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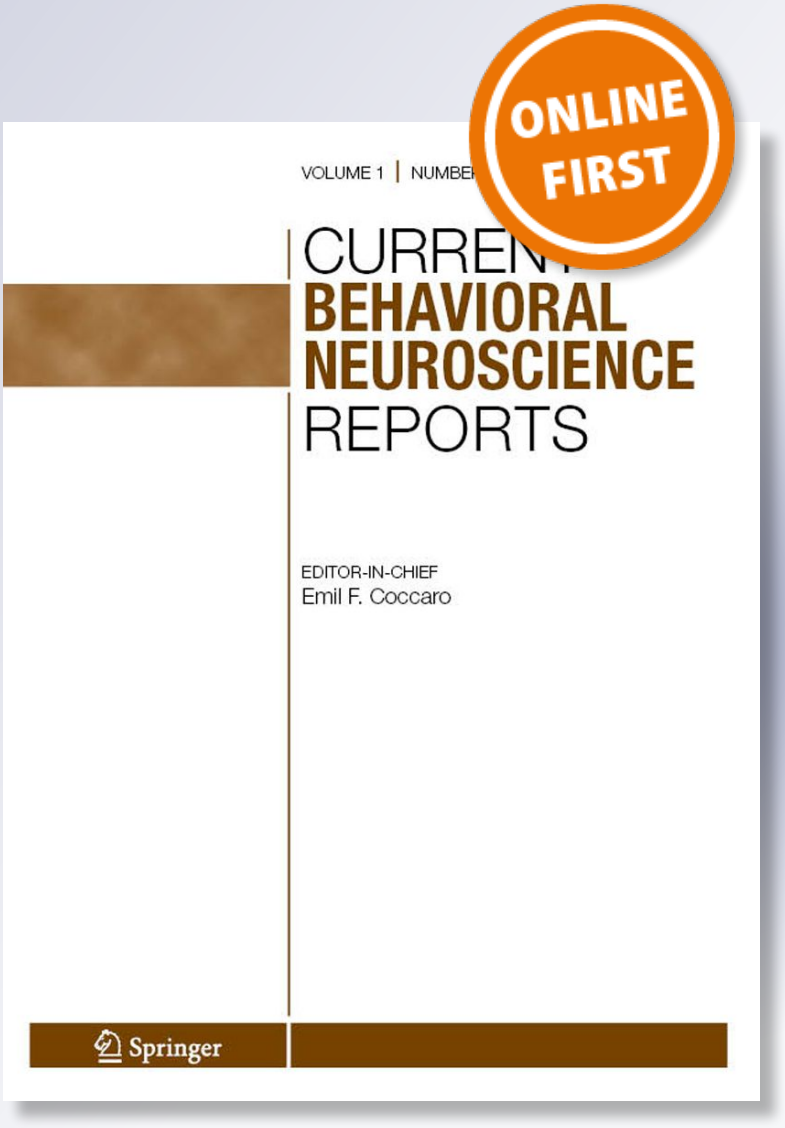

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NEUROMODULATION (C STAGG, SECTION EDITOR)

Modulation of Brain Function and Behavior by Focused Ultrasound

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Abstract

Purpose of Review The past decade has seen rapid growth in the application of focused ultrasound (FUS) as a tool for basic neuroscience research and potential treatment of brain disorders. Here, we review recent developments in our understanding of how FUS can alter brain activity, perception, and behavior when applied to the central nervous system, either alone or in combination with circulating agents.

Recent Findings Focused ultrasound in the central nervous system can directly excite or inhibit neuronal activity, as well as affect perception and behavior. Combining FUS with intravenous microbubbles to open the blood-brain barrier also affects neural activity and behavior, and the effects may be more sustained than FUS alone. Opening the BBB also allows delivery of drugs that do not cross the intact BBB including viral vectors for gene delivery.

Summary While further research is needed to elucidate the biophysical mechanisms, focused ultrasound, alone or in combination with other factors, is rapidly maturing as an effective technology for altering brain activity. Future challenges include refining control over targeting specificity, the volume of affected tissue, cell-type specificity (excitatory or inhibitory), and the duration of neural and behavioral effects.

