Remotely controlled drug release in deep brain regions of non-human primates

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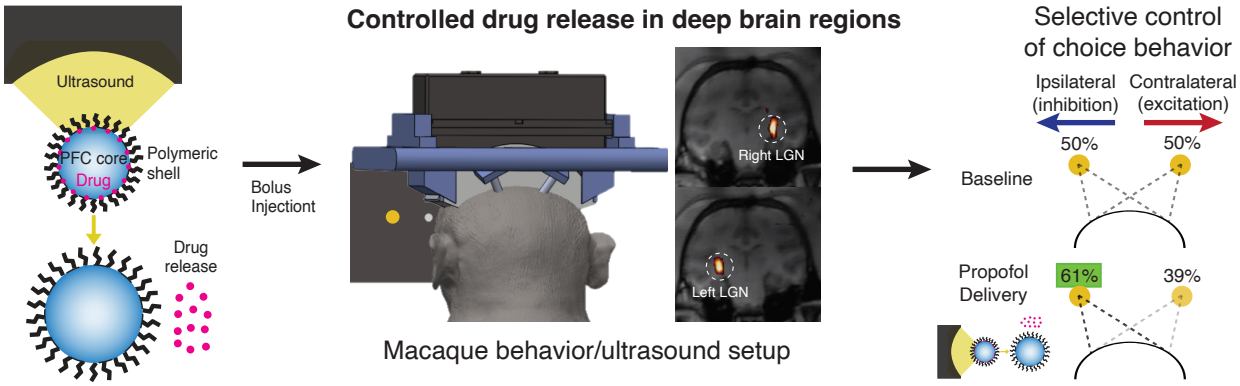
Many areas of science and medicine would benefit from selective release of drugs in specific regions of interest. Nanoparticle drug carriers activated by focused ultrasound—remotely applied, depth-penetrating energy—may provide such selective interventions. Here, we developed stable, ultrasound-responsive nanoparticles that can be used to release drugs effectively and safely in non-human primates. The nanoparticles were used to release propofol in deep brain visual regions. The release reversibly modulated the subjects' visual choice behavior and was specific to the targeted region and to the released drug. Gadolinium-enhanced MRI imaging suggested an intact blood-brain barrier. Blood draws showed normal clinical chemistry and hematology. In summary, this study provides a safe and effective approach to release drugs on demand in selected deep brain regions at levels sufficient to modulate behavior.



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Introduction

Systemic administrations of drugs often carry substantial side effects, which limits the dose that can be delivered, the range of drugs that can be administered safely, and, consequently, the spectrum of patients who can receive effective treatments. An ideal approach would release medication specifically in the target of interest and use remotely applied, noninvasive form of energy as the release trigger.

Ultrasound-sensitive nanoparticle carriers are emerging as a candidate for such a selective approach [1]. A key benefit of this approach is that ultrasound is applied remotely, outside of the body, while being able to penetrate into a selected organ at depth. Drug release from the nanoparticles occurs specifically at the ultrasound focus (Fig. 1A), which can comprise millimeter-sized volumes [1, 2].

Ultrasound-sensitive nanoparticles are commonly filled with a chemically inert perfluorocarbon core [3–11], which bestows them with sensitivity to ultrasound. Proof-of-concept release from such nanoparticles has been demonstrated in rodents [5, 7–10, 12]. However, it has been difficult to determine which combination of perfluorocarbon cores and ultrasound release parameters could mediate both effective and safe applications. This issue has, thus far, precluded translations of this approach to primates and humans.

This article finds that low-frequency ultrasound, in conjunction with stable, high-boiling-point perfluorocarbon nanoparticles, provides release that is both effective and safe. The approach is deployed in non-human primates for targeted neuromodulation of deep brain regions. We targeted deep brain regions to i) demonstrate the non-invasive nature of the approach and ii) realize its promise in selective treatments of mental and neurological disorders, which are commonly associated with malfunctioning deep brain circuits [13–19].

Results

Ultrasound-triggered drug release *in vitro*

We developed ultrasound-sensitive nanoparticle carriers (Fig. 1B) with a core comprising a high boiling point perfluorocarbon—perfluorooctylbromide (PFOB). PFOB has been used safely in large quantities in humans as a blood substitute [20–22], and its high boiling point (142°C) contributes to the stability of the nanoparticles in the blood stream. The nanoparticles are further stabilized using a co-polymer shell (Fig. 1B).

We loaded the nanoparticles with the anesthetic propofol [8, 23]. First, we quantified the effectiveness of the propofol release *in-vitro*, using an approach described previously in which a drug is released into an organic solvent [24]. Ultrasound of increasing pressure increased

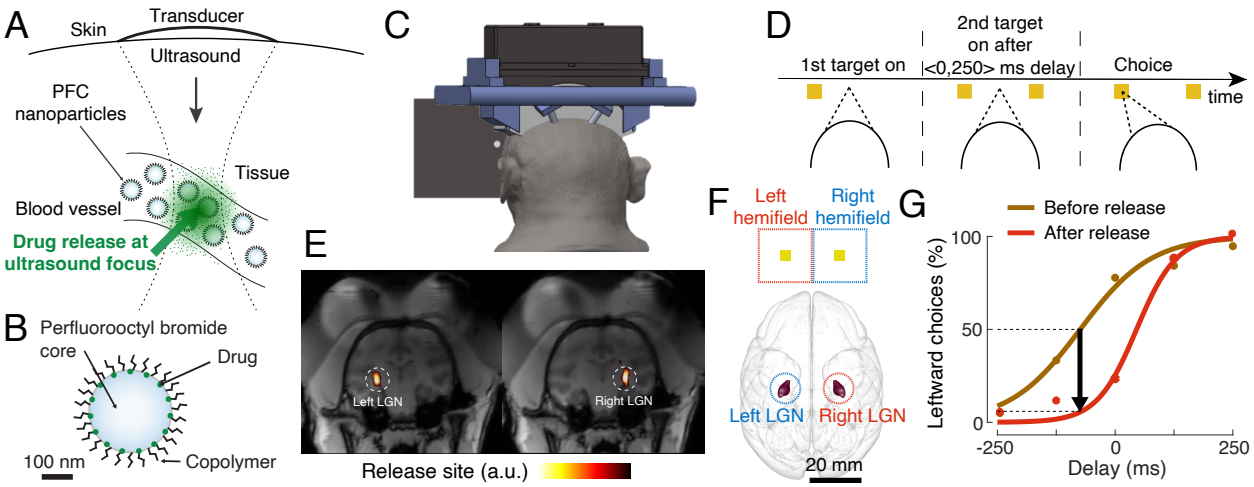

Figure 1. Ultrasound-triggered drug release from nanoparticle carriers in deep brain regions of non-human primates. A) Concept. Remotely applied focused ultrasound enables selective release of drugs from nanoparticle carriers specifically at its focus. B) Nanoparticle formulation. The nanoparticles consist of a perfluorocarbon (PFC) with a high boiling point—perfluorooctylbromide. Perfluorooctylbromide bestows the nanoparticles with high stability and biological safety [20–22]. The nanoparticle is further stabilized using a polyethylene glycol/polylactic acid co-polymer matrix. C) Ultrasound-controlled release in deep brain circuits of task-performing NHPs. A 256-element ultrasonic transducer array [25, 26] delivers ultrasound programmatically into deep brain regions of NHPs, enabling selective drug release is specified brain regions. The array is mounted into implanted head posts to ensure reproducible positioning of the transducer with respect to the head from session to session. D) Visual choice task. One target appears on the left and one target on the right part of the screen, with brief, controlled delay between the onsets. Subjects look at the target that appeared first. E) Validation of ultrasound targeting of the left and right lateral geniculate nucleus (LGN) using MRI thermometry. F) Brain hemisphere-specific representation. The left/right LGN relays visual information about the right/left visual hemifield into primary visual cortex. G) Example psychometric curve during a 3-minute baseline (brown) and a 3-minute period following the release of propofol (red) in the right LGN. The data were fitted with a sigmoid curve. Henceforth, the choice bias following the release is quantified as the proportion of choices at the point of equal preference established during the baseline (black arrow).

the effectiveness of the release, which reached over 60% for a pressure amplitude of 2.1 MPa (Fig. S1). The modulation of the release by the ultrasound pressure was significant (one-way ANOVA: $F(1, 6) = 124.16$, $p < 0.0001$).

