Focused Ultrasound Enhances Decision-Making in Monkeys

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Abstract

Noninvasive brain stimulation using ultrasound has many potential applications as a research and clinical tool. Here, we investigated the effect of focused ultrasound (FUS) with systemically administered microbubbles on perceptual decision-making behavior in monkeys. We targeted FUS to the putamen in one hemisphere to open the blood-brain barrier, and then tested behavioral performance 3-4 hours later. On days when the monkeys were treated with FUS, their decisions were faster and more accurate than days without sonication. The performance improvement was greater for responses made with the hand contralateral to the treated hemisphere. FUS also enhanced the effect of a low dose of haloperidol. The results suggest that a two-minute application of FUS can have a sustained impact on cognitive performance, and can increase the efficacy of psychoactive medications.

Key words

Ultrasound, stimulation, putamen, accuracy, response time.

Introduction

Brain stimulation is an essential tool for establishing causal brain-behavior relationships, mapping brain circuits, and treating neurological disorders. Current methods are either invasive (electrical or chemical stimulation, and optogenetics) or have limited penetrability (TMS) or localizability (TDCS) (Borchers et al. 2012; Calvo et al. 2006; Dubuisson et al. 1977; Kobyashi et al. 2003; Miller 1965; Nitsche et al. 2003). Focused ultrasound (FUS) is emerging as a non-invasive technology capable of penetrating the skull and meninges to reach deep brain structures. FUS with systemically administered microbubbles has been shown to open the blood-brain barrier (BBB) in various animal models, and may also directly modulate neural activity (Marquet et al. 2011; Tung et al. 2011; Downs et al. 2015; Chu et al. 2015; McDannold et al. 2006). A few pioneering studies in monkeys (Deffieux et al.2013) and humans (Hameroff et al.2013; Legon et al. 2014) have provided evidence that FUS alone can be used to modify perception and behavior. Deffieux et al. found that FUS can increase the latency of antisaccades in monkeys. Tactile discrimination was enhanced during FUS stimulation of the somatosensory cortex in human subjects (Legon et al.2014), while overall mood improved when the frontal-temporal cortex was stimulated with FUS (Hameroff et al.2013). Further investigation using different species, brain targets, and behavioral tasks is warranted to establish the effectiveness and range of applications for this approach.

Here, we trained monkeys to perform a common perceptual decision-making task that involves the detection of coherent visual motion (Lappin & Bell, 1976). The advantage of this task is that it allows quantitative measures of response time and accuracy. Such measures have been used to develop sophisticated computational models of decision-making in both humans and monkeys (Luce 1986; Ratcliff 1978). Electrophysiological studies point to a critical role of the striatum (caudate and putamen) in similar tasks (Ding & Gold, 2013). We used the coherent motion task to investigate the effect of FUS on decision-making and motor performance. Rhesus monkeys were treated with FUS and microbubbles to open the BBB 3-4 hours prior to behavioral testing. FUS was targeted to the putamen, a part of the basal ganglia involved in cognition, reward, and motor control. This study also investigated the interaction of FUS with a low dose of haloperidol as this technique could be used to non-invasively facilitate the drug effects, or to deliver drugs that cannot readily cross the intact BBB.

On days when the monkeys received the FUS treatment, their decisions were faster and more accurate, particularly when responses were made with the contralateral hand. A threshold dose of haloperidol also reduced response time, but impaired accuracy. FUS enhanced these drug effects. These results indicate that FUS can be used alone or in combination with psychoactive drugs to enhance or modify cognitive performance.

Results

Effects of FUS on blood-brain barrier

The BBB was successfully opened in the putamen region of the basal ganglia for all FUS procedures. In Fig. 1, the red/yellow areas indicate where the contrast agent was able to pass through the BBB, indicating successful BBB opening. The blue shaded regions indicate the region targeted by the FUS transducer. All openings achieved within this study fell within the

targeted region and no untargeted BBB openings were observed. No damage from the FUS procedure was detected; the T2-weighted MRI and susceptibility weighted imaging scans did not display any hyper- or hypointense voxels in the targeted regions, which could indicate edema (Supplementary Figures 1 & 2).