Keywords Blood-brain barrier permeability · Microbubble · Brain stimulation · Cognition · Drug delivery

Introduction

The ability of high-frequency sound waves to perturb the function of electrically active cells was demonstrated almost 90 years ago [1]. Lynn et al. [2] used focused ultrasound (FUS) to create brain lesions; however, Fry et al. [3] appear to have been the first to apply FUS to *modulate* activity within the central nervous system (CNS). They reported that focusing ultrasound in the lateral geniculate nucleus of the anesthetized cat suppressed visually evoked potentials (VEP) in the primary visual cortex (see also Adrianov et al. [4]). Shortly thereafter, Ballantine et al. [5] demonstrated prolonged (up to 1 h), but reversible, pupillary dilation following sonication of the Edinger-Westphal nucleus in the cat midbrain. These early studies established that FUS could be targeted to specific structures within the brain to modulate physiological responses and behavioral outputs.

Over the past decade, there has been steadily increasing interest in the use of focused ultrasound (FUS) to directly or indirectly manipulate brain function and thereby investigate causal brain-behavior relationships, map brain circuits, and treat neurological disorders (see Table 1). FUS is noninvasive, yet can penetrate deeply within the brain through the intact skull, thereby complementing other neuromodulatory techniques that require surgery (electrical deep brain stimulation, optogenetics, and chemogenetics) or have lower penetrance (transcranial electrical stimulation). Current research seeks to optimize and extend the use of FUS, while elucidating the

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Table 1 Summary pulse parameters for FUS neuromodulation

Reference	Type	Animal model	Brain area	Frequency (MHz)	Duration (s)	Duty cycle %	PRF (kHz)	Ispta (W/cm^2)	Isppa (W/cm^2)	Increase or decrease response	Signal analyzed	Observation
Tufail 2010 [6]	In vivo	Mice	Motor cortex	0.35	0.066	30	1.5	0.036	0.2–0.3	Increase	Single unit recording	Increase in firing rate after FUS stimulation
				0.35	0.030	50	2.5	0.042	0.2–0.3	Increase	EMG	Tail flick, whisker, and limb contraction
Yoo 2011 [7]	In vivo	Rabbit	Motor cortex	0.69	1	50	0.01	6.3	12.6	Increase	EMG	Limb contraction
				0.69	1	50	0.01	1.6	3.3	Increase	fMRI	Increased BOLD signal
King 2013 [8]	In vivo	Mouse	Motor cortex	0.5	0.030	100	–	–	3	Increase	EMG	Tail flick, neck, and limb extension
Kim 2014 [9]	In vivo	Rat	Motor cortex	0.35	0.300	50	0.25	–	2.7	Increase	EMG	Tail twitches
Legon 2014 [10]	In vivo	Human	Primary somatosensory cortex S1	0.5	0.5	36	1	–	23.87	Decrease	EEG	Decrease in peak amplitude of N20–P27 and P27–N33 evoked potentials
Mueller 2014 [11]	In vivo	Human	Primary somatosensory cortex S1	0.25	0.3	50	0.5	–	23.87	Modulation	EEG	Change phase distribution of beta and change in phase rate of beta and gamma band
Lee 2016a [12]	In vivo	Human	Primary somatosensory cortex S1/S2	0.21	0.5	50	0.5	17.5	35	Increase	Tactile	Tactile sensations (palm/back of the hand or individual fingers)
Kamimura 2016 [13]	In vivo	Mouse	Sensorimotor cortex	1.9	1	50	1	–	–	Increase	EMG	Hind limb
Lee 2016b [14]	In vivo	Sheep	Sensorimotor cortex	0.25	0.3	50	0.5	0.9–5.7	1.7–11.3	Increase	EMG	Hind limb
			Visual cortex							Increase	EEG	Increase in visual evoked potential
Yoo 2011 [7]	In vivo	Rabbit	Visual cortex	0.69	> 7.5	5	0.1	1.6	6.4	Decrease	EGG/fMRI	Suppressed P30 in evoked potential/decreased in BOLD signal
Kim 2015 [15]	In vivo	Mouse	Visual cortex	0.35	150	5	0.1	–	3	Decrease	EEG	Decrease visual evoked potential
						8.3				Increase		Increase visual evoked potential
Daniels 2018 [16]	In vivo	Mouse/pig	Auditory cortex	0.23	52	3.4	0.0005	–	2.3–4.6	Decrease	EEG	Decrease the auditory evoked potential
Deffieux 2013 [17]	In vivo	Primate	FEF	0.32	0.1	100	–	4	13.5	Increase	Reaction Time	Delayed anti-saccades

Table 1 (continued)