Ultrasound-triggered drug release in deep brain regions of non-human primates

We next evaluated the release capacity of the approach *in-vivo* in non-human primates (NHPs), testing the ability to release drugs in specific deep brain regions through the intact skull and skin. To do so, we developed a system (Fig. 1C) that enables controlled and reproducible

delivery of focused ultrasound into deep brain targets of awake NHPs [25, 26]. We engaged the subjects in an established choice task that is often used in neurology to evaluate the effects of stroke in visual regions [27, 28] and in neuroscience to quantify neuromodulatory effects [25, 26, 29–31]. In this task, subjects decide whether a left or a right target appeared first (Fig. 1D) and make an eye movement to that target. We specifically targeted the left and right lateral geniculate nuclei (LGN; Fig. 1E), which are the input nuclei of visual information to the brain.

Each LGN represents the contralateral visual hemifield and target (Fig. 1F), and so provides a well-defined framework for interpreting the polarity and magnitude of neuromodulatory effects on visual choice behavior [25, 26]. For instance, if propofol, which is an anesthetic, neuroinhibitory drug, is released in the right LGN, one can expect an impaired perception of the left target. This should lead to an ipsilateral, rightward preference in the visual choice task (Fig. 1D,F).

To test this prediction, we established a behavioral baseline (brown in Fig. 1G). We then injected the nanoparticles into the blood stream using a bolus such that the concentration of the encapsulated propofol was 0.5 mg/kg. Following a 1-minute delivery of pulsed ultrasound into the right LGN, we indeed found a strong ipsilateral bias in the animal's choices (red in Fig. 1G). The animal chose the rightward target more frequently following the release of propofol in the right LGN, consistent with a release of a neuroinhibitory drug in the LGN.

The ultrasonic array enables selective drug release in specified brain regions, which allowed us to evaluate this effect systematically for both the right and the left LGNs. Across all recorded sessions, we found that the released propofol indeed induced an ipsilateral bias in the animals' choices (Fig. 2, blue). This effect was specific to the propofol released from the nanoparticles; ultrasound alone of the same parameters had, if anything, the opposite effect (Fig. 2, red). Moreover, the effect was specific to the insonated LGN side. We quantified these effects using a two-way ANOVA that incorporated all 80 recorded sessions and evaluated the choice behavior during the time window of expected propofol effects (2-5 minutes following the ultrasound onset [32]). The ANOVA detected a highly significant ($F(1, 74) = 15.68$, $p = 0.0002$) interaction between the carrier factor (propofol nanoparticles or saline) and the insonated LGN side (left or right LGN). This significant interaction demonstrates that the ultrasound released propofol selectively in each LGN and was capable of selectively modulating choice behavior.

This inhibitory effect is notable also when we separated the data into the right and left LGN release sessions, and was observed for both levels of tested ultrasound pressure (Fig. 3). A two-way ANOVA, evaluated in the same time window, again detected a significant interaction of the LGN side and carrier type (1.2 MPa: $F(1, 34) = 7.91$, $p = 0.008$; 1.5

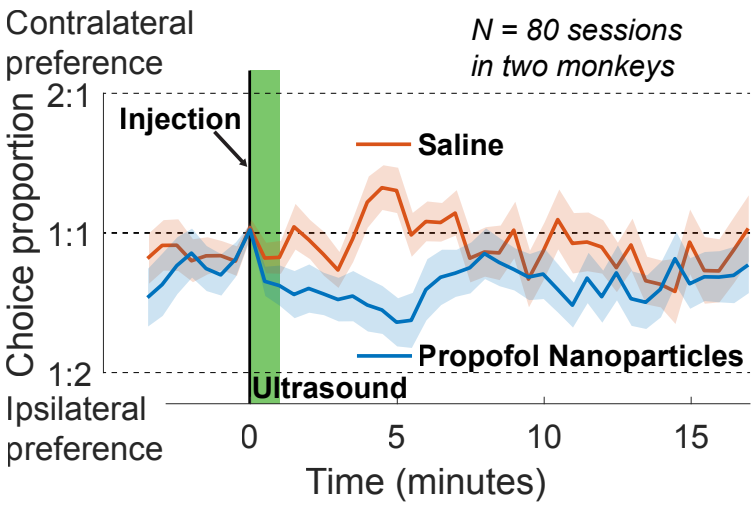

Figure 2. The release of propofol in deep brain regions of NHPs modulates choice behavior. Mean±s.e.m. proportion of choices contralateral to the targeted LGN as a function of time for the propofol-loaded nanoparticles (blue) and saline (red). The choice proportion was quantified in 3-minute moving-average windows, and was evaluated with respect to the point of equal preference obtained during a 3-minute baseline established prior to the injection of the nanoparticles (Fig. 1F). The injection and the ultrasound exposure times are indicated with a vertical black line and green bar, respectively.

MPa: $F(1, 36) = 7.17$, $p = 0.01$). These results confirm that ultrasound-triggered propofol delivery induced transient side-specific neuroinhibitory effects. Moreover, the effect shows the expected polarity given that propofol is a neuroinhibitory drug.

Integrity of the blood-brain barrier

With respect to brain applications, the nanoparticle-based approach is designed to deliver drugs that naturally pass the blood-brain barrier (BBB) without perturbing it. We evaluated the integrity of the BBB following the release using an established approach—gadolinium-enhanced MRI imaging. Specifically, T1-weighted gadolinium-enhanced images have been used to detect the disruption of the blood-brain barrier [33–36]. It has been found that a 10% signal change provides a threshold below which the blood-brain barrier can be considered intact. Across both animals and two independent sessions, in no case did the signal at the target LGN exceed that threshold (representative slices for both monkeys: Fig. 4; all slices for both sessions and monkeys: Fig. S3). As a positive control, blood vessels are clearly identifiable (Fig. 4; arrows). Moreover, T2-weighted contrast-enhanced imaging, which is commonly used to assess potential edema [37, 38] also did not reveal signal changes (Fig. 4, Fig. S4). Together, these analyses suggest that the BBB remained intact and that the

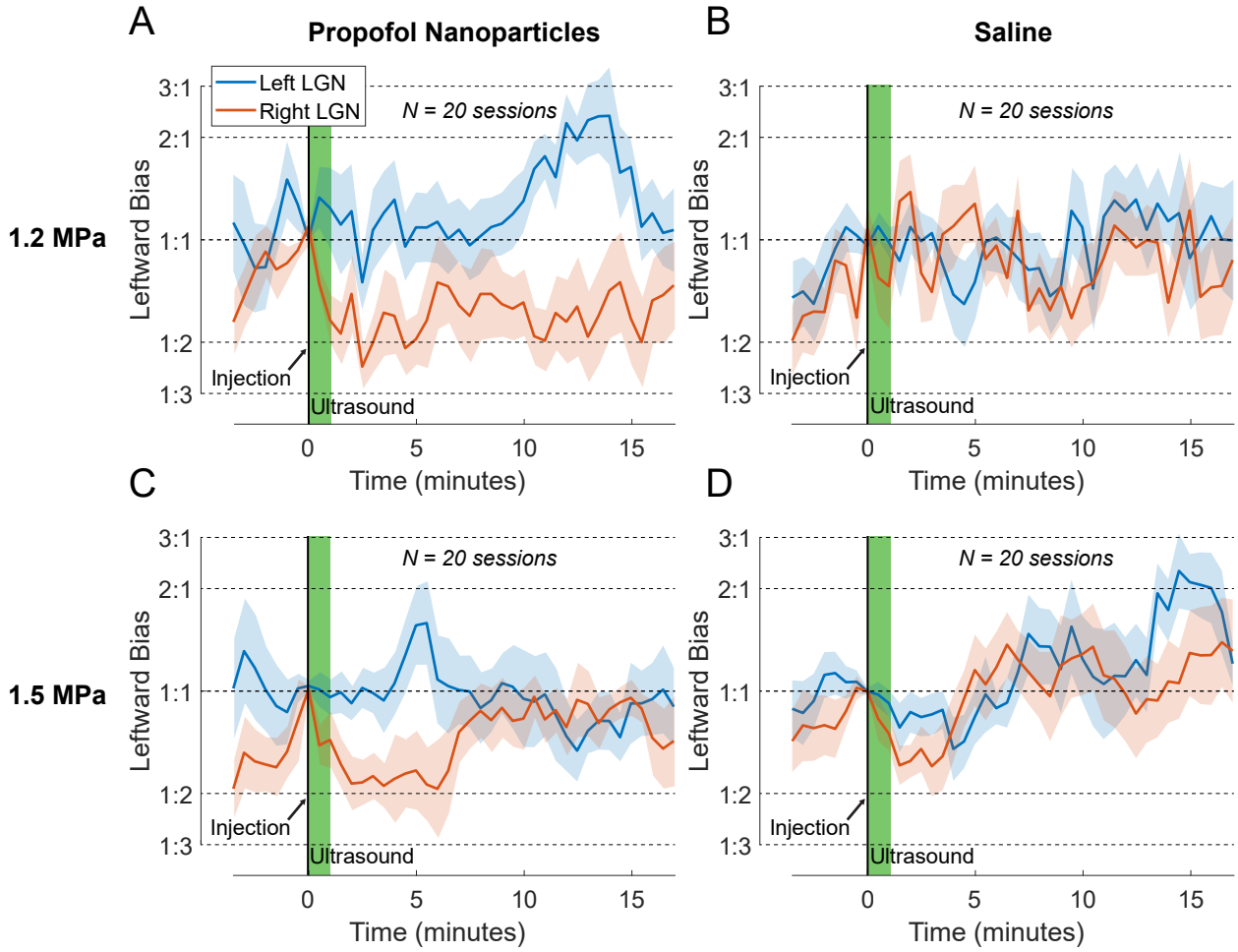

Figure 3. Propofol-induced modulation of choice behavior is target-specific. Same evaluation and format as in Fig. 2, for ultrasound delivered into the right LGN (red) or the left LGN (blue), for the propofol-loaded nanoparticles (A, C) or saline (B, D), and for applied ultrasound pressure of 1.2 MPa (A, B) or 1.5 MPa (C, D). Each plot comprises $n = 10$ sessions per LGN.

