajectory was removed by clipping and applying a chemical depilatory (Nair, [amazon.com](https://www.amazon.com)). The midline and interaural line were drawn with a skin marker.

Then, the animals were taken off isoflurane and anesthetized with ketamine/xylazine. After the respiratory rate recovered to over 30 breaths per minute, the animals were placed in a plastic stereotactic frame (Image Guided Therapy, Pessac, France) coupled to the FUS system, and immobilized with ear bars and a bite bar. The transducer was aligned to 7 mm anterior to the interaural line (= 2 mm caudal to bregma), 2 mm lateral of midline, 2 mm deep to skin for the cortical site and 6 mm anterior to interaural line (= 3 mm caudal to bregma), 1.2 mm lateral of midline, and 5 mm deep to skin for the deep site. These coordinates are summarized in [Table S2](#). The depth of sonication was controlled by adjusting the height of the coupling bag between the transducer face and the rat head. Ultrasound gel was used to couple the transducer to the skin. Then, the frame, including the FUS transducer, was placed within the scanner bore.

At the scan start, 2–2.2 mCi (in 150–500  $\mu$ L) of [18F]-Fluorodeoxyglucose (FDG) was administered through the tail vein catheter. Two minutes later, nanoemulsions were administered intravenously to a total encapsulated propofol dose of 1 mg/kg. Twelve minutes after administration of radiotracer (i.e., 10 min after nanoemulsion administration), FUS was applied while the animal was

inside the PET scanner for 4 min for the given peak negative pressure, with a burst length of 50 ms and a burst repetition frequency of 1 Hz. To account for the skull and soft-tissue attenuation, attenuation coefficients were extrapolated from O'Reilly et al. (2011). Scans were conducted for at least 30 min.

Images were then reconstructed with 0.85 mm (mediolateral) x 0.85 mm (dorsoventral) x 1.21 mm (anteroposterior) voxels, with 2-min frames up to 8 min post radiotracer administration, after which 4-minute frames were used. Further analysis was done with the SimpleITK package in Python and the Fiji distribution of ImageJ (Schindelin et al., 2012).

### In Vivo Characterization of the FUS Field

To determine the maximal extent of the sonication field for each target, and evaluate for any scattering, we used a blood-brain barrier (BBB) opening protocol similar to prior evaluations (McDannold et al., 2013). First, tail vein access was established via cannulation while the animal was anesthetized with 3% isoflurane. Dorsal scalp fur in the sonication trajectory was removed by clipping and applying a chemical depilatory (Nair, amazon.com). The midline and interaural line were drawn with a skin marker.

Then, animals were taken off isoflurane and anesthetized with ketamine/xylazine. After the respiratory rate recovered to over 30 breaths per minute, animals were placed in a plastic stereotactic frame (Image Guided Therapy, Pessac, France) coupled to the FUS system, and immobilized with ear bars and a bite bar. The transducer was aligned to either the cortical or deep site discussed in the PET section above. Ultrasound gel was used to couple the transducer with the skin. Definity Microbubbles (20  $\mu$ L/kg, Lantheus, N. Billerica, MA) were injected through the tail-vein cannula in a 1:10 dilution. Ten seconds after injection, FUS was applied for 5 min with an estimated peak *in situ* negative pressure of 0.4 MPa ( $\sim 3\times$  the estimated threshold intensity for this experiment), derated for the skull based on animal weight (O'Reilly et al., 2011), with a burst length of 10 ms and a burst repetition frequency of 1 Hz.

MRI evaluation was completed as discussed in the prior section. After sonication, axial T1-weighted fast spin-echo (FSE) images were taken (parameters: TR/TE: 720/11 ms; matrix size: 256/252; slice thickness: 1.5 mm) pre- and post-contrast intravenous administration. Post-contrast coronal T1-weighted FSE (TR/TE: 4800/68 ms; matrix size: 256x248; slice-thickness: 1.3 mm), axial T2\*-weighted (same parameters as above), and axial T2-weighted (TR/TE: 5000/68 ms; matrix size: 256 x 248; slice thickness/spacing: 1.3 mm) images were also acquired.