Reference	Type	Animal model	Brain area	Frequency (MHz)	Duration (s)	Duty cycle %	PRF (kHz)	Ispta (W/cm^2)	Isppa (W/cm^2)	Increase or decrease response	Signal analyzed	Observation
Wattiez 2017 [18]	In vivo	Primate	FEF/SEF	0.32	0.1	100	–	–	5.6/1.9	Increase	Single unit recording	SEF activity significantly modulated shortly after FUS stimulation
Hameroff 2103 [19]	In vivo	Human	PFC	8	15	0.01	0.527	0.15	–	Modulation	Psychological test	Improved mood and reduction of pain
Kamimura 2016 [13]	In vivo	Mouse	Superior colliculus/-pretectal nucleus/locus coeruleus	1.9	1	50	1	–	–	Modulation	Visual observation	Eye movements/pupillary dilatation
Tufail 2010 [6]	In vivo	Mice	Hippocampus	0.25	0.325	32	2	0.084		Increase	LFP	Increase in firing rate CA1 and amplitude LFP
Yi 2015 [20]	In vivo	Rat	Hippocampus	2.5	0.160	50	0.5	0.0001–-0.15	1.2–-19.2	Increase	LFP	Increase power alpha, beta and gamma with LUS increase
Rinaldi 1991 [21]	In vitro	Rat	Hippocampus	0.75	2–15 min	90	150	80	–	Decrease	LFP	Dendritic response and cellular discharge were reduced
Bachtold 1998 [22]	In vitro	Rat	Hippocampus	0.5	5 min	–	200	50	–	Modulation	LFP	Enhanced dendritic potential, depressed fiber volley and cell population potentials
Tyler 2008 [23]	In vitro	Mice	Hippocampus	0.44	2.5	–	0.1	2.9	–	Increase	Neural activity by fluores-cence	Activation of sodium and calcium voltage-gated channels
Yoo 2011 [7]	In vivo	Rat	Thalamus	0.65	1.2	5	0.1	0.3	6	Modulation		Decrease effect anesthesia
Min 2011[24]	In vivo	Rat	Thalamus	0.65	0.180	5	0.1	0.13	2.6	Increase	EEG	Epileptic bursts in EEG
Min 2011b [25]				0.65	1.2	5	0.1	0.175	3.5	Modulation	Microdialysis	Increase of serotonin, dopamine and decrease levels of GABA in the frontal lobe
Yang 2012 [26]	In vivo	Rat	Thalamus	0.65	1.2	5	0.1	0.175	3.5	Modulation	Microdialysis	Decrease levels of GABA in the frontal lobe
Dallapiazza 2017 [27]	In vivo	Pig	Thalamus	0.22/0.65-/1.1	40	43.7	0.01	–	25–30	Decrease	EEG	Suppression in somatosensory evoked potential

Table 1 (continued)

Reference	Type	Animal model	Brain area	Frequency (MHz)	Duration (s)	Duty cycle %	PRF (kHz)	Ispta (W/cm^2)	Isppa (W/cm^2)	Increase or decrease response	Signal analyzed	Observation
Legon 2018 [28]	In vivo	Human	Thalamus	0.5	4	36	1	–	14.53			Attenuation of somatosensory evoked potential and reduced perceptual discrimination
Adrianov 2004 [4]	In vivo	Cat	LGN/optic tract	0.98	10–60	30–60	0.5–50	–	7–63	Decrease	EEG	Suppression in visual evoke potential
Mehic 2014 [29]		In Vivo	Mouse	Whole brain	0.5	59	30	1.5	0.1–5	17.5	Increase	EMG
Limb/tail/whisker movements												
Kim 2017 [30]		In Vivo	Mouse	Whole brain	0.43	5	40	0.375/0.-75/1.5	0.129	1.8	Increase	Intrinsic optical imaging
Amplitude change of hemodynamic signal evoked by varying PRF												

PRF pulse repetition frequency, *Ispta* Spatial peak time-average intensity, *Isppa* spatial peak pulse-average intensity, *FEF* frontal eye field, *SEF* supplementary eye field, *PFC* prefrontal cortex

underlying biophysical mechanisms [31–34, 35•, 36–39]. Recent studies in humans and nonhuman primates (NHP) show that FUS alone can affect neural activity as well as performance on perceptual and cognitive tasks. However, neural and behavioral effects may also be obtained by combining FUS with circulating microbubbles to open the blood-brain barrier (BBB), and these effects may have durations much longer than FUS alone. All of these studies underscore how close we are to utilizing FUS as a routine research and clinical tool [40].

