Transcranial focused ultrasound to the thalamus alters anesthesia time in rats

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A pulsed application of focused ultrasound (FUS) to the regional brain tissue alters the state of tissue excitability and thus provides the means for noninvasive functional neuromodulation. We report that the application of transcranial FUS to the thalamus of anesthetized rats reduced the time to emergence of voluntary movement from intraperitoneal ketamine/xylazine anesthesia. Low-intensity FUS was applied to the thalamus of anesthetized animals. The times required for the animals to show distinct physiological/behavioral changes were measured and compared with those times required in a control session without sonication. The sonication significantly reduced the time to show pinch response and voluntary movement. The modulatory effects of FUS on anesthesia suggest potential therapeutic applications for disorders of consciousness such as minimally consciousness states. *NeuroReport* **22:783–787 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Since the pioneering work examining the biological effects of ultrasound in the early 1950 and 1960s [1,2], much effort has been made to establish the safe and effective applications of ultrasound for various therapeutic uses. As a new modality that enables noninvasive functional neuromodulation, ultrasound is gaining momentum in neurotherapeutics, especially in targeting brain disorders [3]. Transcranial application of ultrasound, with the formation of an acoustic focus in region-specific brain tissue, has been achieved using multiarrayed transducers surrounding the head [4,5]. The fundamental frequency used for transcranial ultrasound is typically less than 1 MHz [6], which is lower than the frequency used in diagnostic ultrasound imaging (typically 2–3 MHz). The highly localized delivery of acoustic energy (approximately the size of a rice grain) to the regional brain areas has been demonstrated in humans for thermal ablation of tumor [7] and functional neurosurgery for pain [3].

The application of pulsed-mode ultrasound modulates the excitability of the neural tissue both *ex vivo* [8] and *in vivo* [9]. We have recently demonstrated that pulsed focused ultrasound (FUS) to selected brain areas of anesthetized animals (rabbits and rats) modulated (either elicited or suppressed) its excitability [10,11]. During the study, we observed the unusual shortening of anesthetic duration in the animals subjected to the sonication. We hypothesized the FUS altered the function of neural substrates affected by the anesthetic, and thereby decreased the duration of the anesthesia. This motivated us to examine whether the FUS can indeed decrease the emergence time from anesthesia in a reproducible manner.

Pulsed FUS was delivered to the thalamus of the rats subjected to a general anesthesia of intraperitoneal ketamine (*N*-methyl-D-aspartate receptor antagonist) and xylazine ($\alpha 2$ adrenergic receptor agonist). The thalamus was targeted as ketamine, at an anesthetic dose, which is known to depress activity in the neocorticothalamic axis and central nuclei of the thalamus [12–14]. To assess the effects of FUS on emergence from anesthesia, we measured the time points when animals began to show selective physiological/behavioral features on emergence such as respiratory rates, whisker movement, responses to external stimulations, and initiation of voluntary movement.

Materials and methods

Overview of the procedures

All experiments were conducted under institutional review and approval by the Harvard Medical Area Standing Committee on Animals. Two of a total of 17 Sprague-Dawley rats were used to survey the timing of typical physiological responses emerging from anesthesia. All other animals underwent two sessions of anesthesia, separated by 6.2 ± 2.4 days allowing recovery from anesthesia. In one of the two sessions, the animal was subjected to the sonication condition denoted 'FUS'; the session without sonication was denoted 'control' condition. The sequence of the conditions was randomized and counter-balanced across animals. The weight of the

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animals was also balanced across the conditions. Two animals with atypical hyperventilation ($n = 1$) and incomplete anesthetic induction ($n = 1$) were excluded from further testing and analysis.