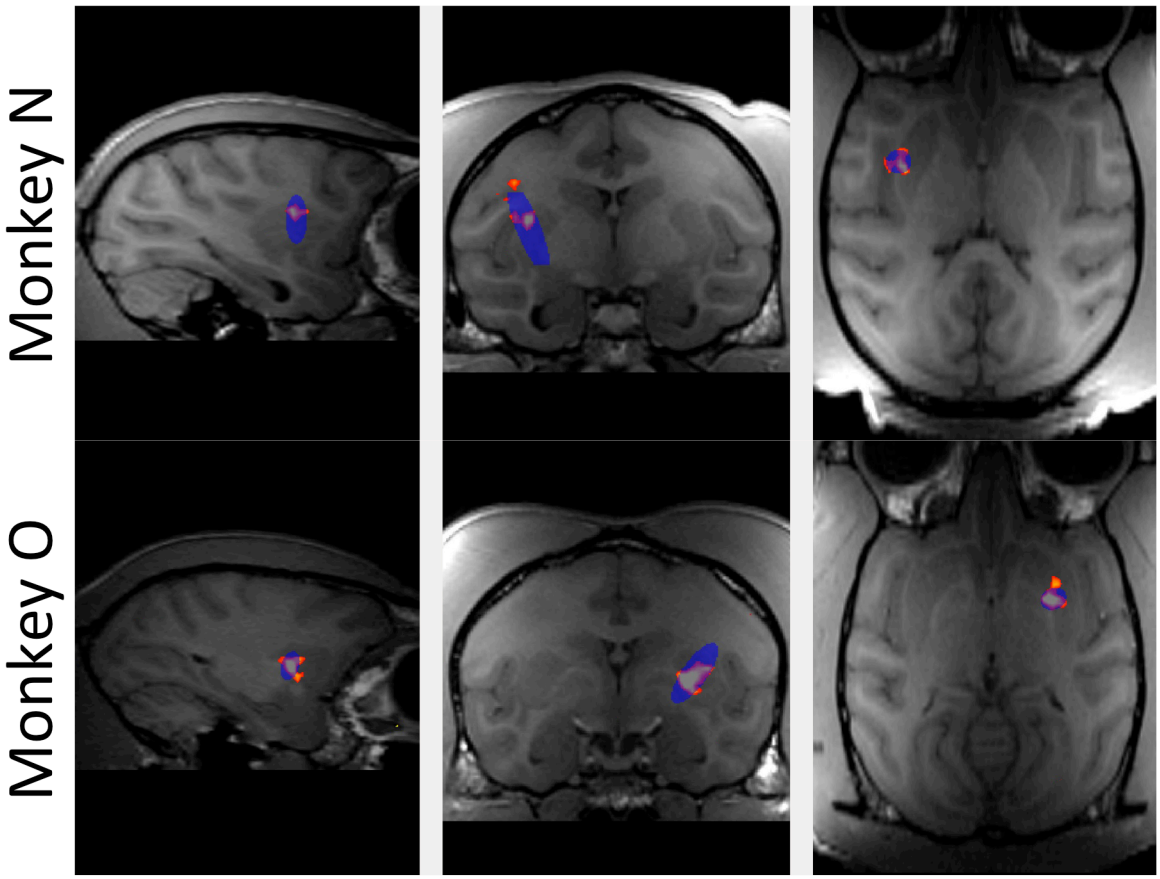

Figure 1. Contrast-enhanced (gadodiamide) MRI showing target of FUS in putamen of a monkey. Top row shows sagittal, coronal and horizontal slices through the brain of monkey N. Blue oval indicates the targeted region. Red and orange voxels indicate BBB opening. Bottom row shows the same for monkey O.

Effects of FUS on decision-making behavior

The two monkeys performed 31 behavioral sessions (16 for monkey N, 15 for monkey O) of the coherent motion detection task. N completed an average of 1385 trials per session (22,154 total trials), while O averaged 931 trials (13,960 total). Behavior was quantified in terms of response

time and accuracy. Response time was measured as the interval between motion stimulus/target onset and the first touch. Decision accuracy was measured as the percent correct choices relative to total correct and incorrect responses. Trials in which the monkey failed to respond were disregarded. Results for the two monkeys were qualitatively similar, except that monkey N (the younger of the pair) tended to respond faster and more accurately overall. The results were therefore combined across subjects after applying a correction for overall response time and accuracy (see Methods).