Efficacy of FUS for Modulating Neural Activity, Perception, and Behavior

FUS Alone

Recent studies in awake monkeys and humans have provided evidence that application of FUS in the CNS can modify perception, behavior, and neurophysiological responses. At low intensities,[1] FUS can be applied to the brain of awake subjects transcranially without any evidence of pain, discomfort, or distress [43]. Deffieux et al. [17] applied FUS to monkeys performing an anti-saccade task [44], which is a prefrontal cortex-dependent test of the cognitive control of eye movements. They targeted the prefrontal cortex (left frontal eye field, FEF) and the premotor cortex (PMC). Latencies for anti-saccades ipsilateral to the sonicated hemisphere were significantly increased while targeting the FEF but not PMC, thus demonstrating both anatomical specificity and the effectiveness of FUS to reduce cortical excitability. There were no significant effects on eye movement amplitude or dynamics (peak velocity). Importantly, there was no significant effect on prosaccade latency, indicating that the mechanism was cognitive rather than motor. This group did a follow-up study [18•] in which they established the feasibility of modulating the rate of action potentials in supplementary eye field (SEF) neurons as FUS was applied simultaneously to the frontal eye field (FEF) in macaques performing the anti-saccade task. SEF activity was significantly modulated in 40% of SEF neurons shortly after FUS onset. Half of the SEF neurons showed a transient increase of activity induced by FUS. The SEF has reciprocal connections with the FEF [45]. Hence, modulation of SEF firing rate could be due to anti-dromic activation of SEF axon terminals or orthodromic activation of FEF neurons projecting to SEF.

Other groups have investigated the effects of FUS on cognitive states in human subjects ([19], Monti 2016). Hameroff and colleagues applied FUS to the frontal-temporal cortex to determine its effects on mood and pain perception. Unlike other studies, which investigated behavioral effects either during or immediately after stimulation, here the effects were determined 10 and 40 min after the end of FUS application. Subjects reported a significant improvement on the Global Affect test, as well as slightly reduced pain levels 40 min after the application of FUS. Whether FUS can have more sustained effects on mood and motivation is an important question if it is to be used in the treatment of psychiatric disorders. In a single case study, Monti et al. [46] reported improvement in the Glasgow Coma Scale rating of a patient with severe traumatic brain injury following 10 applications of FUS to the thalamus. This is an important step for the use of FUS in the treatment of disorders of consciousness.

Tyler and colleagues have performed a series of studies on the effects of FUS on human somatosensation (also explored by Gavrilov and colleagues as reviewed in Gavrilov 1996). The effects of peripheral FUS stimulation applied to the fingertips resulted in activation of human somatosensory cortex recorded by electroencephalographic (EEG) and functional magnetic resonance imaging (fMRI). Fingertip stimulation with pulsed FUS led to increased BOLD (blood oxygen level-dependent) activation in brain regions involved in somatosensory discrimination, including the primary somatosensory cortex and parietal operculum, as well as higher brain regions with somatosensory responses, such as the insula, anterior middle cingulate cortex, and supramarginal gyrus [47]. This group then applied FUS directly to the primary somatosensory cortex (S1, [10]). EEG recordings showed that directing FUS transcranially into S1 significantly attenuated the amplitudes of somatosensory evoked potentials (SSEP) elicited by median nerve stimulation [10]. While FUS was applied to their somatosensory cortex, subjects exhibited enhanced sensitivity to the frequency of air puffs and exhibited improved two-point tactile discrimination. In a follow-up study, neurophysiological recordings using EEG showed that FUS altered the phase distribution of intrinsic brain activity for beta frequencies (12–30 Hz), but not gamma (> 30 Hz). This modulation was accompanied by a change in phase rate (a measure of EEG dynamics) of both beta and gamma frequencies. Additionally, FUS modulated phase distributions in the beta band of early sensory evoked activity but did not affect late sensory evoked activity, lending further support to the spatial specificity of FUS for neuromodulation [11]. When FUS was applied to the thalamus, SSEPs were attenuated and performance was slightly reduced on a two-point tactile discrimination task [28•].

[1] Ultrasound can be characterized by the level of acoustic exposure. The field has defined two terms related to safety: thermal index (TI) and mechanical index (MI). TI is the ratio of the power used to that required to raise the temperature by 1 °C. MI denotes the peak rarefactional pressure normalized by the square root of the center frequency and is a measure of non-thermal effects such as cavitation. The average intensity is defined as the total power delivered divided by beam area (W/cm^2). Spatially averaged intensity can be indicated by Isppa (W/cm^2, spatial peak pulse-average intensity) while temporally averaged intensity, Ispta (spatial peak, time-averaged intensity (W/cm^2)), indicates the rate of energy deposition in the tissue [41, 42].

