Focused Ultrasound Modulates the Level of Cortical Neurotransmitters: Potential as a New Functional Brain Mapping Technique

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ABSTRACT: Regional modulation of the level of cortical neurotransmitters in the brain would serve as a new functional brain mapping technique to interrogate the neurochemical actions of the brain. We investigated the utility of the application of low-intensity, pulsed sonication of focused ultrasound (FUS) to the brain to modulate the extracellular level of dopamine (DA) and serotonin (5-HT). FUS was delivered to the thalamic areas of rats, and extracellular DA and 5-HT were sampled from the frontal lobe using the microdialysis technique. The concentration changes of the sampled DA and 5-HT were measured through high-performance liquid chromatography. We observed a significant increase of the extracellular concentrations of DA and 5-HT in the FUS-treated group as compared with those in the unsonicated group. Our results provide the first direct evidence that FUS sonication alters the level of extracellular concentration of these monoamine neurotransmitters and has a potential modulatory effect on their local release, uptake, or degradation. Our findings suggest that the pulsed application of FUS offers new perspectives for a possible noninvasive modulation of neurotransmitters and may have diagnostic as well as therapeutic implications for DA/5-HT-mediated neurological and psychiatric disorders. Int J Imaging Syst Technol, 21, 232–240, 2011; Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/ima.20284

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I. INTRODUCTION

Noninvasive, region-specific regulation of neural functions in the brain has been pursued by many researchers to explore new ways to map brain functions and to provide therapeutic remedies. Recent advances in image-guided focused ultrasound (FUS) techniques allow for the noninvasive and spatially accurate transcranial delivery of acoustic energy to a focused tissue region (Jolesz et al., 2005). Several investigations of the effects of FUS on the ex vivo animal brain have revealed that ultrasound can significantly influence the neurophysiology of in vitro local neural circuitry (Bachtold et al., 1998; Rinaldi et al., 1991). For example, the ultrasound can temporarily increase (Gavrilov et al., 1996) or decrease the excitability of the neuronal tissue (Fry et al., 1958). We recently demonstrated that the administration of low-intensity FUS (spatial-peak temporal average intensity; $I_{spta} < 165$ mW/cm^2) to a regional brain area, delivered in a train of pulses, modulated (i.e., increased or suppressed) neuronal excitability in vivo (Yoo et al., 2008, 2009) without inducing tissue/vascular damage (Yoo et al., 2011). We also found that neither temperature changes nor the disruption of the blood brain barrier (BBB) contributed to these findings (Yoo et al., 2011).

Other than direct modification of cellular-level excitability, the role of FUS in the modulation of the local concentration of neurotransmitters is still unknown. Noninvasive neuromodulatory techniques such as transcranial magnetic stimulation have been shown to modulate the extracellular level of neurotransmitters (Keck et al., 2002; Zangen and Hyodo, 2002; Kanno et al., 2004); however, due to the magnetically inductive nature of magnetic stimulation, the area of modulation is rather wide (typically on the order of centimeters) and is also limited to the cortical surface (Kobayashi and Pascual-Leone, 2003; Fregni and Pascual-Leone, 2007). FUS sonication, on the other hand, delivers spatially focused acoustic energy (on the order of millimeters) to a regional brain area with the ability to reach deep brain structures. We were motivated to examine the potential of FUS for the noninvasive modulation of neurotransmission as such techniques are virtually nonexistent. The demonstration of such a capability will provide compelling evidence for the role of FUS in functional neuromodulation, as well as its utility as a new method for functional mapping.

Among the different types of neurotransmitters in the brain, we investigated whether or not FUS alters the extracellular level of two representative monoamines, dopamine (DA) and serotonin (5-hydroxytryptamine; 5-HT), by directly assessing extracellular concentrations using the microdialysis technique (Arbuthnott et al., 1990; Zhang and Beyer, 2006). DA, one of principal modulatory neurotransmitters of the mammalian brain, is most closely associated with processes related to motor movement and control, and reward and motivation (Ungerstedt et al., 1982; Koob and Swerdlow, 1988). On the other hand, 5-HT influences processes associated with memory, learning, mood, appetite, and sleep (Schwartz et al., 1990; Ursin, 2002; Wrase et al., 2006; Adams et al., 2008; Nemeroff and Owens, 2009).