Focused ultrasound sonication setup

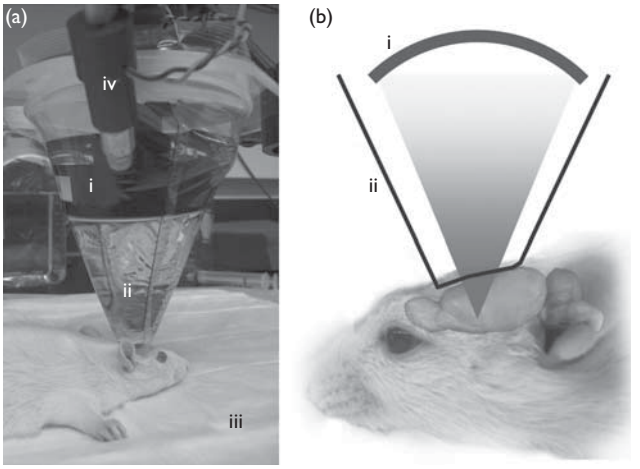
An air-backed, spherical segment ultrasound transducer operating at a frequency of 650 KHz was used. This fundamental frequency is within the 440–700 KHz frequency range known for the optimal transmission through the ex-vivo human skull [15,16]. The transducer had a diameter of 6 cm and a radius of curvature (focal depth) of 7 cm. The method for generating the pulsed FUS, hardware specs, and acoustic power calibration are described elsewhere [17]. The acoustic focus, mapped using a hydrophone (Onda, California, USA) mounted to a triple-axis stage scanner (Velmex, New York, USA), was cigar shaped and measured 3.5 mm in diameter and 6.2 mm in length at full-width half maximum of the acoustic pressure field. For estimating the intensity, the pressure amplitude measured by the hydrophone was corrected for attenuation through the rodent skull *in situ* [18]. I_{sppa} (spatial-peak pulse-averaged intensity) was calculated by a pulse intensity integral, which was estimated from the integral of the square of instantaneous pressure waveforms divided by the acoustic impedance of the media [19].

For sonication parameters, tone burst duration of 0.5 ms and pulse repetition frequency of 100 Hz were used. These pulsing parameters were used previously to provide suppressive effects on cortical excitability, albeit with a lower acoustic intensity, I_{sppa} of 3.3 W/cm^2 [10,11]. A preliminary test on three animals (data not shown) had shown reduction in time to emergence, but with a higher intensity, I_{sppa} of 6 W/cm^2, corresponding to a spatial peak temporal average intensity, $I_{spta} = 300$ mW/cm^2. The peak negative pressure to the brain tissue was approximately 490 kPa, with associated mechanical index (MI) of 0.61.

Anesthesia induction and sonication

All animals were anesthetized with an intraperitoneal ketamine/xylazine mixture of 80 : 10 mg/kg, which was administered by the same experimenter throughout the experiment to minimize bias. The onset time of anesthesia was marked by unresponsiveness to pinching of the hind paw. The animal was then positioned on a temperature-regulated heated pad (T-pump, Gaymar, New York, USA) and was placed in a prone position under the sonication apparatus (Sonomo, Korea; Fig. 1). The ultrasound transducer faced downward, directing the FUS beam through a plastic bag containing degassed water, was placed over the scalp of the animal. Ultrasound gel was applied between the scalp and the bag. Optical stereotactic guidance described in our previous work [17] established the sonication focus (spatial peak intensity) at the thalamus. The craniometric approximation was used to locate the thalamus based on the distance from the eye and ear canal (2–3-mm caudal to the bregma and 7-mm deep from the skull surface) [20]. Sonication began 40 min after the onset of anesthesia and was maintained for 20 min. For consistency, the control session was conducted under the same experimental setting as the sonication session.

Fig. 1



(a) Experimental setup. The animal's head is coupled to an (i) ultrasound transducer by a (ii) bag containing degassed water. The (iii) heat pad regulated the temperature while the (iv) optical imaging system provided the stereotactic guidance to the thalamus. (b) Illustration of the sonication setup (not drawn to scale).

Rectal temperature and respiratory rate of the animal were measured every 5 min. The time it took the animal to display the selected physiological phenomena of increased respiratory rate (10% above baseline under anesthesia), rapid and irregular respiration, and periodic movement of whiskers, were recorded for each. The animal's responsiveness to an air puff to the eyes (delivered through a rubber bulb) and hind-paw pinching, as well as the initiation of voluntary movements of the head, forelimbs or hind paws were monitored. When voluntary movement was detected, the animal was moved back to the cage for full recovery from the anesthetic.