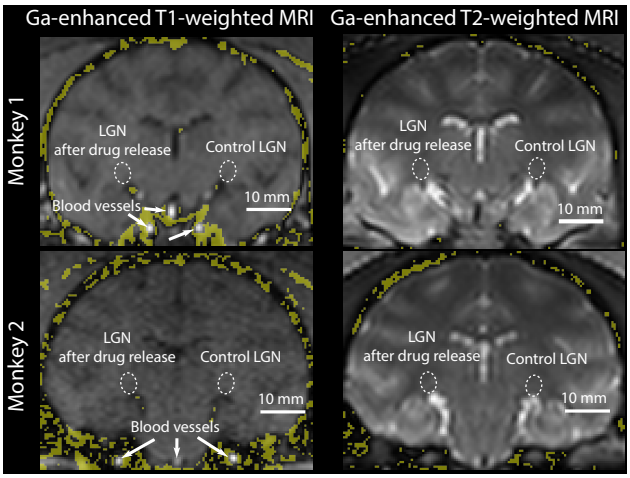

Figure 4. Intact blood-brain barrier following ultrasound-based drug release. Gadolinium-enhanced T1-weighted (left) and T2-weighed (right) MRI contrast images following the administration of propofol-filled nanoparticles (0.5 mg/kg) and one-minute pulsed ultrasound (1.5 MPa) delivered into the left LGN into the two monkeys used in the behavioral task (rows). The ultrasound was delivered using the same hardware and targeting as during the behavioral experiments. Immediately following the release, we injected the contrast agent gadoteridol. The images show the difference before and after the administration of nanoparticles, ultrasound, and gadoteridol. The yellow regions label differences greater than 10% (T1-weighted) and greater than 0% (T2-weighted).

one-minute pulsed ultrasound exposure did not induce detectable harm to the target tissue.

Pharmacokinetics and biodegradability

We investigated the pharmacokinetics of the nanoparticles in the NHPs. To do so, we incorporated in the nanoparticles a fluorescent dye, along with propofol (see Materials and Methods). We drew blood samples at 2, 10, 20, 40, 80, and 120 minutes following the injection. We then quantified the amount of fluorescence from these blood samples relative to the initial time point. The resulting blood clearance curve (Fig. 5A) shows an initial half-life of 3.1 minutes followed by a slow decay with half-life 195 minutes. This clearance characteristic agrees with those reported using perfluoropentane-based nanoparticles in rats [7, 39]. The dual-exponential nature suggests that the clearance of the nanoparticles involved two distinct processes or organs.

We thus evaluated in which organs the PFOB-based nanoparticles degrade. Two monkeys, one marmoset, and 3 rats were sacrificed two hours after the injection of dye-loaded nanopartices, and their major organs were extracted for analysis. The majority of the dye-loaded nanocarriers were found in the liver, again in line with previous studies in rats [7, 39]. Appreciable amounts were also detected in the kidneys and lungs (Fig. 5B).

Clinical chemistry and hematology

The vascular access ports enabled us to repeatedly draw blood following the administration of the nanoparticles into the blood stream, and thus conduct detailed clinical chemistry and hematology evaluations. Since the nanoparticles were found to degrade primarily in the

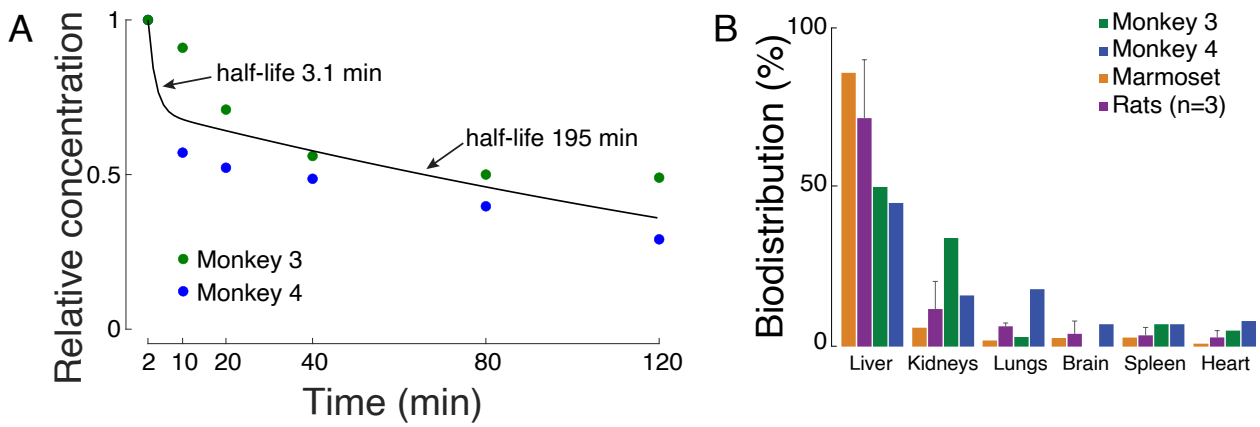

Figure 5. Blood clearance kinetics and organ biodegredation. A) Relative fluorescence as a function of specific sampling times indicated on the abscissa. The nanoparticles were loaded with an infrared dye administered at time 0. The data were fitted with a double exponential function. One exponential showed a fast and the other a slow time constant (see inset). B) Distribution of the nanoparticles in major organs. The figure shows the relative dye accumulation in the respective organs as a percentage of total fluorescence. The rat data are presented as means±standard deviation. The brain for Monkey 3 was not available for this analysis.

liver (Fig. 5B), we evaluated key markers of liver function—alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate aminotransferase (AST) (Fig. 6A). ALP and AST remained within normal ranges (green). ALT was mildly elevated during the course of the study and returned to baseline levels in both animals after a two-week period.

To assess the response of the immune system, we also evaluated the white blood cell count (Fig. 6B). A detected increase in the white blood cell count was well within the normal range (green). Complete clinical chemistry and hematology analyses are provided in Tables S1 and S2.

Discussion

This article finds that stable nanoparticles with a high boiling point core can be used to safely release propofol in specific deep brain regions of NHPs. The release of propofol was substantial in that it manifested in specific changes in visual choice behavior. The effect pointed in the expected, neuroinhibitory direction and was reversible. The release is found to be safe at the behavioral, anatomical, and hematological levels. At the behavioral level, we found that the release in visual regions did not impair NHPs' ability to successfully perform visual discrimination. At the anatomical level, there was no detectable disruption of the NHP blood brain barrier. At the hematological level, a key marker of immune reaction—white blood cell count—remained within normal values. The average half life of the nanoparticles