In this study, FUS was administered to the thalamus of rats. The thalamus provides the main input to the striatum, which is one of important neural substrates implicated in the transmission and function of DA (Smith et al., 2004, 2009; Goff et al., 2009). Although serotonergic neurons are located predominantly in the raphe nuclei, the serotonergic transmissions project diffusely over the central nervous system (Halbach and Dermietzel, 2006b) with relatively higher concentrations of innervations in the thalamus and cerebral cortex. Before, during, and after the sonication session, the extracellular levels of both DA and 5-HT were measured in micromolar concentrations in the frontal lobe of the rat brain. Direct in vivo microdialysis from the rat brain (Ungerstedt et al., 1982) was used for the quantification of endogenous neurotransmitter in the extracellular space of the central nervous systems (CNS).

II. MATERIAL AND METHODS

A. Study Overview. All procedures were carried out in accordance with the rules set forth by the Institutional Animal Care and Use Committee. Male Sprague–Dawley rats (250–400 g; $n = 22$) were randomly divided into two groups. Those in Group 1 were treated with FUS sonication on the brain during microdialysis ($n = 12$; 289.1 $\pm$ 7.3 g, ranging from 264 to 323 g; noted as ''FUS,'' all error notations are presented in standard error: s.e.). This group allowed us to investigate the effects of sonication on the extracellular level of DA and 5-HT. Group 2 underwent microdialysis without sonication ($n = 10$; 315.8 $\pm$ 5.0 g, ranging from 297 to 337g; noted as ''Control'') and provided a control condition to evaluate the extracellular level of the neurotransmitters in the absence of sonication.

B. Microdialysis Setup and Animal Preparation. Prior to the experiment, the recovery rate of a microdialysis probe was examined in vitro using known concentrations of neurotransmitters. The probe was immersed in a standard solution containing 10 ng/ml of DA and was then perfused with artificial cerebrospinal fluid (CSF; CMA Microdialysis, Chelmsford, MA) at a rate of 2 μl/min, which was driven by a microinjection pump (NE-300, New Era Pump, Farmingdale, NY). The artificial CSF consisted of NaCl (147 mM), KCl (2.7 mM), $CaCl_2$ (1.2 mM), and $MgCl_2$ (0.9 mM).

Rats were anesthetized with urethane (1.5 g/kg; U2500, Sigma, St. Louis, MO) via an intraperitoneal injection and were placed in a stereotaxic frame (ASI Instruments, Warren, MI). Urethane anesthesia is preferred over other inhalant and injection-based anesthetic agents due to its minimal interference/interaction with the neurotransmitters (Maggi and Meli, 1986; Hedenqvist and Hellebrekers, 2003). An intracerebral guide cannula was then surgically implanted prior to insertion of the microdialysis probe. A burr-hole (2 mm in diameter) was drilled on the skull above the frontal lobe (2 mm anterior to bregma, 3 mm lateral to the middle suture), and the dura was carefully penetrated by a syringe needle. The hemispheric position of the burr-hole was randomized and counter-balanced across the animals. Through this burr-hole, the guide cannula (CMA Microdialysis, Chelmsford, MA) was administered to the frontal lobe (3 mm ventral from the dura and at a 60° angle to the horizontal line passing through the burr hole in the skull; cf., Fig. 1A). Here, two supplementary screws were also attached to the skull to firmly fixate the guide cannula using epoxy glue. A microdialysis probe (12 Elite 14/03, CMA Microdialysis, Chelmsford, MA) was then inserted through the guide cannula under stereotactic guidance (Paxinos and Watson, 2004), reaching a depth of 3–4 mm ventral from the dura. The probe was of the concentric design (0.5 mm in diameter, 20 kDa cut-off) consisting of a semipermeable membrane (3 mm in length). We ensured that the entire probe membrane was contained in brain parenchyma under the dura. The probe was intentionally positioned away from the sonication path as direct sonication on the probe itself alters the membrane permeability (and introduces confounds to the data). This potential alteration in membrane permeability was tested by focusing the sonication directly on the microdialysis probe ($n = 4$) and measuring the recovery rate of known concentrations of DA.

After the insertion of the probe, the animal was laid prone on a thermostatically controlled heating pad (T-pump, Gaymar, Orchard Park, NY). The temperature of the animal was measured every 20 min to ensure the maintenance of core body temperature. The animal's head was gently immobilized using a cushion around the head while an additional custom-made cushion was placed below the animal to keep a patent airway while lying prone under anesthesia. To allow equilibration with the tissue and to diminish possible acute effects from probe implantation on the microenvironment (e.g., BBB disruption), microdialysates were sampled starting 2 h after insertion of the probe.