### Calculation of Heating Induced by Focused Ultrasound Protocols

To compute the estimated heating during each ultrasound pulse, we compute the intensity of ultrasound  $I$  as

$$I = \frac{P^2}{2Z}$$

where  $P$  is the peak negative pressure and  $Z$  is the acoustic impedance (Haar and Coussios, 2007). Additionally, we can derive from the ultrasound heat equation (Nyborg, 1988) the change in temperature per unit time  $dT/dt$  as

$$\frac{dT}{dt} = \frac{2\alpha I}{\rho C}$$

where  $\alpha$  is the tissue attenuation coefficient,  $\rho$  is the density of the material, and  $C$  is the specific heat. In the case of brain tissue, we set our constants as

$$Z = 1.60 \times 10^6 \frac{\text{kg}}{\text{s} \cdot \text{m}^2}$$

$$\rho = 1.03 \frac{\text{g}}{\text{cm}^3}$$

$$\alpha = 0.44 \frac{\text{dB}}{\text{cm}}$$

$$C = 3630 \frac{\text{J}}{\text{kg} \cdot ^\circ\text{C}}$$

as taken or derived from published values for each (Azhari, 2010; Hasgall et al., 2015). Computing with these constants give an estimated temperature rate of rise during sonication at  $P = 1.2$  and 1.8 MPa peak negative pressure of 1°C/s and 2°C/s respectively. Given that we sonicate with pulses that are maximally 0.1 s at 1.2 MPa and 0.05 s at 1.8 MPa, we expect temperature rises of up to 0.1°C per pulse in either condition.

## QUANTIFICATION AND STATISTICAL ANALYSIS

All data were analyzed and plotted using custom code written in Python.

### EEG Recordings

#### EEG Preprocessing

Raw EEG traces were digitally filtered with a 4th order bandpass Butterworth filter with cutoff frequencies of 1–100 Hz. Notch filtering for 60 Hz noise and its higher harmonics consisted of 2nd order digital Chebyshev filters with cutoff frequencies of 58–62 Hz, 118–122 Hz, 178–182 Hz, and 238–242 Hz. VEP traces were computed by averaging over all presented VEP stimuli over a 60 s period with a Gaussian kernel with a standard deviation of 20 s. The N1P1 amplitude for averaged VEP traces was quantified by finding the first local minimum 40 ms after stimulus onset, then finding the next local maximum, and then taking the difference. Traces consisting of N1P1 amplitudes that swung between adjacent presentations of more than 30  $\mu\text{V}$  in either direction were excluded because they were indicative of VEP traces that were too unstable to quantify due to spiking electrical artifacts. All traces were then normalized by dividing by the averaged N1P1 amplitudes over the period from 0 to 120 s.

Within a single experiment, we have multiple traces for which we applied sonication after injection of nanoparticles. First, we average over all of the traces within that single experiment to generate a single averaged trace per experiment. Then we average over all of these averaged traces for all experiments with a given condition. We define  $N$  in this case to be the number of recording sessions conducted.

#### Electrophysiologic Effect Quantification

For quantification of effect size and recovery time, we use the piecewise model

$$VEP(t; k_{on}, k_{off}) = \begin{cases} 1, & t < 180 \\ 1 - k_{on}(t - 180), & 180 \leq t < 240 \\ 1 - 60k_{on}e^{-k_{off}(t-240)}, & 240 \leq t \end{cases}$$

where  $t$  represents the experiment time (in minutes),  $k_{on}$  represents the rate of suppression by propofol, and  $k_{off}$  represents the rate of recovery. To fit against our experimental data, we first sample the model at the same time points as from our quantification of the N1P1 amplitude, then digitally apply a Gaussian Kernel to our model over a period of 60 s with a standard deviation for 20 s to mimic the averaging of N1P1 amplitudes from our analysis (Figure S2A). We then fit our two parameters by least-squares regression with the Trust Region Reflective variant of the Levenberg-Marquardt method (Figure S2B). We report root mean square errors (RMSE) in Table S1. With our fit parameters, we can now define our effect size and recovery half-life time as

$$\text{Effect Size} = \text{N1P1 Difference} = 1 - VEP(240)$$

$$\text{Recovery Time} = \frac{\ln(2)}{k_{off}}$$

To estimate the errors of our estimated parameters, we utilize a boot-strap technique. Let  $N$  be the number of experiments for a given condition. For 500 times, we sample  $N$  experiments, with replacement, and generate the trace using the double averaging method described above. Then, we estimate our parameters. We take the standard deviation of our trials and divide by  $\sqrt{N}$  to compute the SEM of the estimated parameters.