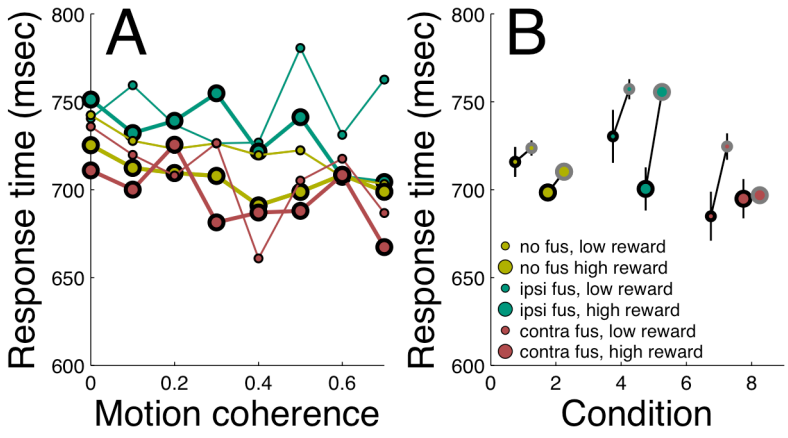

Figure 2. Effects of motion coherence, reward, sonication, and haloperidol on response time (RT) for single target trials. A) Response time for single target trials as a function of motion coherence, offered reward, and sonication. Error bars omitted for clarity. B) RT averaged over coherence levels. Each pair of circles connected with a line compares average RT during haloperidol sessions (black border) with saline sessions (grey border). Error bars represent ±1 s.e.m. Legend applies to both subplots.

Figs. 2a and 3 summarize the behavioral results for all 31 sessions, including those with and without sonication and with haloperidol or saline (effects of drug and sonication are considered separately below.) We first consider performance on trials with only one target. The monkeys were 100% correct on these trials because there was only one choice and trials without a response were not counted; hence, only response times were analyzed. Statistical results (ANOVA and GLM) are given in Supplementary Table 1 and main effects are plotted in Fig. 2a. Mean response time was faster for large reward trials than small rewards. There was also an effect of motion strength even though the motion stimulus was irrelevant; response times were faster on trials with high coherence stimuli and increased with decreasing coherence. Haloperidol ("drug") tended to shorten response time across all conditions (Supplementary Table 1 and Fig. 2b; symbols with black borders are from haloperidol session, symbols with gray borders are from saline

sessions). The effect of haloperidol was not significant in sessions without sonication (Supplementary Table 2.)

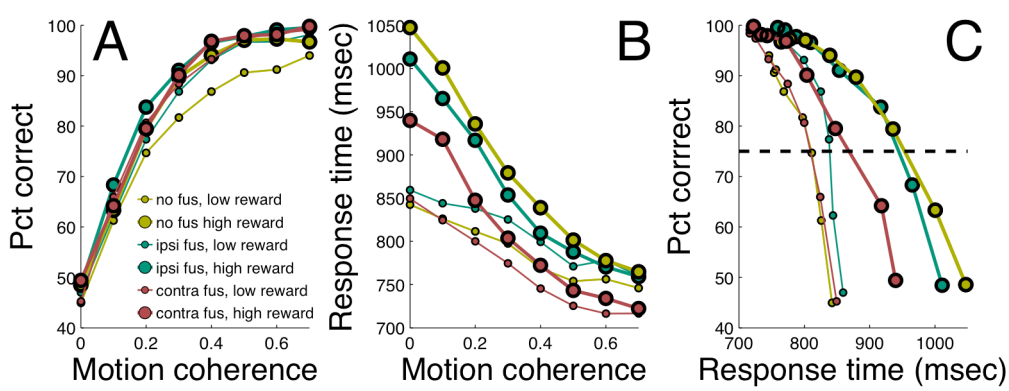

Figure 3. Effects of motion coherence, reward, and sonication on response time and accuracy for choice (2-target) trials. A) Accuracy (percent correct) for two-target trials. B) Response time for two-target trials. C) Accuracy vs. response time for two-target trials. Dashed horizontal line is 75% correct level. Legend in A applies to all subpanels. Error bars are omitted for clarity.

Sonication did not have a significant main effect on response time for all sessions (Supplementary Table 1), but did have a significant effect for sessions without haloperidol (Supplementary Table 3.) Furthermore, there was a significant interaction of sonication and drug (Supplementary Table 1.) Post-hoc analysis showed that the effect of haloperidol was significant only on days with sonication (Supplementary Table 4). Haloperidol-associated reduction of response time on sonication days appeared to be greater for responses with the ipsilateral than contralateral hand (Fig. 2b), possibly because responses with the contralateral hand were already as fast as possible (floor effect).

For trials with two targets, both performance accuracy and response time were analyzed. Accuracy improved with increasing motion coherence (Supplementary Table 5 and Fig. 3a) and response times were reduced (Supplementary Table 1 and Fig. 3a), as shown in previous studies (Roitman & Shadlen 2002). Offered reward size was associated with increased response time, i.e. the monkeys were slower to respond when there was a larger reward at stake (Supplementary Table 1). This was in contrast to their behavior on single-target trials

where responses tended to be faster on large reward trials. Larger offered reward size was associated with slightly better performance accuracy, particularly on days without sonication (Supplementary Table 5.)