To examine the time course of potential sonication effects, the levels of neurotransmitters for a series of consecutive time intervals were assessed by collecting the microdialysates every 20 min using a fraction collector (CMA142, CMA Microdialysis). As the microdialysates were collected only during 10 min in every 20-min interval, its perfusion rate (i.e., 2 μl/min) was maintained at the same level of the in vitro recovery. The collection schemes are shown in Figure 2: two baseline intervals (noted ''B1''—from 40 to 20 min prior to sonication, and ''B2''—from 20 min presonication to the onset of sonication), a sonication interval (noted ''FUS''), and five

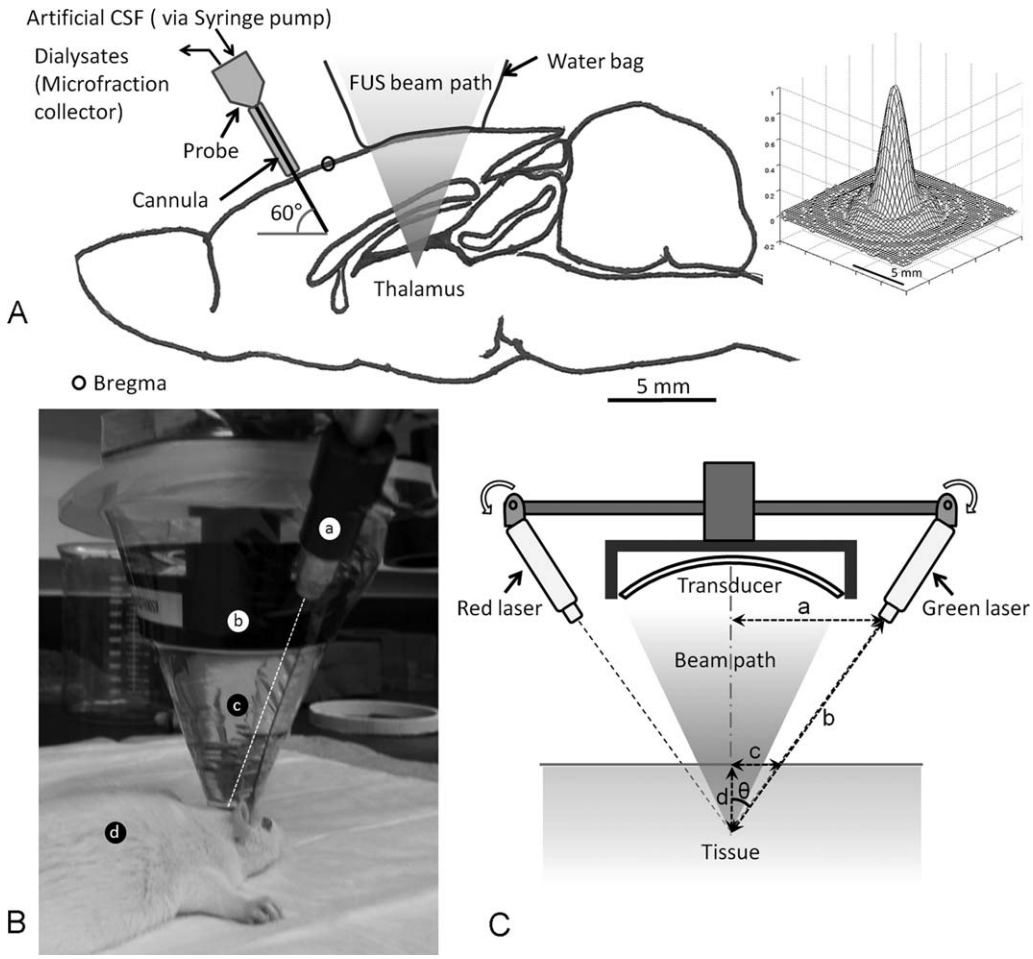

Figure 1. (A) Schematic diagram of the sonication path and the location of the microdialysis probe in relation to the rat brain anatomy. The inset indicates the mesh profile of acoustic pressure (normalized to the maximum pressure at the focus) perpendicular to the incident sonication beam. (B) A photo of the experimental setup: (a) laser device, (b) transducer, (c) water bag, and (d) rat. (C) Trigonometric diagram of the estimation of the ultrasound focus: $c = d \times \tan(\arcsin(a/b))$, whereby a = half of the distance between the laser pointer; b = the distance between the focus and the laser pointer; c = half of the distance between the laser spots on the tissue surface; d = depth of the focus from the tissue surface (measured values: $a = 6.5$ cm; $b = 11$ cm; thus $\theta \approx 36.2^{\circ}$).

postsonication intervals (noted ''P1'' through ''P5''). Based on our preliminary experiments, some of the animals manifested a degree of head movement after long anesthesia (>120 min postsonication). Such movement has shown to deteriorate the data quality, and thus, limited the observation period to 120 min postsonication in this study. The sampled microdialysates were subsequently frozen at -80°C for further analysis.