### PET Imaging

#### Quantification of PET Effect Size

To determine the effect size, we first identified the midline and the interaural line on the image taken from 12–16 min (i.e., during sonication). To define the sonication region of interest, we used the same landmarks as when physically positioning the transducer (using the cerebellum/cerebrum border as the interaural line) and measured our coordinates as given in Table S2. We then determined the voxel with the minimal FDG uptake (under an assumption of detecting a decrease, as we are uncaging an anesthetic) in a  $3 \times 3$  mm region centered on that measured target and used that to define the center of the measured effect. This procedure was completed exactly in the same fashion for all groups, including for blank nanoparticle sonication.

We normalized by using the same quantification method on the contralateral side (4 mm lateral to sonication for the cortical site, 3 mm for the deep site). We then report the ratio of the sonication area to the contralateral site. For our time activity curve (TAC) analysis, we carry out the same quantification method for each frame.

#### Effect Full-Width Half-Max (FWHM) Quantification

We quantify the effect FWHM from the cortical sonication experiments. First, we interpolated our images via cubic interpolation. Then, the ROI from the quantification of the effect size was identified on the sonication image. 1-D curves were generated to identify the local minima and its adjacent local maxima along the right-left and ventral-dorsal axis. For the right-left axis, FWHM was defined as the total distance for FDG avidity to recover to half of the distance between the local minima at the focus and its local maxima. For the ventral-dorsal axis, because our focus site was cortical, we instead defined the half-max distance as the distance over which FDG

avidity reached half of its maximum value moving ventrally beyond the focus, and computed the FWHM by dividing this distance by 60%, as 60% of the distance covered by the FWHM is beyond the focus distal to the transducer according to simulations and hydrophone recordings of the transducer sonication field.

### **Generation of Statistical Parametric Maps**

First, all images were manually cropped to include only the brain to ease coregistration. Images were then coregistered against one of the scans taken with sonication of blank nanoemulsions for each site. Coregistration was performed in Simple ITK, with first a rigid-body transformation and then an affine optimization. Interpolation was performed with a Hamming windowed sinc function. Similarity measures were computed by thresholding the images against the median and then taking the root mean square error. For the affine optimization, the Nelder-Mead method was used, with a function convergence tolerance of  $10^{-6}$ . After coregistration, the cerebellum was identified for the fixed blank image used for coregistration, and this ROI was used for all coregistered images for normalization as discussed below.

To compute statistical parametric maps, for each scan within the condition we selected the sonication frame and the frame taken from 24–28 min (i.e., the final frame taken). We subtracted the final frame from the sonication frame and then divided by the mean value from the cerebellum ROI derived for normalization. We then repeated this process across all scans for the same condition. Then for each voxel, we computed the z-score by taking the average normalized difference and then dividing by the standard error across all experiments for that specific voxel, with  $N$  defined as the number of imaging sessions conducted under that specific condition. We thresholded our images by the voxel-wise Bonferroni corrected alpha value of 0.05 (i.e.,  $p \leq 10^{-5}$ ,  $z \leq -4.25$ ).

### **DATA AND SOFTWARE AVAILABILITY**

All data and code are available upon reasonable request to Raag D. Airan ([rairan@stanford.edu](mailto:rairan@stanford.edu)).