Time to each transition was noted. A repeated-measures analysis of variance was performed to examine differences in anesthetic emergence time with and without sonication. The animals were kept alive after the second session and their food uptake, defecation, and movement behavior were assessed for abnormality on days 1, 3, 7, and 14 before killing.

Results

The animals' body weight, temperature, and baseline respiratory rate were similar (Table 1); and all showed normal behavior and weight gain (approximately 50%) after sonication.

Figure 2 shows the box plot of the measured physiological parameters comparing the two conditions. The intraperitoneal injection of anesthetic produced distinct physiological responses. The changes in the respiratory rate (RR) were similar between FUS and control conditions [$F(1,12) = 1.03$ for increase in RR, $F(1,12) = 2.24$ for rapid irregular RR; both $P > 0.1$]. The onset of whisker movement also showed the same trend across the two conditions [i.e., $F(1,12) = 1.27$; $P = 0.28$]. However, the time to initiation of responses to the air puff (noted as 'eye blink R') started to show a larger difference between the conditions [$F(1,12) = 4.33$; $P = 0.06$]. Remarkably, the sonicated rats responded to hind leg pinch approximately 55 min after onset of anesthesia, whereas the control condition rats did not respond until 67 min [$F(1,12) = 12.5$; $P = 0.004$]. Furthermore, the rats began to move their limbs much earlier under sonication (69.8 ± 9 min) compared with the control condition [88.2 ± 12.9 min; $F(1,12) = 18.5$; $P = 0.001$].

Table 1 The weight, respiratory rate, and body temperature of the rats measured from control and focused ultrasound sessions

	Control session	FUS session
Weight	303.2 ± 34.4 g	304.2 ± 33.4 g
Respiratory rate	65.5 ± 3.8/min	66.4 ± 2.9/min
Body temperature	36.1 ± 0.5°C	35.9 ± 0.3°C

All these values were indifferent between the two conditions.
FUS, focused ultrasound.

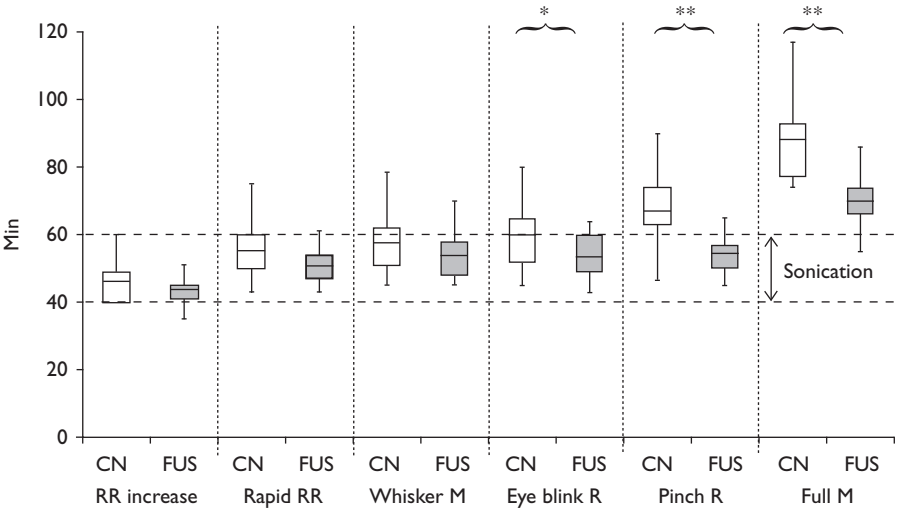
Discussion

Pulsed ultrasound sonication of the rat thalamus significantly reduced the time to emergence from anesthesia (as much as 20 min) as measured by the time to voluntary movement. Although an acoustic intensity (I_{sppa}) of 3.3 W/cm^2 had suppressive effects on brain function in previous studies [10,11], it is notable that this intensity failed to decrease the duration of the anesthetic state; whereas a higher intensity of 6 W/cm^2 (in I_{sppa}, corresponding $I_{spta} = 300$ mW/cm^2) significantly decreases anesthetic duration. As pulse parameters tone burst duration and pulse repetition frequency were unchanged, this suggests that the acoustic intensity is important in determining the direction of the neural modulation. It is conceivable that with identical pulse parameters, increasing the acoustic intensity could shift the direction of neuromodulation from suppression to stimulation. Further study is required to elucidate how the various sonication parameters affect neuromodulation.