C. Focused Ultrasound Sonication Setup. An air-backed, spherical segment ultrasound transducer (6 cm in diameter; 7 cm in radius-of-curvature), operating at a fundamental frequency of 650 kHz was used in this study. This frequency is within the frequency range of 440–700 kHz which has an optimal transmission gain through the ex vivo human skull (White, 2006; White et al., 2006). The transducer was driven by electrical sinusoidal waveforms that were produced by a function generator (Agilent, Santa Clara, CA) and amplified by a linear power amplifier (403LA, ENI, Rochester, NY). Prior to the experiment, the relationship between the amplitude of the electrical signal and the corresponding acoustic power of the transducer was measured by a calibrated needle hydrophone (HNR500, ONDA, Sunnyvale, CA). The hydrophone was mounted to a high-resolution three-axis robotic stage (BiSlides, Velmex, Bloomfield, NY) to map the received power characteristics of the acoustic fields in 3D space. The acoustic focus was roughly cigar-shaped and measured 3.5 mm in diameter and 6.2 mm in length at the full-width-at-half maximum of the acoustic pressure field (cf. Fig. 1A, inset). It is notable that the closest distance between the probe and the sonication focus was approximately 5 mm (cf. Fig. 1A), which was sufficiently far away to each other so that the acoustic pressure virtually fell under the detectable range (see the normalized pressure profile of the acoustic pressure field at the focus, Fig. 1A inset).

The sonication parameters were regulated by software that alters the output pattern of the electrical signals from the function generator (Sonomo, SensMed, Framingham, MA). Based on our previous investigation regarding the suppressive effects of sonication (Yoo et al., 2008, 2009), the following sonication parameters were used in this study: 0.5 ms for the tone burst duration (TBD) and 100 Hz for the pulse repetition frequency (PRF). The sonication lasted for 20 min. Sonications given at a longer TBD and higher acoustic energy may generate sustained increases in neural cell excitability (potentially inducing seizures), and thus were not used in this study.