Sonication was associated with slightly improved performance accuracy (Supplementary Table 5 and Fig 3a). Sonication also had significant effects on response time (Supplementary Table 1). These effects were significant when considering all sessions (Supplementary Table 1: response time, Supplementary Table 5: accuracy), or only sessions without haloperidol (Supplementary Table 3: response time, Supplementary Table 5: accuracy.) Sonication was associated with faster response times for large rewards (Fig. 3b). For small rewards, sonication sped up responses with the contralateral hand but slowed responses for the ipsilateral hand (Fig. 3b).

Accuracy is plotted against response time in Fig. 3c to show the amount of time taken to reach a given level of performance. This can be taken as a measure of decision-making efficiency. For small rewards, monkeys achieved 75% correct performance in less time than for large rewards; i.e. when a large reward was at stake, their responses were slowed even though this gained them little in accuracy. Sonication alone made the monkeys more efficient for large rewards with the contralateral hand and less efficient for small rewards with the ipsilateral hand.

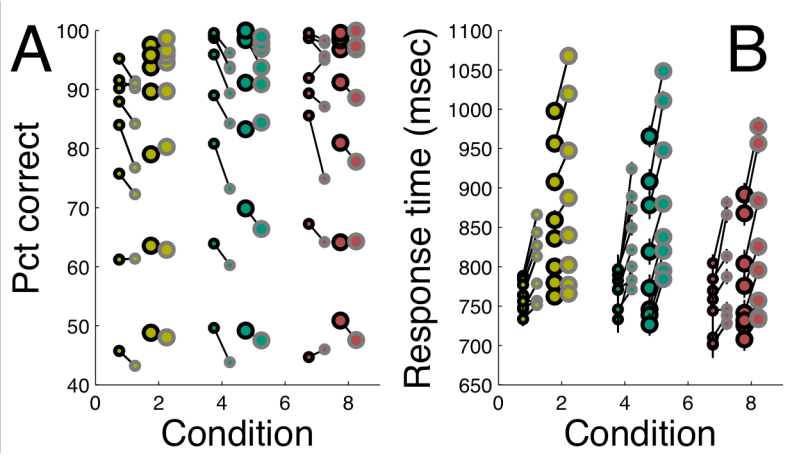

Figure 4. Effects of sonication and haloperidol on performance for two-target trials. A) Effects on accuracy. B) Effects on response time. Error bars represent ±1 s.e.m. Black borders are sessions with haloperidol, grey borders are saline controls. All conventions same as **Fig. 3**. Data in each column are plotted separately for each coherence level.

Haloperidol also had significant effects on accuracy and response time compared to saline controls (Supplementary Tables 1 and 5). Haloperidol was

generally associated with higher accuracy on trials with small rewards, but had mixed or equivocal effects on trials with large rewards (Fig. 4a). Sonication enhanced the negative effects of haloperidol on accuracy (Supplementary Table 6.) Haloperidol was associated with faster response times for both large and small rewards, with or without sonication (Fig. 4b). However, sonication and drug had a significant interaction for response times (Supplementary Table 1.) Sonication and haloperidol were both associated with reduced response times and their interaction was strongest for the ipsilateral hand (Supplementary Table 4), though response times were fastest overall for the contralateral hand (Fig. 4b).

Overall, sonication tends to improve accuracy and shorten response time, while haloperidol speeds responses but reduces accuracy. When used in combination, sonication enhances the effects of haloperidol.

Discussion

Targeting FUS to the putamen of monkeys resulted in significant improvements in decision-making performance. Monkeys responded faster and more accurately when tested on days with sonication than on days without. The physiological and psychological mechanisms underlying this effect are not known. The effects depended on the hand used to respond and are therefore unlikely to be due to general arousal. The effects also depended on reward size. When monkeys had to make a choice and a large reward was available, their response times in the absence of sonication were up to 200 ms slower than on small reward trials. Sonication reduced this difference to 100 ms while slightly improving accuracy. Thus, sonication appears to have improved the efficiency of decision-making. The performance enhancement was found even though animals were tested 3-4 hours after sonication, suggesting that there may be a persistent effect on the activity or responsiveness of putamen neurons, which, in turn, may be

due to a direct effect of ultrasound or an indirect effect of opening the blood-brain barrier. Further experiments are needed to ascertain the temporal window within which performance improvements are obtained. Such experiments should be done by sonicating subjects while they are alert (Downs et al. 2015) to avoid any confounding effects of anesthesia.