**Neuron, Volume 100**

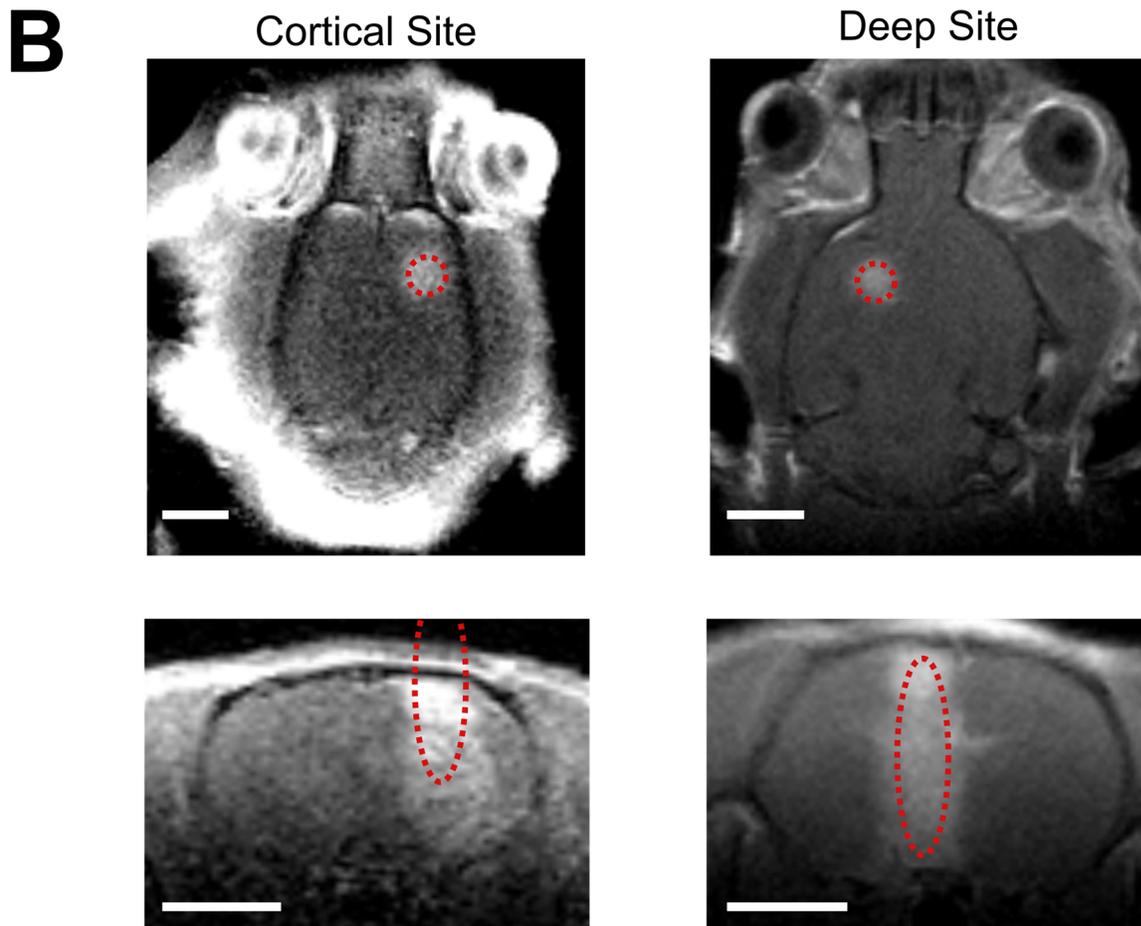
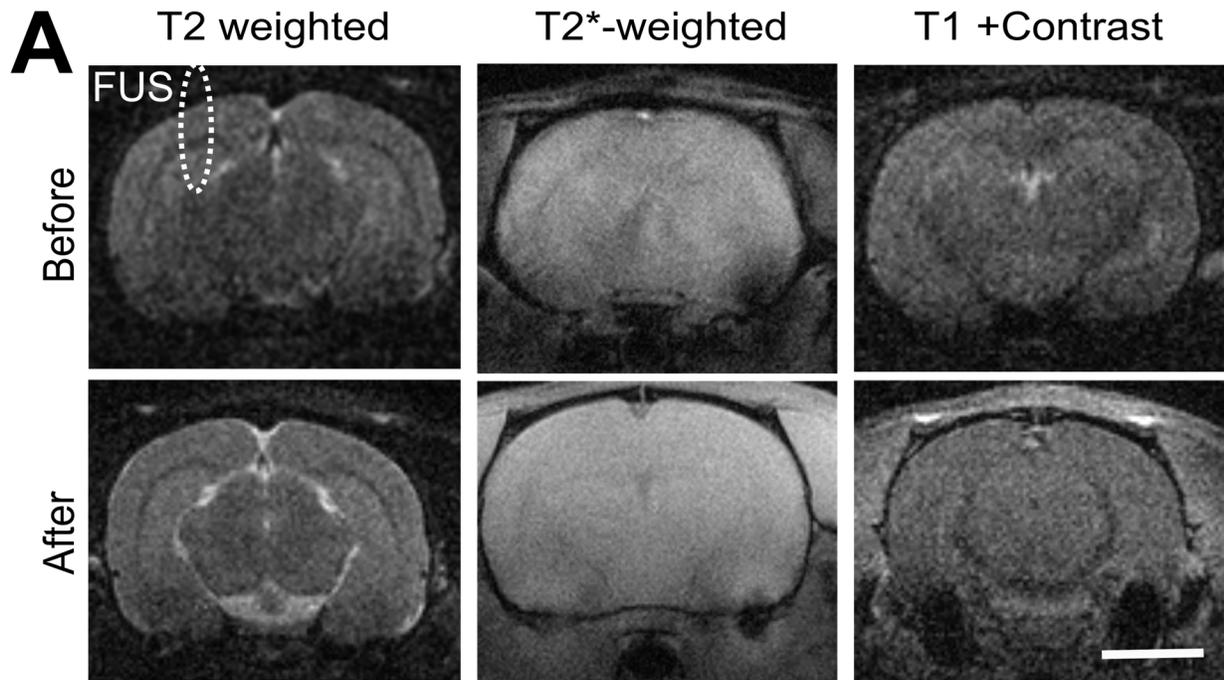
**Supplemental Information**