The prevailing hypothesis as to how pulsed, FUS effects neuromodulation is that the pressure transmitted to the tissue creates a time-varying, nanometer-scale deformation of the neural cell membrane, possibly modulating the function of ion channels and mechanoreceptors embedded within the membrane, thereby affecting cellular excitability [21,22]. Cascading events might affect neurotransmitter release, uptake, and a general modification of neural circuits.

As to the mechanism by which sonication reduces emergence time from the anesthetic, several hypotheses

Fig. 2



A comparison box plot indicating the range, first/third quarter percentile, and the mean of the time to reach the measured physiological parameters after the onset of effective anesthesia. Focused ultrasound (FUS) was applied from 40 to 60 min after the onset of anesthesia (indicated with dashed lines). White boxes: control condition (denoted by 'CN') without sonication, gray boxes: FUS sonication (noted as 'FUS'). Acronyms for the parameter: Eye blink R, response to the air puff to the eyes; Full M, the onset of the voluntary movement; Pinch R, response to the hind-paw pinching; Rapid RR, rapid and irregular respiration; RR increase, increase in respiratory rate; Whisker M, movement of the whisker. *Marginal significance ($P = 0.06$), **significance ($P < 0.005$).

can be proposed. First, FUS may modulate levels of neurotransmitters, which antagonize the action of xylazine on $\alpha 2$ receptors. Our previous studies have already shown that FUS directed to the thalamus affects extracellular serotonin and dopamine levels [17], and neurotransmitter-mediated alteration in anesthesia remains a viable mechanism. Second, the thalamocortical connectivity that is dissociated by the action of ketamine, may be re-established by FUS stimulation of the thalamus, analogous to the way electrical stimulation of the thalamus by deep brain stimulation shows promise in improving chronic disorders of consciousness [23–25].

In terms of safety, the acoustic intensity and MI used in this study were sufficiently below the maximum limits for ultrasound imaging in the Food and Drug Administration guidelines (i.e., $MI = 1.9$ and $I_{spta} = 720\,mW/cm^2$ for most of the soft tissue imaging [10]), and this conforms with the normal behavior we observed in all animals after sonication.

The possibility that FUS can modulate anesthetic effects may have implications for the treatment of chronic disorders of consciousness, for example, minimally consciousness states or vegetative states caused by trauma or stroke, and for studying the mechanisms of consciousness and general anesthesia. Already, deep brain stimulation and dopaminergic or gabanergic drug therapy have shown promising results on these disorders [23–25]. Transcranial magnetic stimulation may also provide potential noninvasive alternatives to the therapy [25]; however, it does not provide adequate stimulation for small regions of the brain that are located deep in the brain, such as thalamus. FUS, with its advantages in exquisite control on depth and focal size, which have been demonstrated through transcranial application in humans [3], may thus provide unprecedented opportunity for studying and treating disorders of consciousness.

The anesthetics used in this study belong to a subset of agents that affect consciousness. Therefore, to generalize the effects of FUS on anesthesia and consciousness, further studies involving different classes of anesthetics will be necessary. In addition, the scoring techniques for the evaluation of anesthetic states were somewhat arbitrary. For example, a threshold for responses to pinch and for voluntary movements of whiskers and limbs may vary slightly depending on the evaluator. Adoption of functional neuroimaging techniques to assess the transition in metabolic states during FUS stimulation and the use of less subjective and nonblinded assessment of the anesthetic states, would also help to provide the key to unlocking our understanding of consciousness and its disorders.

Conclusion

Pulsed ultrasound focused at the thalamus significantly decreased time to emergence from ketamine/xylazine anesthesia in rats. Although the mechanism behind this observation is still unknown, the neuromodulatory potential of this noninvasive and spatially specific tool warrants further investigation.

Acknowledgements

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Conflicts of interest

None declared.

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