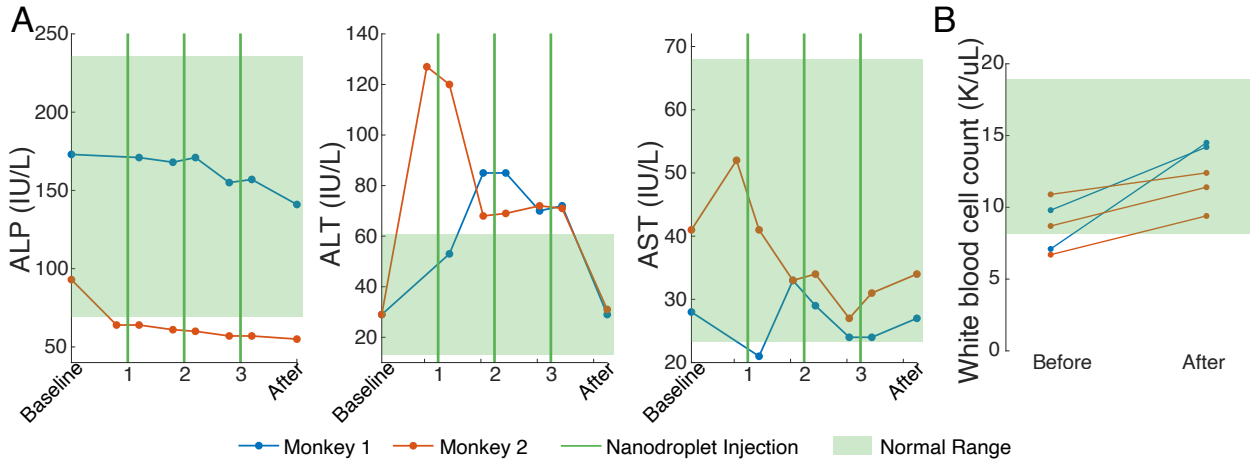

Figure 6. Clinical chemistry and hematology. A) Liver function-relevant serum chemistry: alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate aminotransferase (AST), across 3 independent sessions. Green lines indicate the time points of the nanoparticle administration. Blood draws were completed immediately before and 1.5 hours after for each administration. For Monkey 1, a preinjection draw in the first session was not available. The first injections for each animal used nanoparticles with propofol concentration of 1.0 mg/kg dose, followed by 0.5 mg/kg for subsequent injections. Baseline was taken prior to any intervention and the final measurement was taken two weeks after the last nanoparticle dose. B) White blood cell counts before and 1.5 hours after the administration.

in the NHP blood was found to be about 30 minutes, which provides a practical time window for applications in humans.

A hallmark of this remotely-controlled approach is its ability to flexibly deliver a drug into spatially circumscribed regions of interest. Our finding of target-specific effects on choice behavior (Fig. 2) supports the notion of a spatially-specific release. Specifically, the centers of the left and right LGNs are separated by about 22 mm [40]. Therefore, the approach used in this study has a spatial precision of at least that order. Previous studies in rodents have showed that achieving a sub-cm precision is feasible [8, 11]. In addition, the effect was specific to propofol-filled nanoparticles; saline administered with ultrasound of the same parameters produced no significant effect.

Another key feature of this approach is the low systemic dose of drug required to elicit strong local effects. Anesthesia of macaques typically requires around 2 mg/kg of propofol when administered as a bolus [41]. In comparison, the dose used in our study was just 0.5 mg/kg. This indicates that the approach can be used to release a drug at a target at a high concentration while maintaining a relatively low systemic level. The approach may thus improve current systemically toxic or harmful treatment regimens, such as chemotherapy or treatments of brain circuits with psychedelics.

For brain applications, the nanoparticle-based approach for localized drug delivery differs

fundamentally from a related approach, which uses ultrasound combined with blood-circulating microbubbles to transiently disrupt the blood brain barrier (BBB) [42–44]. The nanoparticle-based approach aims to deliver drugs that naturally pass the BBB while preserving its integrity (Fig. 4). Moreover, in the nanoparticle-based approach, drugs are encapsulated within the nanoparticles and shielded by a polymeric shell (Fig. 1B). This design prevents the drug from interacting with tissues and organs until exposed to ultrasound or broken down. In comparison, in the microbubble-based approach, a full dose of a systemically injected drug immediately interacts with all vascularized tissues, thus limiting the specificity of that approach.

All components of the nanoparticles used in this study have been used in humans, which is expected to facilitate regulatory approval. PFOB is well tolerated by humans [45–47], and due to its oxygen-binding capacity has been used as a blood substitute [20–22]. Moreover, the hydrophobic block of the copolymer used—the polylactic acid (PLA)—is generally recognized as safe by the FDA and has been used broadly in modern medicine [48, 49]. Likewise, polyethylene glycol (PEG) has been used extensively for its ability to circumvent activation of the immune system and thus extend the lifetime of drug carriers in the circulation [50, 51]. Indeed, we found that our PEGylated nanoparticles were present in the blood two hours following administration (Fig. 5A). The predominant breakdown of the nanoparticles in the liver suggests an engagement of the reticuloendothelial system [52]. The detected elevations in alanine aminotransferase were below the levels that could indicate hepatocellular damage [53]. Furthermore, the initial increase dropped by the second and third injection and returned to normal following a two-week washout [54, 55].

The approach reported in this study builds on ultrasound-responsive nanoparticles initially developed for localized chemotherapy in rodents using perfluoropentane (PFP) [12, 24] and perfluoro-15-crown-5 ether (PFCE) drug carriers [5]. PFP has a boiling point below the body temperature and thus has raised safety concerns. PFCE has a much higher boiling point—and thereby stability—but its biological half-life of nearly 8 months limits clinical deployment [56]. The perfluorocarbon core used in this study, PFOB, addresses both issues, but difficulties have remained in regard to its relatively high activation pressures [57], as activation pressure appears to correlate with the boiling point [58, 59]. We have resolved this issue using relatively low ultrasound frequencies (300-480 kHz). In this ultrasound frequency range, we observed effective release *in vitro* (Fig. S1) and *in vivo*, finding a strong and selective modulation of choice behavior of NHPs (Fig. 2, Fig. 3).

This study has two limitations. First, although the study uses an established behavioral framework and the well-characterized visual system to evaluate neuromodulatory effects [25–31], there is a lack of a modality to image the released propofol. Microdyalisis was not

used due to concerns of a needle disrupting the blood-brain barrier, which would artificially boost the release. Second, the reported behavioral effects are subject to adaptation and potentially other higher-order cognitive influences [26]. This issue is mitigated by contrasting the release across the two brain sites and by contrasting propofol-filled nanoparticles with saline.

These NHP data are expected to lead to an accelerated adoption of this targeted approach in humans. For instance, the propofol-containing nanoparticles could be used for virtual lesioning of individual candidate regions involved in epilepsy, pain, or mental disorders. Longer-acting drugs could subsequently be delivered into the identified malfuncioning circuits and thus provide personalized, targeted therapy. Targeted delivery of drugs for other indications such as cancer, and organs other than the brain, could fuel additional important applications.

Conclusions

In summary, we have developed an approach that enables ultrasound-triggered delivery of drugs into circumscribed regions in the brain or the body as demonstrated by robust modulation of choice behavior and a favorable safety profile in NHPs. This targeted pharmacomodulation approach has the potential to provide treatments to individuals for whom current drug treatments cause unbearable or undesirable side effects.

Materials and Methods

Materials

Methoxy poly(ethylene glycol)-b-poly(D,L-lactide) (PEG-PDLLA) co-polymers with 2 : 2.2 kDa molecular weights, respectively, were obtained from PolyScitech (USA). Perfluorooctyl bromide were obtained from Tokyo Chemical Industry Co. (Japan). Propofol was obtained from Sigma Aldrich (Millipore Sigma, Canada). Infrared dye IR800RS NHS Ester was obtained from LI-COR Biosciences (USA). HPLC-grade tetrahydrofuran (THF), n-hexane, and methanol were obtained from Fisher Scientific (USA). Sterile phosphate buffer solution (PBS) was obtained from Cytiva Life Sciences (USA).

Animals

All procedures conformed to approved protocols by the Animal Care and Use Committee of the University of Utah. For all behavior studies and safety studies not requiring euthanasia,

we used two male rhesus macaques (*macaca mulatta*) with weights 10.0 kg and 13.8 kg, both aged 8 years (monkeys 1 and 2). Two adult male rhesus macaques (*macaca mulatta*, monkeys 3 and 4), an adult male common marmoset (*callithrix jacchus*), and 3 adult male Sprague-Dawley rats participated in the safety and pharmacokinetics experiments. The macaques weighed 10.8 and 16.1 kg (age 6 and 13 years). The rats weighed 830-900 g (age 1 year). The marmoset weighed 288 g (age 7 years). Sample sizes were chosen based on animals' availability following completion of other studies.