**Noninvasive Ultrasonic Drug Uncaging**

**Maps Whole-Brain Functional Networks**

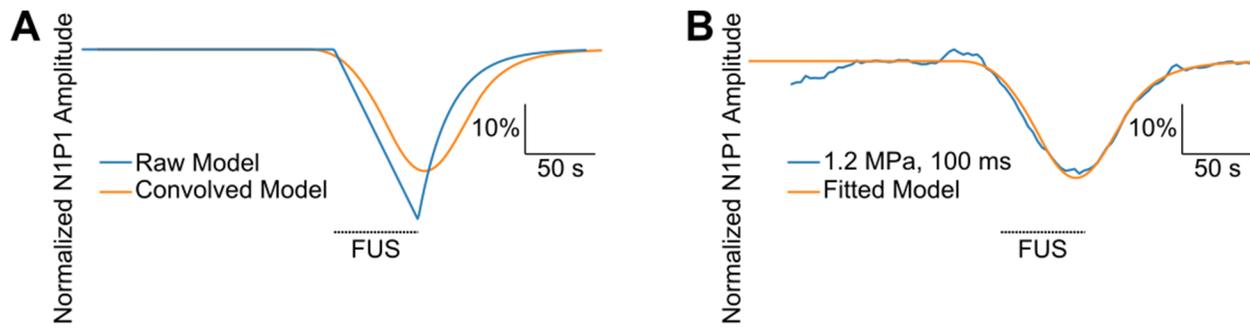
**Jeffrey B. Wang, Muna Aryal, Qian Zhong, Daivik B. Vyas, and Raag D. Airan**

SUPPLEMENTAL DATA



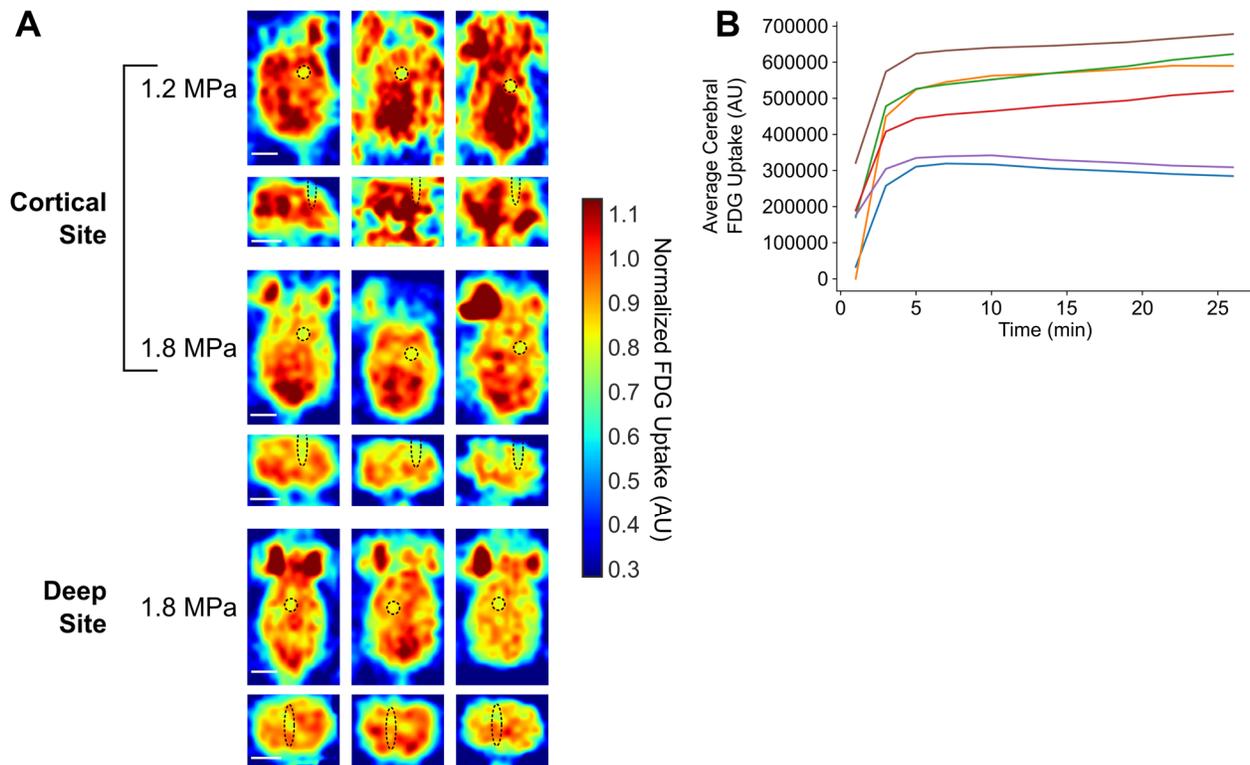
**Fig. S1: Representative MRI evaluation of the effects of ultrasonic drug uncaging on the brain parenchyma. Related to Figures 1, 4, and 5.**