Lee et al. [12] found that FUS could elicit subjective sensations of touch in humans. Their use of multiple FUS transducers allowed simultaneous stimulation of the primary (S1) and secondary (S2) somatosensory cortex in the same hemisphere and elicited various tactile sensations in the absence of any external sensory stimuli. Stimulation of the S2 area alone could also induce perception of tactile sensations. The sensations could be localized to different parts of the hand. There were no sensations from the scalp underlying the transducer. The same group applied FUS to the primary visual cortex (V1) in humans. This resulted in BOLD activation not only from the sonicated brain area but also from the network of regions involved in visual and higher-order cognitive processes. Accompanying visual phosphene perception was also reported [14].

These studies demonstrate that FUS is capable of affecting brain function in a wide array of brain regions; however, the duration of these effects often differs between studies. In the study conducted by Deffieux et al., there was an increase in anti-saccade latency only during the brief (100 ms) application of the FUS, not after it had ceased. In the other studies, it also seems that perceptual and neuromodulatory effects were observed only during stimulation. Hameroff et al. observed effects up to 40 min after application of FUS, but did not test at later time-periods. Whether the duration of FUS effects are due to the nature of the effect (perceptual vs. affective), the brain area targeted, or other factors remains an open question. It is possible that FUS acts by altering cortical plasticity, which might explain longer term effects [19].

Animal studies have shed light on the biological mechanisms underlying neuromodulation by FUS alone. An early study by Velling and Shklyaruk [48] established that FUS could affect electrical activity in the CNS. They applied FUS to different cortical regions in the anesthetized cat and measured the electrocorticogram (ECoG) in nearby cortex using blunt intracranial electrodes. FUS activated cortical activity at low intensities (0.001–0.1 W/cm^2, the method of characterizing intensity was not stated), but caused depression at higher intensities (1–100 W/cm^2). Price and colleagues [21, 22] found that FUS modulated electrically evoked field potentials in slices of rat hippocampal dentate gyrus. Fiber volley and cell population potentials were reduced in amplitude, while dendritic field potentials were enhanced. More recently, Tyler et al. [23] demonstrated that FUS could evoke calcium and sodium transients in mouse hippocampal slices and ex vivo brains. The same group [6] showed that FUS applied transcranially to intact mouse primary motor cortex and hippocampus evokes an increase in action potential firing with short latency, i.e., firing rate increased within 50 ms of FUS onset. Yoo et al. [7] stimulated motor cortex in rabbits and observed an increase in the BOLD response and muscle contraction measured by EMG. When they stimulated in visual cortex, there was a decrease in the P30 component of the visual evoked potential and suppression of the BOLD response.

One insight into the molecular mechanism is that FUS application in Xenopus oocytes revealed a marked increase in potassium currents in members of mechanically sensitive K2P family of ion channels, which are expressed in the brain [49]. Additionally, a study in *Caenorhabditis elegans* demonstrated a behavioral response to FUS application that disappeared when mutants that misexpressed these channels lost sensitivity and failed to respond to sonication. This further showed how some mechanosensitive ion channels are targets for stimulation by FUS [50].