Nanoparticle production

The process of manufacturing the drug-encapsulating, ultrasound-responsive PFC particles is described in detail in previous studies [12, 24]. The process converts small (tens of nanometers in diameter) micelles into much larger (hundreds of nanometers) PFC nanoparticles. First, the PEG-PDLLA polymer constituting the basis of the nanoparticle shell is dissolved in THF at a ratio of 1mL THF : 20 mg polymer. For the biodistribution and blood clearance studies, infrared dye is added at a ratio of 1:32 (dye:polymer) for the rats and marmoset and 1:110 or 1:89 for the monkeys 1 and 2, respectively. THF is then allowed to evaporate overnight until a gel-like layer remains. PBS is added at a ratio of 1 mL PBS : 20 mg polymer and placed on a shaker table at 120 rpm to dissolve for 15 minutes. The addition of PBS orients the hydrophilic copolymer, PEG, toward the water and the hydrophobic copolymer, PDLLA, away from the water, forming micelles. Next, the PFOB core and drug are added and emulsified. A 2:1 ratio of polymer to drug was used. The nanoparticles' diameter can be controlled by the ratio of PFC to polymer, as reported previously [5]. We used a ratio of 3 μL PFOB : 1 mg polymer for all experiments except the 1.5 MPa behavior studies, for which the ratio was adjusted to 2.75 : 1. The PFC and drug are added to 15 mL centrifuge tubes and gently shaken to combine before adding 8 mL of the micelle solution. A 20 kHz, 500W sonicator with a cup horn attachment (VCX500, Sonics) emulsified the PFOB and drug, forming stable nanoparticles. The samples were sonicated in a 10°C water bath at 20% power for 3 minutes, inverting the tubes halfway through to ensure even distribution. A custom temperature-controlled cooling system maintained the bath temperature during sonication. We found this controlled temperature approach to maximize the consistency of the nanoparticle sizes, drug encapsulation, and release properties. The resulting solution contains the desired nanoparticles in addition to remaining micelles, dissolved polymer, and free propofol. Nanoparticles are isolated from micelles and free drug using three cycles of centrifugation at 3,000 g relative centrifugal force (RCF) at 4°C. After each cycle, the supernatant is discarded and the pellet dissolved in fresh PBS.

Nanoparticle characterization

Nanoparticle sizes were measured using a Zetasizer Nano S (Malvern Panalytical, UK), which reports the intensity-weighted size distribution. The size values reported in the Supplementary Materials section describe the z-average diameter ± standard deviation of the distribution of the intensity values measured by the device. To quantify the amount of drug encapsulated, 25 μL of nanoparticle solution was added to 225 μL of methanol to dissolve all components. A UV-vis spectrophotometer (NanoDrop 2000, Thermo Scientific) was used to quantify the concentration by comparing the absorbance at 276 nm to a propofol standard curve.

Sterilization and endotoxin testing

For animal studies, nanoparticles were sterilized with either ultraviolet light or filtration. UV light was selected for the acute studies for its efficacy of sterilization while it is considered unlikely to disrupt polymeric drug carrier structure and function [60]. Samples in glass vials were exposed to an 8W UV lamp (Philips, USA) in a custom chamber for 3 hours. For the longer-term studies in monkeys, we sterilized the micelle solution by 0.2 micron filtration, then used sterile reagents for the remainder of the production. Filtration of the finished product was not possible because the nanoparticles are mostly larger than the filter pore size.

To confirm that the process does not introduce unwanted contamination, we tested for the presence of bacterial endotoxin, an FDA-required step for any drug product [61]. We used gel-clot lyophilized amebocyte lysate tests (ToxinSensor, Genscript) to detect the presence of endotoxin above the detection threshold. This semi-quantitative method was used to ensure that the nanoparticles contained a level of endotoxin lower than the FDA allowable limit of 5.0 EU/kg when diluted at doses to be administered to animals.

Nanoparticle dosing

Long-term study animals were implanted with vascular access ports to enable rapid intravenous infusions and blood draws. The vascular access port system consists of the port itself (Swirl-Phantom, Access Technologies) and a polyurethane catheter (Hydrocoat, Access Technologies) inserted via the saphenous vein into the inferior vena cava. Dosing was ramped up over four sessions to minimize potential safety issues: 0.1, 0.33, 0.66, and 1.0 mg/kg of propofol. We chose to use a dose of 0.5 mg/kg, at which animals showed no signs of drowsiness.

Behavior experiments

The system for delivering ultrasound to the lateral geniculate nucleus and monitoring behavior effects is described in previous publications [25, 26] and illustrated in Fig. 1. Two non-human primates were trained in a visual discrimination task which has commonly been used in neurology and neuroscience [25, 26, 28, 62]. In this task, the subject fixates on a central target presented on a screen as detected using an eye tracker (Eyelink, SR Research). After a randomized delay, the fixation point disappears and a target is presented in either the left or right visual hemifield. Following another delay, a second target is presented in the opposite visual hemifield. The subject is rewarded with a probability between 50% and 100% with a drop of juice from a lick spout for selecting the target presented first. In each session, there were 5 possible delays between the onset times of the two targets, with the delays calibrated to each subject. Delays are selected such that the subjects are near 100% accuracy in the longest delays and near 50% accurate at the middle delay (0 ms), with some degree of uncertainty at the delay in between. Using these delays, we fit a sigmoid function to each subject's behavior to quantify the magnitude and the polarity of neuromodulatory effects.

In this study, we quantified behavior by fitting a sigmoid curve to 3 minute segments of data. The subjects first performed at least 3 minutes of behavior before any intervention to establish a baseline. We use this baseline behavior to establish the point of equal preference, which is the time delay at which the sigmoid curve crosses the 50:50 choice proportion line. Animals were not cued to perform the task while we administered propofol-loaded nanoparticles or saline. The task performance was resumed upon the delivery of the ultrasound. In 3-minute segments following sonication onset, we measured effects on choice behavior as the percent of leftward choices at the delay of equal probability established during the baseline. Plots in Fig. 2 are an average of results from 5 sessions for each condition for each subject, for a total of $n = 40$ sessions. One saline session was excluded due to a lack of trials in the 3 minute baseline period. We interleaved sessions with nanoparticles and saline and the sonicated side. Sessions were completed on a nearly daily basis.

Pharmacokinetics studies

Rodents were anesthetized with 2.5-3% isoflurane, and dye-loaded nanoparticles were administered at a dose of 1 mg/kg propofol followed by an equal volume of sterile saline. After one hour, the animals were euthanized by exsanguination under 5% isoflurane anesthesia.

The primates were preanesthetized with ketamine (25 mg/kg intramuscularly) and intubated with endotracheal tubes. They were artificially ventilated and anesthesia maintained with 1-4% isoflurane throughout the procedure by veterinary staff. The animals were placed

on heated operating table pads to maintain body temperature. For the marmoset, dye-loaded nanoparticles were injected through the tail vein at doses of 1 mg/kg propofol for each of two injections separated by 45 minutes. A total volume of 2 ml of nanoparticle solution was administered, followed by an equal volume of sterile saline. The marmoset was euthanized by an overdose of sodium pentobarbital and perfused transcardially with 4% paraformaldehyde 82 minutes after the first injection. For Monkey 3, one injection of dye-loaded nanoparticles was administered in the right saphenous vein at 1 mg/kg propofol and a volume of 5 mL, followed by an equal volume of sterile saline. Blood samples were taken from the left saphenous vein 2, 10, 20, 40, 80, and 120 minutes, at a volume of 1 mL each. For Monkey 4, the right (injection) and left (blood draws) cephalic veins were used with a 1 mg/kg propofol dose and 10 ml volume of dye-loaded nanoparticles. Following 120 minutes of monitoring, the monkeys were euthanized by an overdose of sodium pentobarbital and perfused transcardially with 4% paraformaldehyde.

The procedures were painless and wholly consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

IVIS imaging

Major organs were extracted and subjected to infrared fluorescence analysis using an IVIS system (Perkin Elmer) with methods similar to those previously demonstrated [7]. Excitation was set to 745 nm and emission to 820 nm, with 5 seconds of exposure. Extracted organs were placed directly on a dish in the field of view of the camera. For monkeys, the same organs as in the smaller animals were available, with the exception of the brain for Monkey 1. Blood samples were pipetted into drops of 100 uL on a dish. Total fluorescence for each of these samples was quantified using Aura software (Spectral Instruments), defining regions of interest incorporating the whole organ or blood sample and subtracting a background region of the same size. Percent biodistribution was computed as the total fluorescence of the region containing the organ divided by the sum of the fluorescence of all organs. Nanoparticle concentration in the blood was computed as the amount of fluorescence from each sample relative to the first sample, obtained at 2 minutes.

Ultrasound parameters

The ultrasound carrier frequency in the *in vitro* release experiments was 300 kHz. Continuous pulses 100 ms in duration were repeated once per second for a total of 60 seconds. This pulsing sequence was previously used to activate PFC nanoparticles in rodents [7, 24]. The pressure levels at the vial location, measured in degassed water, were 0, 0.5, 0.9, 1.3, 1.7, 2.1, and

2.5 MPa. The pressure fields were measured using a capsule hydrophone (HGL-0200, Onda) calibrated between 250 kHz and 40 MHz and secured to 3-degree-of-freedom programmable translation system (Aims III, Onda).