**(A)** No effects such as edema (which would be bright on T2-weighted MRI, left), hemorrhage (which would be dark on T2\*-weighted MRI, middle), or blood-brain barrier disruption (which would be bright on post-contrast T1-weighted MRI, right) were noted in the parenchyma by MRI with and without contrast when comparing before (top) and after (bottom) focused ultrasound (FUS; 60 x 100 ms bursts at 1 Hz burst frequency with 1.8 MPa peak in situ pressure) application to a single focus in visual cortex (dashed ellipse, top left: expected sonication focus full-width at half-maximum) after intravenous administration of propofol-loaded nanoparticles (bolus of 1 mg/kg encapsulated propofol). Scale bar: 5 mm. **(B)** To visualize the maximal *in vivo* sonication field in the rat and provide a positive control for the analysis in **A**, we performed microbubble-mediated blood-brain barrier disruption with a sonication pressure ~3 times the estimated threshold for this effect (0.4 MPa with 10 ms pulses delivered every second for 5 minutes) (McDannold et al., 2013). Depicted are representative post-contrast T1-weighted 3T MRI images for each target used for PET experiments. It is estimated that areas exposed to sonication of at least ~0.12 MPa = 30% of maximum would appear bright on these images (McDannold et al., 2013). This is supported by the extent of gadolinium uptake beyond the sonication FWHM (red dashed ellipse). Scale bars: 5 mm



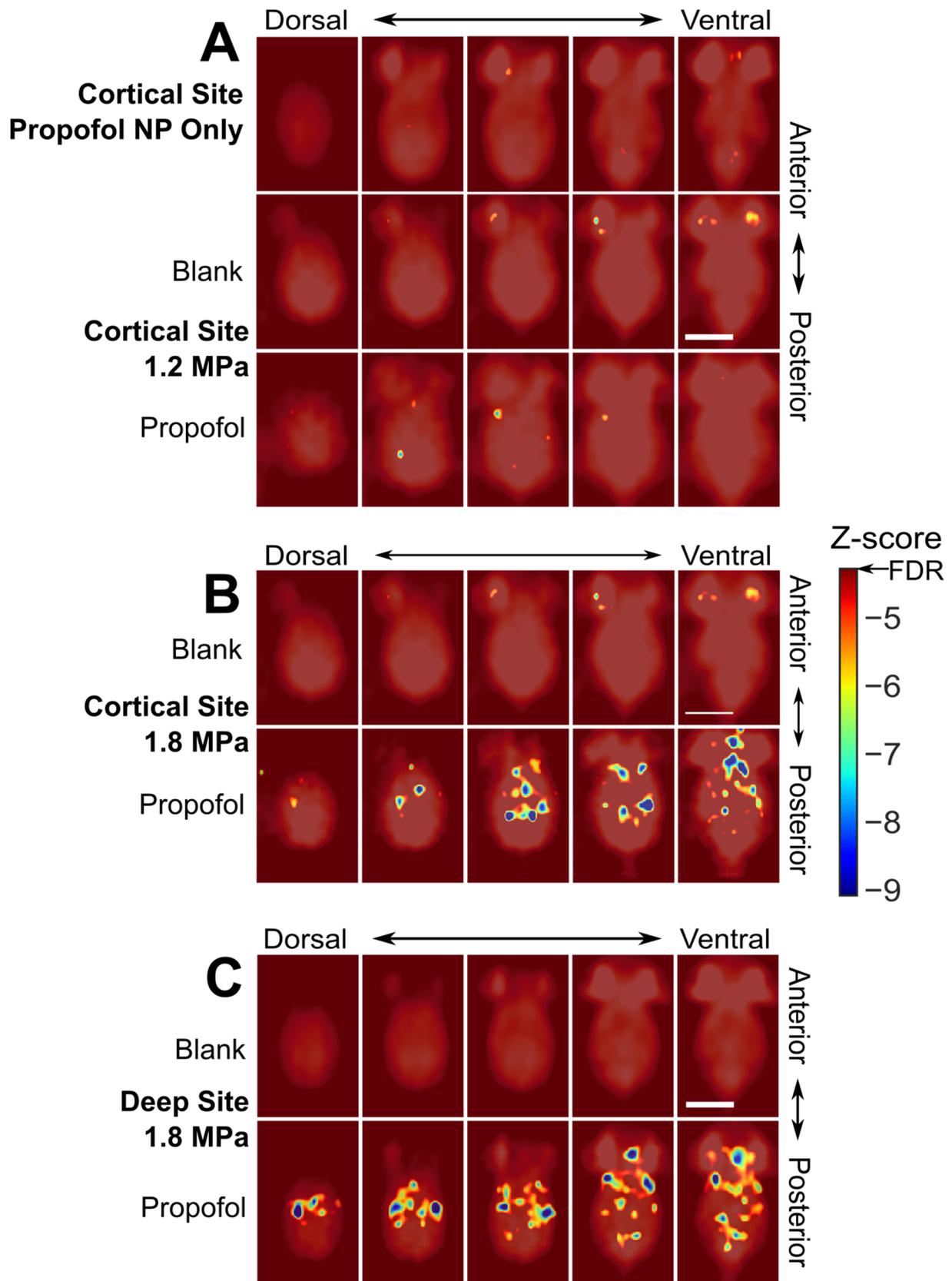
**Fig. S2: Modeling of anesthesia of visual evoked potentials (VEPs) with ultrasonic propofol uncaging. Related to Figure 2.**