Recent studies have explored the efficacy of FUS neuromodulation as a function of ultrasound parameters such as frequency and intensity. [20] measured local field potentials in the hippocampus of anesthetized rats. They found that LFP power in all frequency bands (1–100 Hz) increased virtually monotonically with FUS intensity (1.2–19.2 W/cm^2). In the same context, Daniels et al. [16] showed in mice that increasing the intensity from 2.3 to 4.6 W/cm^2 (SPPA) boots the suppression in auditory evoked potential (AEPs) from 40 to 60% at 0.23 MHz. Kim et al. [9] explored the set of acoustic parameters required to induce a neural response in the motor cortex and produce an associated tail movement in rats (see Table 1 for parameters). However, there was not a proportional response when combining different parameters; the response was all or nothing. Similarly, King et al. [8] investigated the effective parameters to induce neuromodulation. Stimulation success increased as a function of both acoustic intensity and acoustic duration. Interactions of intensity and duration indicate that successful stimulation results from the integration of stimulus amplitude over a time interval of 50 to 150 ms. The motor response elicited appeared to be an all-or-nothing phenomenon. Kim et al. [15] found that the application of pulsed FUS not only suppresses excitability (measured as visual evoked potentials) but can also enhance the excitability depending on the acoustic intensity and the rate of energy deposition. Ye et al. [51] investigated how neurostimulation efficacy varied with FUS frequency by measuring forelimb EMG in mice as they stimulated motor cortex transcranially. Lower frequencies were most efficient with 0.3 MHz evoking a response on 50% of trials at intensities < 1 W/cm^2 (all intensities reported as SPPA). Higher frequencies (up to 2.9 MHz required higher intensities, e.g., at 1.4 MHz, 50% success required between 20 and 60 W/cm^2, while 2.9 MHz barely evoked a response at 90 W/cm^2. Kamimura et al. [52] investigated the dependence of mouse cortical (hindlimb EMG) and subcortical (pupillary dilation and motion) response depended on FUS pressure at 1.9 MHz. They found thresholds of peak rarefactional pressure at 1.45 MPa (megapascals) for hindlimb responses and 1.2 MPa for pupillary responses.

The question of whether FUS acts through the same mechanism as electrical stimulation was addressed by Gulick et al. [53]. They found that both FUS and epidural electrical stimulation of motor cortex evoked hindlimb movement. Immediately after FUS stimulation, the cortex was refractory to a second FUS stimulus for up to 3 s, but was not refractory to electrical stimulation, suggesting that the two kinds of stimuli act through different mechanisms. The authors speculate that FUS stimulation may depend on the creation of endogenous microbubbles, or some mediating factor that is not required for electrical stimulation. In the other hand, Han et al. [54] reported that ketamine inhibits ultrasound stimulation by blocking cortical neural activity.

The possible combinations of FUS parameters (center frequency, pulse sequence, pressure/intensity) by brain region, response type, and species are vast. Furthermore, it is possible that different cell types are selectively responsive to certain parameters and can thereby be selectively activated or suppressed. The potential of this tool has thus barely been tapped.

Effects of FUS with Microbubbles

Ballantine et al. [5] noted that the application of FUS alone could increase the permeability of the blood-brain barrier (BBB). It was subsequently found that using intravenous lipid-shell microbubbles would allow the BBB to be opened at lower FUS pressures (Hynynen et al. [55], [56]). While FUS has been demonstrated to open the BBB without microbubbles, the acoustic intensities needed are near or at the range of tissue ablation (Bakay et al. [57]; Vykhodtseva et al. [58]). Thus, for safe BBB opening, a combination of microbubbles and FUS is required. While the exact mechanisms of the BBB opening are unknown, acoustic cavitation of the microbubbles in the focal area of the FUS has been determined as a major factor [59–61]. This acoustic cavitation causes the microbubbles to oscillate, exerting mechanical forces on the surrounding vascular walls [61–63]. Increasing the permeability of the BBB can potentially affect neural activity and behavior due to changes in the extracellular milieu near the location of the opening. Because the BBB remains open for up to 48 h after treatment [64], the duration of modulatory effects that are linked to BBB opening may last much longer than for FUS alone.

To determine the safety of opening the BBB in a large animal model, McDannold et al. [65] performed repeated BBB openings in the visual cortex and lateral geniculate nucleus (LGN) of rhesus monkeys using FUS with microbubbles. They found that these treatments neither impaired nor enhanced the animals' ability to perform a visual-motor task. However, Downs et al. [43, 66, 67•] demonstrated cognitive improvements when monkeys were tested on a motivated reaching task and a visual motion detection task.

For the motivated reaching task, the NHP were given light ketamine sedation in order to place an IV catheter. They were then seated in a behavioral testing apparatus and began performing the task. After about 20 min, microbubbles were injected intravenously and FUS was delivered for 2 min unilaterally to the putamen, a basal ganglia nucleus involved in cognition, reward, and movement control. The NHP continued to perform the reaching task without interruption throughout the FUS with microbubble application. The outcome measure, reaction time to touch a visual target presented on a touch-panel display, was significantly shorter after sonication compared to before sonication within the same behavioral session.

For the motion detection task, FUS with microbubbles was applied unilaterally to the putamen under anesthesia. The monkeys were tested 3–4 h after treatment. Motion detection thresholds were significantly lower following BBB opening than in sessions on days without treatment. Reaching reaction times were faster when monkeys used the arm contralateral to the side of the FUS treatment than when they used the ipsilateral arm.