Sonication also enhanced the effect of a low dose of haloperidol, a D2 dopamine antagonist, given 5 minutes prior to behavioral testing. Previous studies of the effects of haloperidol on response times have reported mixed results depending on species, task and dosage (Brockel et al, 1995; Blokland & Honig 1999; Kern et al. 1998.) In the current study, low dose haloperidol tended to shorten response time and reduce decision accuracy. Both effects were enhanced by sonication. This result indicates that FUS can be used to significantly enhance dopaminergic medications to modulate cognitive performance. The results also demonstrate that the systemic dose of a drug necessary to achieve a desired pharmacological effect can be reduced by increasing BBB permeability through the application of FUS to a targeted brain region, even if the drug in question readily crosses the BBB. This would allow for smaller systemic doses, and thus reduction of potential side effects of already available drugs for therapies to treat neurological diseases and disorders.

There are few previous studies investigating the effect of FUS without BBB opening on alert subjects completing behavioral tasks. Deffieux and colleagues applied FUS to monkeys performing an antisaccade task by targeting the left frontal eye field (FEF) and the premotor cortex (Deffieux et al, 2013). Ipsilateral antisaccade latencies were significantly slowed while targeting the FEF but not the premotor cortex. Two other groups investigated the effects of FUS on human subjects (Legon et al, 2014; Hameroff et al, 2013). Subjects tested by Legon et al. exhibited enhanced sensitivity to the frequency of air puffs and discrimination at a two-point touch test while FUS was applied to their somatosensory cortex. FUS was applied to the frontal-temporal cortex in subjects of the Hameroff et al. study and unlike the other two studies with simultaneous/immediate behavioral testing, results were determined 10 and 40 minutes after

application. Subjects reported a significant improvement on the Global Affect test, as well as slightly reduced pain levels 40 minutes after the application of FUS. These studies demonstrate that FUS is capable of affecting the function of the brain depending on the targeting area, while the Hameroff et al. study shows the effects could be time sensitive. A key difference from the current study is that in the aforementioned studies the BBB remained undisrupted in the targeted region to the knowledge of the experimenters.

Recently, our group applied the FUS BBB opening procedure to awake, behaving monkeys performing a reaching task with variable reward magnitude (Downs et al. 2015). That study found a slight increase in response time as well as a significant improvement in the accuracy of reaching to visual stimuli during a 2-minute application of FUS and throughout the remaining 2 hours of behavioral testing. Another group, Chu et al, investigated the effects of BBB opening via FUS opening on somatosensory evoked potentials (SSEPs) and blood-oxygen-level dependent (BOLD) responses when targeting the left primary somatosensory cortex in anesthetized rats (Chu et al.2015). Results showed both a decrease in SSEP and BOLD signals within 10 minutes after finishing the FUS procedure. Their results highlighted the impact of sonication parameters utilized, as lower acoustic pressures resulted in little to no neurological effect, while higher acoustic pressures created sustained neurological effects. Our study utilized an acoustic pressure found to be safe during prior studies conducted within our lab, which was greater than the pressure used by Chu and colleagues. The exact mechanisms behind the excitation or inhibition of neurons via FUS is currently unknown, but a current theory is that mechanical forces emitted by the transducer during sonication affect mechanoreceptors in the cell membrane (Ostrow et al.2011; Wahab et al.2012; Tyler et al.2008). However, this explanation seems to be limited to cases in which the sonication is applied simultaneously during the behavioral testing. Our results, along with the studies conducted by the Hameroff and Chu studies, demonstrate that the effects of FUS sonication can persist after the time of application. Further studies plan to determine the optimal time after FUS application to open the

BBB for maximal behavior modulation. Understanding the relationship between treatment time and behavioral effects will help distinguish the mechanical effect of the sonication from the other potential neurological effects of the BBB being opened at the target region.

In conclusion, opening the BBB via FUS with microbubbles can have a significant effect on the behavioral responses of monkeys 3-4 hours after the end of the sonication. The BBB opening also facilitated the delivery of a low dose of haloperidol demonstrating that therapeutic doses of a drug can be reduced to mitigate the potential side-effects after opening the BBB at the target region for therapy. Overall, our results demonstrate the potential for FUS BBB opening to enhance behavioral performance in monkeys.

Methods

The procedures with monkeys were approved by the Institutional Animal Care and Use Committees (IACUC) of Columbia University and the New York State Psychiatric Institute (NYSPI). Two adult male Macaca mulatta (N, O) were used in all experiments (9 and 20 years old, 5.5 and 9.5 kg). Monkeys were provided daily rations of vitamin enriched dry primate biscuits, as well as enrichment toys and allowed access to play modules. Monkeys were trained using operant conditioning to perform a decision-making task. On behavioral testing days, monkeys performed the task for fluid reward until satiated. After behavioral testing, Monkeys were given a fruit treat (banana, apple, or orange). On days when behavioral testing was not conducted, monkeys were given a liter of water.