(A) In the raw model, before sonication, the VEP is assumed to be unchanging from its normalized baseline value. Upon sonication, the VEP amplitude decreases linearly during sonication as a first-order approximation of the net effect of uncaged propofol supply, redistribution, and metabolism. After sonication stops, the VEP N1P1 amplitude recovers back to its baseline with exponential decay. In the convolved model, we convolve with a Gaussian kernel that matches the kernel used to compute our N1P1 amplitude from the raw data. Depicted here is the model with the same parameters fit for our propofol nanoparticles when exposed to 1.2 MPa peak negative pressure and a burst length of 100 ms. The dashed bar is the time for which sonication (FUS) is applied. (B) Comparison of our fitted model with the N1P1 amplitudes recorded during ultrasonic propofol uncaging with 1.2 MPa peak negative pressure and 100 ms burst length (N=6). Here, root mean square error is 2.16% (in the units of normalized N1P1 amplitude) and model  $R^2$  is 90.2%.



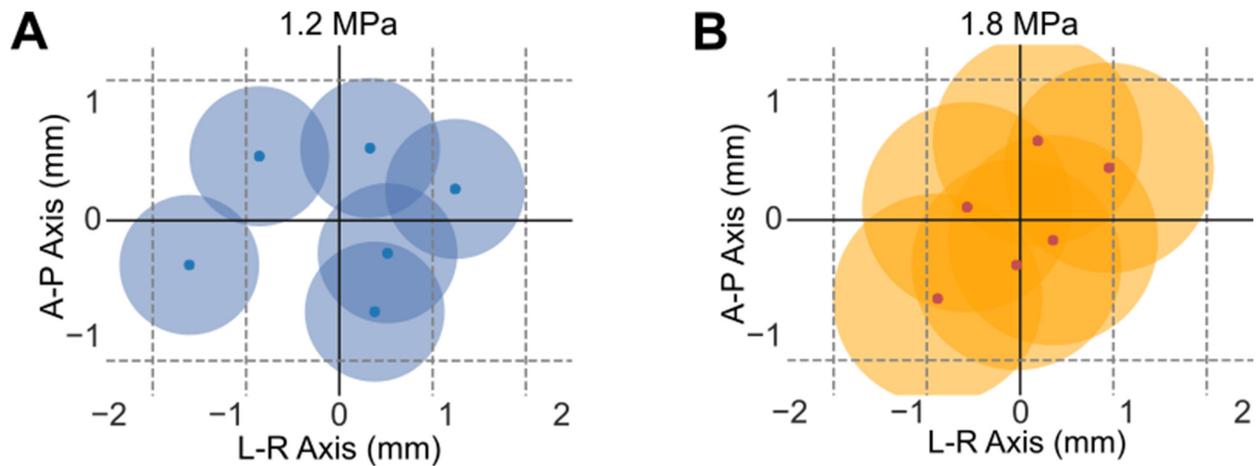
**Fig S3: Reproducibility of neuromodulation induced by ultrasonic drug uncaging and diversity of FDG uptake profiles between animals. Related to Figures 3 and 4.**