In the pharmacokinetics experiments, the same transducer was operated at 248 kHz in 60-second blocks of 100 ms pulses. The animals were shaved and the transducer was coupled to the scalp using a 2% agar cone and ultrasound gel. The maximum pressure was estimated to be 1 MPa for all animals. The pressure was estimated from free-field measurements and a correction for the skull which indicates a transmission rate of 76% through the skull of a 780g rat [63] and 66% through a macaque skull [64]. Transmission through the marmoset skull has been less thoroughly studied but was predicted to be similar to rats since the species are comparable in weight. Ultrasound was applied to the rats at the midline, 2 mm posterior to the eyes for 60 seconds 5 minutes after administration of nanoparticles. For the marmoset, ultrasound was applied at the posterior surface of the skull for 60 seconds 5 minutes after each of the two administrations. The right and left visual cortex were targeted independently for Monkey 3. Ultrasound was applied in 60-second blocks over 90 minutes for a total of 4 sonication blocks per side starting 2 minutes after nanoparticle administration. Sonication of the left and right sides was interleaved. For Monkey 4, the right and left visual cortex were targeted simultaneously using two ultrasound transducers to maximize the sonicated volume. In this monkey, sonications were repeated for two 60-second blocks separated by two minutes. In both of these cases, sonication was repeated to represent upper bounds on ultrasound to be delivered.

In the behavioral, MRI, and blood draw components of the investigation, we used a custom 256-element phased array (Doppler Electronic Technologies) to deliver ultrasound to lateral geniculate nuclei, as described in detail in our previous publications [25, 26]. Briefly, the scalp is shaved and the transducer is affixed to the animal's head via implanted titanium pins (Gray Matter Research) and coupled using a 6% polyvinyl alcohol (Fisher Scientific) cryogel. The transducer was driven by the Vantage256 controller (Verasonics, Kirkland, WA) at 480 kHz. This corresponds to the lower corner frequency of the transducer's bandwidth and was selected to maximize the mechanical effects on the nanoparticles. Ultrasound pressure and focal location were determined using MR thermometry. Details on this procedure are published [25, 26]. Ultrasound at the frequency of 480 kHz was pulsed at 10% duty cycle for one minute for all experiments. In the behavioral experiments, we delivered into the targets a peak pressure of 1.2 MPa in 10 ms pulses or 1.5 MPa in 30 ms pulses. In the MRI sessions, we used a higher exposure to capture the potential blood brain barrier opening—peak pressure of 1.5 MPa delivered in 30 ms pulses.

MR imaging

All studies were performed on 3T MRI scanners (Trio and Vida, Siemens, Erlagen, Germany). The MR imaging was used to assess the potential disruption of the blood-brain barrier (BBB) or tissue damage. Two sessions of propofol-based nanoparticle release were performed in each monkey under the MRI. Potential disruption of the BBB is assessed using gadolinium-enhanced T1-weighted imaging, which is a common approach since gadolinium does not cross an intact BBB [33, 65, 66]. T2-weighted images were taken to inform on a potential edema formation [35]. We followed a previous NHP protocol to evaluate these effects [33]. Specifically, in each experiment, we first collected a T1-weighted scan (3D Volumetric Interpolated Breath-hold Examination (VIBE), TR/TE=4.46/1.42 s, 192x132x80 mm field of view, 1 mm isotropic voxels, readout bandwidth 490 Hz/pixel, 5 averages, 3:55 minutes) and a T2-weighted scan (Sampling Perfection with Application optimized Contrast using different flip angle Evolution (SPACE), TR/TE=4000/179 s, 192x136x80 mm field of view, 1 mm isotropic voxels, readout bandwidth 789 Hz/pixel, Turbo factor 165, 1 averages, 3:30 minutes) to use as baselines. We then administered a solution of nanoparticles at a concentration of 0.5 mg/kg of propofol followed by 10 mL of sterile saline via an intravenous catheter placed in the subjects' arm. Ultrasound was delivered starting two minutes after completion of the nanoparticle injection. Immediately following the ultrasound delivery, gadolinium-based contrast agent was administered at a dose of 0.15 ml/kg (ProHance Gadoteridol, 279.3 mg/mL, Bracco Diagnostics). Repeated T1 and T2 scans were taken 30 minutes following contrast administration, which allows gadolinium in larger blood vessels to diffuse away.

MR images were processed in MATLAB by performing subtraction of the baseline image from the post-treatment image. In the BBB study, signal contrast of more than 10% would be considered to indicate presence of gadolinium and therefore BBB opening [33]. This threshold is high-enough to circumvent the noise caused by lingering gadolinium in blood vessels [33]. Threshold of 0% was used for the T2-weighted images. In both cases, we analyzed 5 slices (5 mm) in each direction from the location of the LGN.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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CRediT authorship contribution statement

Matthew Wilson: Writing - original draft, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization. **Taylor Webb:** Writing - review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization. **Henrik Odéen:** Writing - review & editing, Investigation, Methodology. **Jan Kubanek:** Writing - review & editing, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization.

Data Availability

Data will be made available on request.

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Supplementary Information

Nanoparticle characterization

The mean±SD diameter of the nanoparticles was 473±28 nm for *in vitro* drug release experiments, 550 ±83 nm in MRI studies, 809 ±328 nm for behavior studies at 1.2 MPa, and 526 ±109 nm at 1.5 MPa. The somewhat larger average size of the nanoparticles prepared for the behavioral studies is likely due to the higher quantities of all materials necessary for the injection into a large animal. Endotoxin tests revealed endotoxin levels lower than the FDA allowable limit of 5.0 EU/kg.

Drug release efficacy was assessed *in vitro* using a solvent extraction method similar to that described in [7]. In this procedure, nanoparticle solutions are placed in contact with an organic sink (hexane) in a 1.5 mL centrifuge tube while ultrasound is applied from a single-element transducer coupled to the tube with degassed water. Following sonication, the hexane was extracted to measure the concentration of propofol released using UV-vis spectrophotometry. The time hexane was in contact with the nanoparticles was held constant at 105 seconds to control for the time course of drug release. Percent drug release is reported as the amount of drug released into hexane relative to the drug encapsulated.

The results shown in Fig. 2 contain sessions with baseline behavior periods which were highly biased. To assess the impact of these sessions, we have also analyzed the results by excluding any sessions in which the monkeys were biased more than 70% in either direction. Excluding these sessions removes the offset in baselines shown in Fig. 3, while retaining a clear bias induced by propofol delivery at the 1.5 MPa pressure. A two-way ANOVA with factors of LGN sonicated and injection type (the same analysis as the main text) detected a significant effect at 1.5 MPa 2-5 minutes after sonication ($F(1,29) = 4.31$, $p = 0.047$) but not 3-6 minutes after sonication ($F(1,29) = 1.92$, $p = 0.18$). However, the effect was not significant at 1.2 MPa with this analysis 2-5 minutes after sonication ($F(1,29) = 3.52$, $p = 0.071$). This suggests that for immediate effects to be substantial enough to modulate behavior, a pressure higher than 1.2 MPa may be necessary.

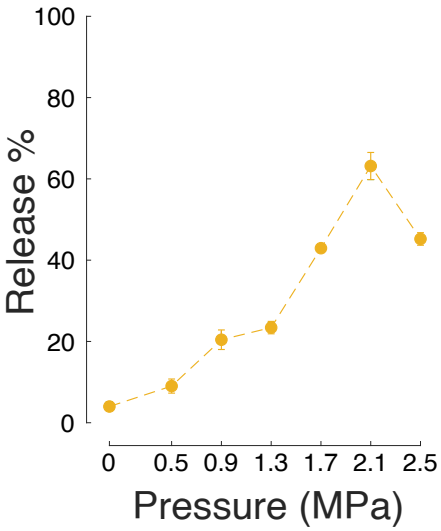

Figure S1. PFOB nanocarriers effectively release propofol in response to ultrasound *in vitro*. Mean±s.e.m. percentage of propofol released into hexane in response to 300 kHz ultrasound of ultrasound pressure amplitude indicated on the abscissa. The data comprise $n = 4$ independent vials for 0 MPa, and $n = 3$ for all other pressures.