Focused Ultrasound and Drug Delivery

On selected days, monkeys received a FUS with microbubble treatment 3-4 hours prior to behavioral testing. For the FUS procedures, subjects were sedated with ketamine (10 mg/kg) and atropine (0.04 mg/kg) and placed into a stereotaxic positioning frame under general anesthesia (isoflurane 1-2%) to ensure accurate targeting. Microbubbles (4-5 um, in-house prepared, Feshitan et al. 2009) were administered intravenously at the onset of the FUS application (single element transducer, 500 kHz, 400 kPa, 10 ms pulse length, 120 second duration; H-107, Sonic Concepts, WA, USA). The putamen region of the basal ganglia was targeted for all experiments. Throughout the procedure, the monkeys' vital signs were continuously monitored (heart rate, SPO2, mean arterial pressure, respiratory rate and end tidal CO2). After the FUS procedure there was a 3 to 4 hour recovery period allowing the monkeys to fully recover from anesthesia.

Haloperidol, a D2 antagonist (R&D Systems, Inc., Minneapolis, MN), was used to augment neuromodulation. Haloperidol powder was dissolved in saline and titrated to the concentration of 0.01mg/kg. On selected days, before the task began, monkeys were administered either saline or haloperidol (0.01mg/kg) intramuscularly. The injection was given 5 minutes prior to the start of behavioral testing. The threshold dose of haloperidol was determined as the maximum dose that had a minimal effect on behavioral results when the BBB was intact. The timing of events during the FUS procedure, recovery, drug injection and behavioral testing is shown in Fig. 5a.

MRI Analysis

One day after the FUS procedure, BBB opening and safety of the procedure was verified with contrast enhanced T1-weighted as well as T2- weighted MRI and susceptibility weighted imaging scans respectively. All MRI scans (3T, Philips Medical Systems, MA, USA) were acquired 36 hours after the FUS procedure. T2-weighted (TR = 10ms, TE = 27ms, flip angle = 90°, spatial resolution = 400 x 400 μm2, slice thickness = 2mm with no interslice gap) and

susceptibility-weighted image (TR = 19ms, TE = 27ms, flip angle = 15°, spatial resolution = 400 x 400 µm2, slice thickness = 1 mm with no interslice gap) scans were used to verify the safety of the procedure. Contrast enhanced T1-weighted (TR = 19ms, TE = 27ms, flip angle = 15°, spatial resolution = 400 x 400 μm^2, slice thickness = 1 mm with no interslice gap) scans were acquired 30 minutes after IV administration of 0.2ml/kg gadodiamide (Omniscan®, 573.66 DA, GE, Healthcare, Princeton, NY, USA). Gadodiamide was selected as it does not cross the intact BBB. All acquired scans were aligned with a previously acquired stereotactically aligned structural T1-weighted MRI scan to verify opening in the targeted region (Marquet et al, 2015). The contrast enhanced T1-weighted scans were then post processed to quantify the volume of opening. This process has been thoroughly discussed elsewhere (Downs et al. 2015).

Behavioral Testing

Monkeys sat in a custom-made polycarbonate primate chair that allowed them to reach out to visual stimuli presented on a 20-inch LCD touchscreen monitor (NEC 2010x with 3M SC4 resistive touchscreen) placed directly in front of the chair. The resolution of the LCD was 1280 horizontal x 1024 vertical pixels with a refresh rate of 60 Hz. The touchscreen had a resolution of 1024 x 1024 pixels and a sampling rate of 60 Hz. The primate chair incorporated a polycarbonate midline divider so that stimuli presented on the right side of the touchscreen could only be reached by the right hand, and likewise for the left side. The viewing distance was 12 inches. Behavior was reinforced with drops of fluid delivered by a juice tube mounted on the chair. The monkeys were free to move their head and eyes, though they tended to face directly forward so that they could continuously lick the juice tube in anticipation of the reward.

The behavioral task was presented as discrete trials lasting roughly 5 seconds each. Each trial began with a cue stimulus presented on the left or right side of the monitor (Fig. 5b, “Cue”). The cue was a vertically or horizontally oriented yellow bar. The monkey touched the cue with the corresponding hand to initiate the trial. After a short delay, the cue was replaced by

a random dot motion stimulus (Fig 5b, “Choice.”) The motion stimulus consisted of 100 dots moving within a circular aperture. Some of the dots moved in random directions while others moved coherently in a single direction. The coherent direction, either leftward or rightward, varied from trial to trial. The proportion of coherently moving dots defined the motion strength. The strength of the motion stimulus (aka motion coherence) varied from 0 to 0.7 in steps of 0.1. A particular coherence level was presented randomly on each trial. The motion stimulus was flanked on either side by two target stimuli that appeared simultaneously with the motion stimulus. The target stimuli were yellow bars that had the same orientation as the cue. The direction of the coherent dots indicated which target would be rewarded. The monkey was reinforced with drops of water for touching the appropriate target (Fig 5b, “Reward.”) There was no punishment for incorrect responses or failure to respond. There was no signal instructing the monkey when to respond; rather, he was allowed to touch at any time after the motion stimulus and targets appeared.