The mechanism by which FUS with microbubbles affects neural activity and/or behavior is unknown. However, the observation that FUS led to improvements in decision-making performance, even though animals were tested 3–4 h after sonication, suggests that there may have been a persistent effect on the activity or responsiveness of neurons in the putamen, which, in turn, may be due to a direct effect of ultrasound or an indirect effect of opening the blood-brain barrier. It is likely that BBB opening alters the local extracellular milieu, possibly by enriching the availability of oxygen and glucose. Ultrasound without microbubbles may also directly affect the permeability of mechanically or thermally sensitive ion channels [7], though it is unknown how long such effects could persist after sonication.

To investigate neural correlates of BBB opening, Chu et al. [68] explored the functional effects of BBB opening in rat somatosensory cortex using FUS with microbubbles. They found reductions in SSEPs and BOLD responses that lasted up to 7 days at the highest FUS pressures (mechanical index = 0.8). The reduction of SSEPs found by Chu et al. [68] is concordant with the results of Legon et al. [10], who reported reduced SSEPs with FUS alone. This reduction in SSEPs was correlated with an improvement in sensory discrimination [10]. Hence, it is possible that the performance improvements noted by Downs et al. [66, 67•] were associated with reduced activity in the putamen, though this has not been established. Further studies are necessary to fully ascertain the relationship between FUS with microbubble neuromodulation and changes in behavioral outcomes. Does it depend on opening the BBB, and, if so, how does BBB opening affect neural activity in the short (immediately after opening) and longer (hours to days after opening) term? Furthermore, the duration of

neuromodulatory and behavioral effects as a function of FUS parameters needs to be carefully studied.

Ultrasound and Drug Delivery

A third avenue by which neuromodulation may be achieved is by enhancing drug delivery to the central nervous system. Increasing the permeability of the BBB allows molecules that are too big to cross the intact BBB to enter the brain. In addition, for compounds that do not cross the intact BBB, entry is limited to the region in which the BBB is opened, thus improving the targeting specificity of drug delivery and potentially reducing undesirable side effects. Two major challenges in FUS-based drug delivery are control over the size of the BBB opening as well as the concentration of the drug in the brain. In NHP, it has been established that the volume of brain within which BBB permeability is increased depends on FUS pressure and can range from less than 200 to over 800 mm^3 [69]. The amount of drug delivered, as well as the kinetics of transport also scale with FUS pressure. There have been a number of studies on drug delivery for therapeutic purposes in specific disease models (reviewed by [70, 71]), which are outside the scope of the current review.

Recently, McDannold et al. [72] showed that opening the BBB facilitated the blockade of neural activity by the inhibitory neurotransmitter GABA in somatosensory cortex of rats. GABA is generally thought not to cross the intact BBB (but see [73]). McDannold et al. tested the effects of GABA by measuring suppression of SSEPs which scaled linearly with GABA dosage. The suppression lasted up to 2 h after a single bolus injection. No suppression was seen without BBB opening. This study establishes that BBB disruption can gate the efficacy of a neuroactive substance and achieve neuromodulation at a specific brain locus.

There is also evidence that FUS alone can alter neurotransmitter release. Min et al. [25] were able to manipulate dopamine and serotonin levels in rat frontal cortex by sonicating (parameters in Table 1) the thalamus for 20 min. Yang et al. [26] found that sonication of the thalamus in rats reduced frontal lobe GABA levels by 20% as measured with microdialysis. Glutamate levels were not significantly affected, suggesting the FUS selectively reduced the activity of GABAergic (presumed inhibitory) neurons. The same group also showed that thalamic FUS reduced the frequency of chemically induced EEG bursts [24].

Concerns over potential immune responses, mainly triggered by multiple, overlapping regions of BBB opening and/or higher pressures [74], may be alleviated by using FUS to release drugs sequestered in micelles, liposomes, or nanoparticles at frequencies and intensities that do not disrupt the BBB [75, 76]. To avoid drug effects at untargeted locations, nanoparticles could be used, which allow for the noninvasive uncaging of neuromodulatory drugs. Nanoparticles are composed of biodegradable and biocompatible constituents and are activated using sonication parameters that are readily achievable by current clinical transcranial focused ultrasound systems [77]. Wu et al. [78] compared the use of octafluoropropane (OFP) and decaflurobutane (DFB) nanodroplets in mice. They found that OFP nanodroplets successfully delivered 40 kDa molecules (dextran) at pressures of 300 and 450 kPa without cavitation-associated damage. However, this approach is limited to drugs that are able to cross the intact BBB, whereas increasing BBB permeability allows delivery of a much wider range of compounds.