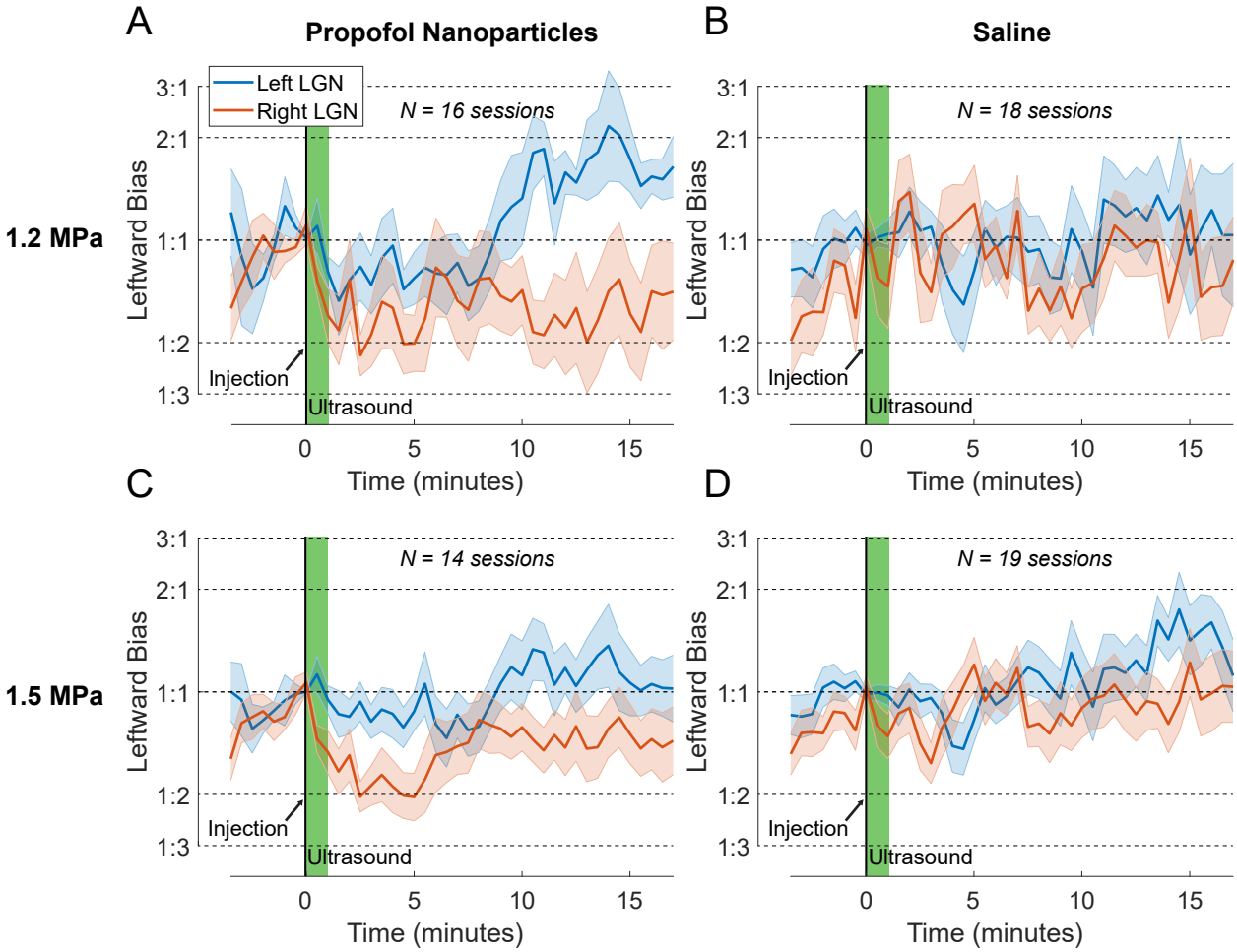

Figure S2. Propofol-induced modulation of choice behavior is target-specific also during sessions with a stable baseline. Same format as in Fig. 3, specifically using data that showed stable baseline, i.e., only including sessions with baseline bias less than 70% in either direction. The post-ultrasound effect is preserved and there is no tendency for a pre-ultrasound effect.

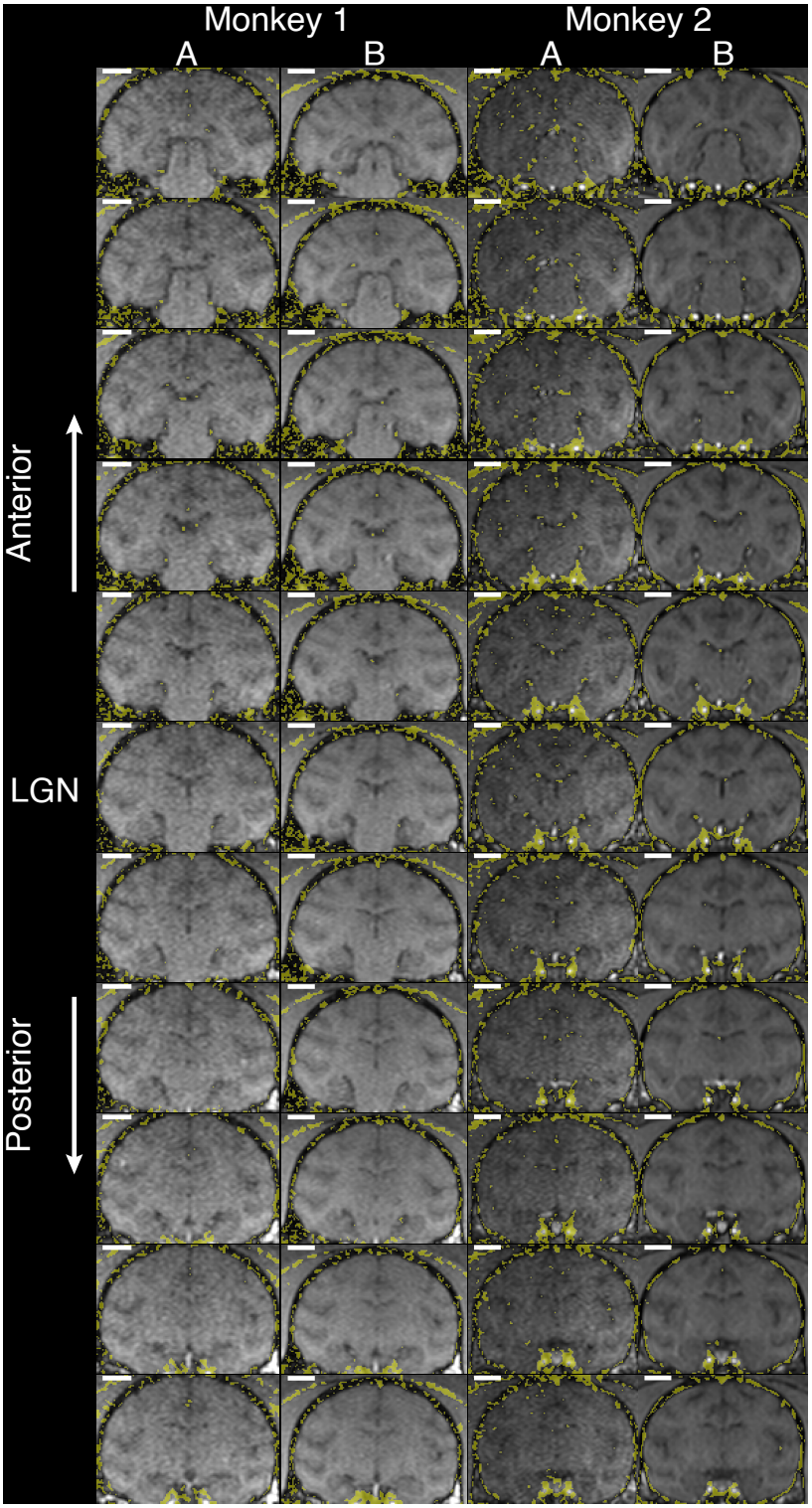

Figure S3. Gadolinium-enhanced T1-weighted MRI detected no sign of blood brain barrier disruption. Same analysis as in Fig. 4. MRI slices centered on the LGN (middle) and moving in 1 mm steps anteriorly (top) or posteriorly (bottom). The data are provided separately for the two monkeys and sessions A and B in each monkey (columns).