There were two reward conditions: small offered reward (1 drop of water) and large offered reward (5 drops). Offered reward level on each trial was signaled by the orientation of the cue and target stimuli. Horizontal indicated large reward, vertical indicated small reward.

One seventh of the trials were controls that were identical to the other trials except that the target for the incorrect response was not presented. On these trials, no decision was required; the monkey could ignore the motion stimulus and simply touch the correct target to receive a reward.

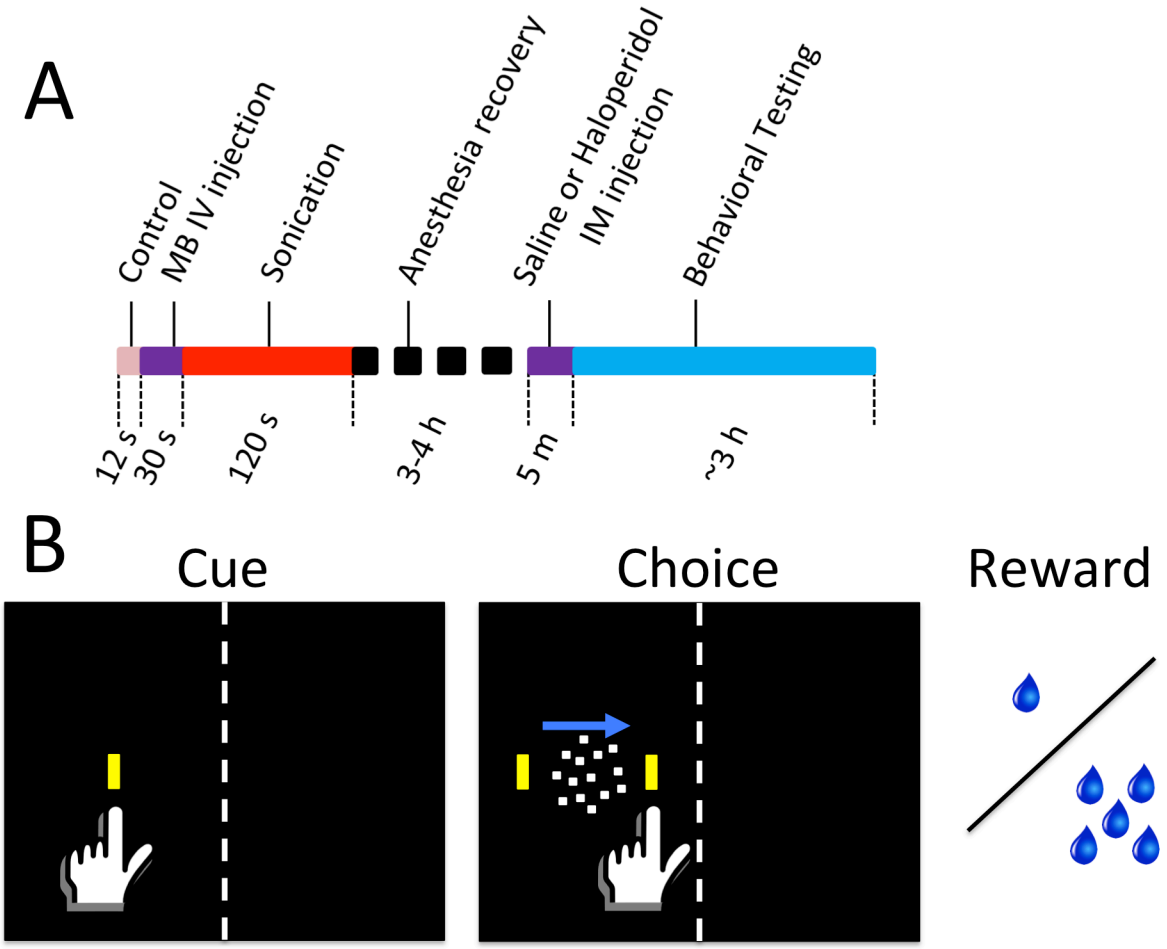

Figure 5. Behavioral task and experimental timeline. A) Timeline of sonication and behavioral testing. B) Decision task sequence. The monkey initiates a trial by touching the cue. A random dot motion stimulus appeared moving to the left or right, flanked by two targets. The monkey touched the target toward which the dots were moving to receive a reward. Stimuli were displayed on the right or left of the screen. A physical barrier forced the monkey to respond with the corresponding hand. Only the yellow bars and dot stimulus were visible to the monkey, not the blue arrow, dotted line, or hand, which are used to indicate the motion of the dots, the physical barrier separating the two halves of the screen, and the manual response, respectively.