In addition to delivery of conventional drugs, BBB opening via FUS with microbubbles allows the delivery of viral vectors for gene transfer, as used in optogenetics or DREADDs (designer receptors exclusively activated by designer drugs). For these approaches, the chief concerns are the targeting accuracy and specificity, as well as the level and cell-type specificity of gene expression.

Thévenot et al. [79] opened the BBB with FUS using a standard protocol (either 0.558 MHz, 0.3 MPa or 1.18 MHz, 0.53–0.6 MPa for 120 s with Definity microbubbles). They then found that adeno-associated virus serotype 9 (AAV9) with green fluorescent protein (GFP) injected through the tail vein was expressed in the striatum and hippocampus of mice. The expression was not cell-type specific; neurons, astrocytes, and oligodendrocytes all expressed GFP after a 12-day incubation period. This approach might be also be used in NHP as AAV9 has been found to be an effective vector for transfection in marmosets [80]. Hsu et al. [81] were able to deliver intravenously administered AAV2-GFP to the mouse forebrain and achieved expression levels comparable to direct injection. Again, GFP was expressed in both neurons and glia. Cell-type specificity might be improved by using a neuron specific promoter.

Alonso et al. [82] opened the BBB with FUS to deliver AAV2/1 in rat and express bacterial β-galactosidase. This group achieved transduction rates up to 90%, mostly in neurons with very few astrocytes. Wang et al. [83] demonstrated that AAV9 can be used to transduce GFP and localize it in neurons at 95% rates using a synapsin promoter. Based on that, Wang et al. [84] developed a less invasive form of optogenetics that used AAV9 to express the light sensitive protein channelrhodopsin-2 (ChR2) in mouse hippocampus and showed that the transduced cells in the region of the BBB opening were electrically activated by blue light as effectively as cells that were transfected by direct injection.

Szablowski et al. [85] in a recent preprint showed that application of FUS allowed infection and expression of DREADDs in the hippocampus of mice. Expression was limited to hippocampus and highly specific for excitatory neurons. The targeted cells were activated by injection of CNO (clozapine N-oxide). Animals also showed inhibition of fear memory formation behaviorally.

As an alternative to viral gene delivery, Mead et al. [86] administered DNA-bearing nanoparticles coated with polyethylene glycol, thus avoiding immune responses against the virus. They tested this by delivering the gene for glial-derived growth factor (GDNF) to the striatum in a rat model of late-stage Parkinson's disease [87]. The FUS-GDNF treated rats were less susceptible to 6-OHDA, as determined by behavioral assays.

Further studies are needed to fine-tune the spatial targeting, extent, degree, and duration of BBB opening as a function of FUS parameters. For neural circuit-tracing studies, it is desirable to deliver a high concentration of drug within a small volume of tissue (e.g., a single cortical layer) with sub-millimeter targeting accuracy. To achieve this will likely require a precise 3D model of each subject's skull, as well as computational methods that model the interaction of the FUS beam with the skull and soft tissues [88–91].

Conclusion

Neuromodulation with ultrasound has a long history (over 50 years), yet there are significant challenges to overcome as it evolves into a refined experimental and clinical technique. By definition, it has several advantages: being noninvasive, deeply penetrating, and spatiotemporally precise. Neuromodulation with ultrasound can be used at the moment with three different approaches. Direct neurostimulation using FUS alone is able to induce neural activation or suppression in thalamic, subthalamic, and cortical brain regions. However, further studies are necessary to optimize the parameters for controlling the desired outcome (e.g., excitation or inhibition). Previous studies were oriented more toward proof of principle than systematic application as a tool to investigate brain function. Second, FUS with microbubbles, which may require BBB opening as an intermediary mechanism, has recently evidenced the capability of modulating neural responses [66, 67•, 68]. A benefit here may be the long duration of the effect in comparison with ultrasound alone, despite its unknown mechanism. A third category of FUS neuromodulation is via drug delivery [91, 92], viral vectors to express distinct type of proteins (optogenetics or DREADD), and gene delivery (DNA-bearing nanoparticles). This approach has the key advantage of not only delivering naturally blocked compounds into the brain but also targeting the anatomical region they can access. Effects mediated by BBB opening may have a longer duration than FUS alone, which is a benefit for certain therapeutic applications.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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