**(A)** Representative examples for individual animal PET images during ultrasonic propofol uncaging directed to the target indicated by the black dashed ellipses. Images are normalized by uptake of the contralateral region from the target. Note that the first column is repeated from **Figs. 3 and 4**. Scale bars: 5 mm. **(B)** Average FDG uptake in the cerebrum for each of the six animals in the 1.2 MPa cortical sonication cohort with propofol-loaded nanoparticles demonstrating the variability of uptake across animals, as expected given that FDG uptake is dependent on factors like depth of anesthesia, animal body weight, and resting glucose level.



**Fig. S4: Unlabeled statistical parametric maps. Related to Figures 4 and 5.**

**(A)** Statistical parametric maps from data generated after nanoparticle administration with no sonication (top), or with sonicating at the cortical target with a peak negative pressure of 1.2 MPa, with blank (middle) or propofol-loaded (bottom) nanoparticles. Note that the anterior paired structures outside the brain are the Harderian glands. Changes in these glands represent the noise floor of this technique. **(B)** Statistical parametric maps generated from sonicating at the cortical target with a peak negative pressure of 1.8 MPa. Note that the top row is repeated from **A**. **(C)** Statistical parametric maps generated from sonicating at the deep site with a peak negative pressure of 1.8 MPa, with blank (top) or propofol-loaded (bottom) nanoparticles. Scale bars: 5 mm.



**Fig. S5: Overlap of sonication sites following whole-brain coregistration. Related to Figures 4 and S4.**

(A) Relative coordinates of the point with minimal FDG uptake at the primary sonication target (for cortical sonication at 1.2 MPa) in the transverse plane, as identified by tricubic interpolation, following the whole-brain registration procedure used for **Fig. S4A**, bottom. Points are centered around the mean of all interpolated coordinates. Grid lines represent the non-interpolated voxel dimensions (not necessarily the exact voxel boundary locations), solid dots represent the sonicated target coordinate for each animal in the cohort, and the larger shaded circles represent the FWHM size of the anesthesia effect (from Fig. 3F). (B) Relative placement of coordinates for the 1.8 MPa cortical sonication condition following the whole-brain coregistration completed for **Fig. S4B**, bottom. All conventions are the same as in A.

| Drug     | Sonication Site | Peak Negative Pressure (MPa) | Burst Length (ms) | Number of experiments | RMSE  |
|----------|-----------------|------------------------------|-------------------|-----------------------|-------|
| Propofol | V1              | 0.8                          | 50                | 6                     | 2.71% |
|          |                 | 1.2                          | 10                | 4                     | 3.70% |
|          |                 |                              | 50                | 9                     | 1.83% |
|          |                 |                              | 100               | 6                     | 2.16% |
|          |                 | 1.8                          | 50                | 5                     | 5.20% |
|          | V1 (First)      | 1.2                          | 50                | 4                     | 3.20% |
|          | M1              |                              |                   | 5                     | 1.82% |
|          | V1 (Second)     |                              |                   | 4                     | 3.07% |
|          | LGN             |                              |                   | 7                     | 2.69% |
|          | Blank           | V1                           |                   |                       | 7     |

**Table S1: Errors for modeling anesthesia of visually evoked potentials (VEPs). Related to Figure 2.**

Root mean square error of fitted model for computing VEP effect sizes and recovery times under different conditions as shown in **Fig. 2**.

| Protocol | Sonication Site | Caudal from Bregma (mm) | Lateral of Midline (mm) | Depth from Bregma (mm) |
|----------|-----------------|-------------------------|-------------------------|------------------------|
| VEP      | V1              | -6.5                    | 3.5                     | -2                     |
|          | M1              | -2                      | 1.5                     | -2                     |
|          | LGN             | -4                      | 3.5                     | -5                     |
| PET      | Cortical        | -2                      | 2                       | -2                     |
|          | Deep            | -3                      | 1.2                     | -5                     |

**Table S2. Stereotactic coordinates of sonication sites. Related to STAR Methods and Figures 2, 3, and 4.**