The complete task design thus had the following variables: display side (left or right), cue/target orientation (vertical or horizontal, corresponding to small and larget reward), motion

direction (left or right), motion coherence (0.0 to 0.7), and number of targets (1 or 2). This resulted in a balanced design comprising 48 conditions per block of trials. All conditions were randomly interleaved.

Statistics

Quantitative analyses were performed using Matlab 8.3 with the Statistics 9.0 toolbox (Mathworks, Natick MA.) Response times were analyzed with multivariate ANOVA and generalized linear model regression. Performance accuracy or outcome (correct, incorrect) was analyzed with multivariate ANOVA and logistic regression. The explanatory variables used in all analyses were: motion coherence (0 to 0.7, 8 levels), offered reward (1 or 5 drops), presence of sonication, sonicated hemisphere (ispsilateral or contralateral to responding hand), and drug treatment (saline or haloperidol).

Response times were normalized by subtracting from the response time for each trial the difference between the average overall response time for each monkey and the average across both monkeys (the size of this correction was +/-55 ms, i.e. all of N's response times were decreased by 55 ms, while O's were increased by the same amount). Performance accuracy (percent correct) was similarly normalized by calculating the correction needed to equalize the accuracy at 0 coherence across monkeys (this correction was +/-2.2%), and adjusting the accuracy at all coherences by that amount. The accuracy correction was not applied for logistic regression analyses in which the dependent variable was trial-by-trial success or failure.

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Acknowledgements: NIH R01MH059244 (VPF); Kavli Foundation (VPF); NIH R01AG038961 (EK); NIH R01EB009041 (EK); Wallace H. Coulter Foundation (EK); FUS Foundation (EK); Alfonso Martin Escudero Foundation, Spain (CS)

Contributions: V.P.F. and E.K. conceived the study. V.P.F. and M.E.D. processed the behavioral and MRI data. M.E.D. conducted all ultrasound and behavioral experiments. T.T., A.B., and M.K., assisted with ultrasound and behavioral experiments. S.C. assisted with behavioral experiments. C.S. manufactured the microbubbles and assisted with ultrasound experiments. All authors participated in manuscript editing.

Competing financial interests:

The authors declare no competing financial interests.

Figures

Figure 1. Contrast enhanced (gadodiamide) MRI of BBB opening in putamen. Top row shows sagittal, coronal and horizontal slices through the brain of monkey N. Blue oval indicates the targeted region. Red and orange voxels indicate BBB opening. Bottom row shows the same for monkey O.

Figure 2. Effects of motion coherence, reward, sonication, and haloperidol on response time (RT) for single target trials. A) Response time for single target trials as a function of motion coherence, offered reward, and sonication. Error bars omitted for clarity. B) RT averaged over coherence levels. Each pair of circles connected with a line compares average RT during

480 haloperidol sessions (black border) with saline sessions (grey border). Error bars represent ±1
481 s.e.m. Legend applies to both subplots.
482
483 **Figure 3.** Effects of motion coherence, reward, and sonication on response time and accuracy
484 for choice (2-target) trials. A) Accuracy (percent correct) for two-target trials. B) Response time
485 for two-target trials. C) Accuracy vs. response time for two-target trials. Dashed horizontal line
486 is 75% correct level. Legend in A applies to all subpanels. Error bars are omitted for clarity.
487
488 **Figure 4.** Effects of sonication and haloperidol on performance for two-target trials. A) Effects
489 on accuracy. B) Effects on response time. Error bars represent ±1 s.e.m. Black borders
490 sessions with haloperidol, grey borders are saline controls. All conventions same as **Fig. 3**.
491 Data in each column are plotted separately for each coherence level.
492 **Figure 5.** Behavioral task and experimental timeline. A) Timeline of sonication and behavioral
493 testing. B) Decision task sequence. The monkey initiates a trial by touching the cue. A random
494 dot motion stimulus appeared moving to the left or right, flanked by two targets. The monkey
495 touched the target toward which the dots were moving to receive a reward. Stimuli were
496 displayed on the right or left of the screen. A physical barrier forced the monkey to respond with
497 the corresponding hand. Only the yellow bars and dot stimulus were visible to the monkey, not
498 the blue arrow, dotted line, or hand, which are used to indicate the motion of the dots, the
499 physical barrier separating the two halves of the screen, and the manual response, respectively.
500