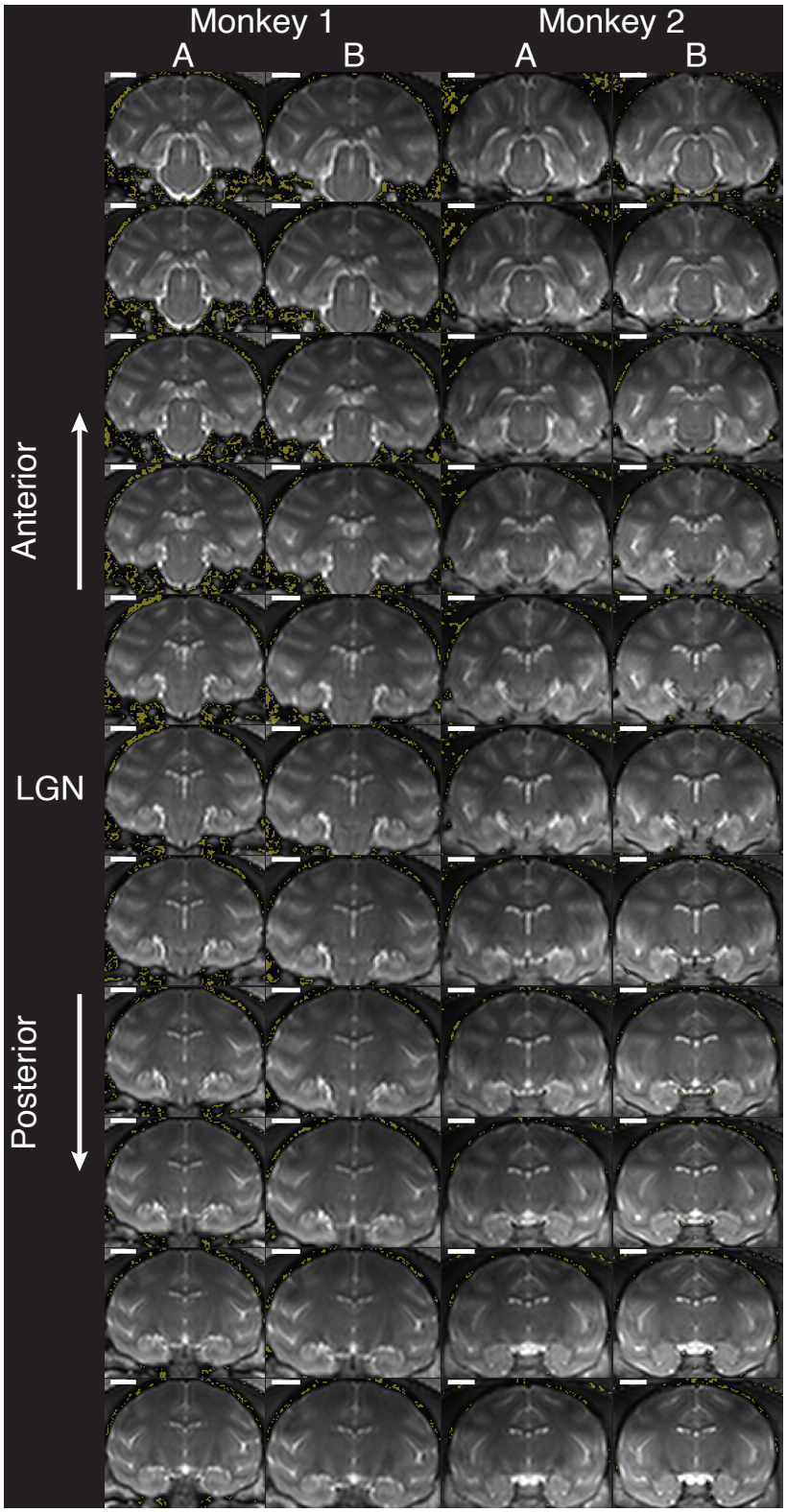

Figure S4. Gadolinium-enhanced T2-weighted MRI detected no sign of edema. Same format as in Fig. S3, using T2-weighted MRI.

		Session 1		Session 2		Session 3		
Clinical Chemistry	Baseline	Before	After	Before	After	Before	After	After
ALP (U/L)	93	64	64	61	60	57	57	55
AST (U/L)	41	52	41	33	34	27	31	34
ALT (U/L)	29	127	120	68	69	72	71	31
Creatine kinase (U/L)	616	76	81	74	119	62	87	904
Albumin (g/dL)	3.9	4.1	3.9	3.8	3.8	3.8	3.8	3.6
Globulin (g/dL)	3.3	3.3	3.2	3.4	3.3	3.3	3.2	2.9
ALB/GLOB ratio	1.2	1.2	1.2	1.1	1.2	1.2	1.2	1.2
Total Bilirubin (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Bili.-Conjugated (mg/dL)	0	0	0	0	0	0.1	0.1	0
Bili.-Unconj. (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2
Total Protein (g/dL)	7.2	7.4	7.1	7.2	7.1	7.1	7	6.5
BUN (mg/dL)	10	14	13	14	13	13	13	12
Creatinine (mg/dL)	1.1	0.9	1	1	1	0.9	1	1.1
BUN/Creatinine Ratio	9.1	15.6	13	14	13	14.4	13	10.9
Cholesterol (mg/dL)	122	115	108	107	105	107	104	99
Glucose (mg/dL)	84	76	87	91	72	113	86	53
Calcium (mg/dL)	9.4	9.2	9.1	8.6	8.8	8.6	8.6	8.7
Phosphorus (mg/dL)	6.9	3.2	2.6	3.3	2.7	3.5	2.7	4.1
Bicarb. TCO2 (mmol/L)	13	23	25	24	24	23	24	26
Chloride (mmol/L)	108	110	108	109	108	109	106	108
Sodium (mmol/L)	149	148	145	146	145	149	150	144
Potassium (mmol/L)	3.5	3.9	3.7	3.3	3.4	4.1	3.9	3.7
NA/K Ratio	43	38	39	44	43	36	38	39
WBC (K/uL)	7.4	10.9	12.4	6.7	9.4	8.7	11.4	12.5
RBC (M/uL)	6.55	6.67	6.59	6.26	6.31	6.54	6.39	6.12
HGB (g/dL)	15.8	16.3	15.6	14.9	14.9	16.1	15.7	14.9
HCT (%)	51.2	52.1	51.3	49.6	50.8	49.6	48.9	47.5
MCV (fL)	78	78	78	79	81	76	77	78
MCH (pg)	24.1	24.4	23.7	23.8	23.6	24.6	24.6	24.3
MCHC (g/dL)	30.9	31.3	30.4	30	29.3	32.5	32.1	31.4
Platelet Count (K/uL)	291	264	250	240	274	365	315	372

Table S1. Clinical chemistry and hematology; Monkey 1. Clinical chemistry and hematology test outcomes based on blood draws taken during baseline, immediately before the injection of the PFOB-based nanoparticles, and 1.5 hours following the injection (columns). The data are provided separetely for the 3 sessions (columns). The blood was analyzed and values provided by IDEXX (Westbrook, Maine).

		Session 1	Session 2		Session 3		
Clinical Chemistry	Baseline	After	Before	After	Before	After	After
ALP (U/L)	173	171	168	171	155	157	141
AST (U/L)	28	21	33	29	24	24	27
ALT (U/L)	29	53	85	85	70	72	29
Creatine kinase (U/L)	125	64	58	61	61	66	1564
Albumin (g/dL)	4.7	4.3	4.2	4.4	4.1	4.2	4.3
Globulin (g/dL)	2.9	2.9	3	3.1	2.8	2.9	2.7
ALB/GLOB ratio	1.6	1.5	1.4	1.4	1.5	1.4	1.6
Total Bilirubin (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Bili.-Conjugated (mg/dL)	0	0	0	0	0	0.1	0
Bili.-Unconj. (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.1	0.2
Total Protein (g/dL)	7.6	7.2	7.2	7.5	6.9	7.1	7
BUN (mg/dL)	10	15	16	14	18	17	14
Creatinine (mg/dL)	1.3	1.3	1.2	1.3	1.2	1.4	1.2
BUN/Creatinine Ratio	7.7	11.5	13.3	10.8	15	12.1	11.7
Cholesterol (mg/dL)	161	162	165	170	149	152	177
Glucose (mg/dL)	55	60	78	60	75	63	51
Calcium (mg/dL)	10.2	10.3	9.8	10.4	9.2	10	9.6
Phosphorus (mg/dL)	3.2	1.7	2.4	1.8	2.9	2.2	3.8
Bicarb. TCO2 (mmol/L)	17	20	23	23	22	23	24
Chloride (mmol/L)	113	109	111	111	110	108	112
Sodium (mmol/L)	149	145	148	149	145	145	148
Potassium (mmol/L)	3.8	3.8	3.9	3.7	3.8	4.3	4.5
NA/K Ratio	39	38	38	40	38	34	33
WBC (K/uL)	8.4	14.1	9.8	14.2	7.1	14.5	6.3
RBC (M/uL)	5.94	5.88	6.2	6.11	5.89	5.73	6.03
HGB (g/dL)	14.1	13.7	14.4	14.2	13.8	13.6	14.1
HCT (%)	45.2	45	46.5	45.7	44.4	43.3	45.2
MCV (fL)	76	77	75	75	75	76	75
MCH (pg)	23.7	23.3	23.2	23.2	23.4	23.7	23.4
MCHC (g/dL)	31.2	30.4	31	31.1	31.1	31.4	31.2
Platelet Count (K/uL)	339	280	362	343	361	368	351

Table S2. Clinical chemistry and hematology; Monkey 2. Same format as in Table S1 for Monkey 2.