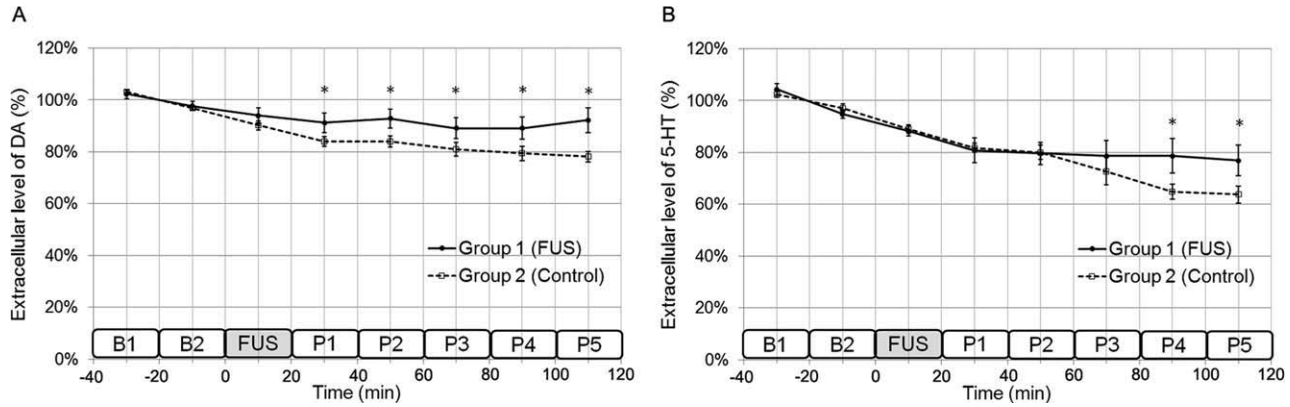

Figure 2. Comparison of the extracellular levels (percentage-wise) of (A) DA and (B) 5-HT between the FUS-treated and untreated groups. The values which were collected during a 20-min period are represented at the center of each time interval. The solid line indicates Group 1 (the FUS-treated group), and the dotted line indicates Group 2 (the untreated group). *X*-axis indicates time course; time zero is the onset of the 20-min sonication interval. The time intervals corresponding to each analyzing period are as follows: −40 to −20 min (B1), −20 to 0 min (B2), 0 to 20 min (FUS), 20 to 40 min (P1), 40 to 60 min (P2), 60 to 80 min (P3), 80 to 100 min (P4), and 100 to 120 min (P5). A gray box represents the sonication period. Error bars indicate standard errors of the mean, and asterisks indicate statistically significant differences ($p<0.05$).

For the calculation of acoustic intensity, the pressure amplitude was estimated after taking into account ultrasound attenuation through the rodent skull in situ (~87% of incoming sonication pressure; Kinoshita et al., 2006). I_{spta} was calculated by a pulse intensity integral (PII) at the PRF while taking duty factors into consideration. The duty factor was computed by the fraction of sonication duration with respect to the continuous sonication. PII was estimated from the integral of the square of instantaneous pressure divided by the characteristic acoustic impedance (NEMA, 2004). The mechanical index (MI) of given sonication, an index of the first-order limit for the safety of ultrasound devices, was derived from the maximum peak negative pressure ($^{Max}P_n$) of an ultrasound longitudinal wave propagating in a uniform medium divided by the square root of its center frequency.

The FUS sonication apparatus (Sonomo, SensMed) is displayed in Figure 1B. The animal was placed in a prone position under the sonication system. The ultrasound transducer faced downward, and the FUS beam was directed through a plastic bag containing degassed water over the scalp of the animal. The direction of the sonication was important as it was difficult to affix a microdialysis probe and associated tubing onto the rat that is lying supine. The coordinates of the sonication focus were positioned well within the tip of the cone-shaped plastic bag.

To align the FUS focus into the targeted region-of-interest, a visual guidance system (Sonomo, SensMed) was used to estimate the spatial location of the focus. As shown in Figures 1B and 1C, two sets of low power (<5 mW) laser beam pointers (generating red and green laser spots with 1 mm diameters) were mounted to the transducer holder in opposite directions equidistant from the center of the sonication path. Assuming that the target surface is relatively flat and the sonication path is applied perpendicular to the surface, the user positions the FUS transducer with the aid of the laser pointers for visual guidance, which can then be used to approximate the location and depth of the sonication focus according to simple trigonometric relationships (Fig. 1C). That is, the depth of the ultrasound focus (noted ''d'' in Fig. 1C), which was targeted to the thalamus (about 5 mm below the surface, along the midline, and 7 mm anterior to the lambda), was adjusted by positioning the transducer to have a distance of ~7 mm between the two laser spots on the rat skull (corresponding to 5 mm × tan 36.2°). The middle coordinate between the two spots was directed 7 mm anterior to the lambda along the midline. After targeting the focus, the lasers were turned off for the rest of the experiment. The size of the focus was large enough to sonicate a significant portion of the thalamus bilaterally. To secure consistent experimental conditions, both the experimental and control animal groups underwent identical experimental procedures and timing, including the application of the FUS setup over the scalp.

D. Dopamine and Serotonin Determination in Microdialysis Samples. Microdialysates (20 μl) were collected in Eppendorf microcentrifuge tubes which contained 5 μl of protective solution comprising 0.1 M acetic acid, 1.0 mM oxalic acid, and 3.0 mM L-cysteine(Kankaanpaa et al., 2001) to prevent oxidative degradation of the monoamines in alkaline solution. The changes in local concentrations of these neurotransmitters were analyzed with high-performance liquid chromatography (HPLC). The HPLC system used in this analysis consisted of an ESA 542 autosampler (ESA, a Dionex Company, Chelmsford, MA), an ESA 582 dual-piston pump, a Capcell PAK MG C18 column (50 × 1.5 mm^2, 3 μm particles, Shiseido, Tokyo, Japan), an ESA Coulochem III detector with a 5020 guard cell and a 5041 amperometric analytical cell with a glassy carbon target electrode and a 25 μm gasket. The guard cell was set at a potential of 275 mV, and the 5041 analytical cell was set to 220 mV. Data were acquired from the 5041 analytical cell and analyzed with EZChrom Elite software configured for the Coulochem III system by ESA. The mobile phase contained 150 mM sodium dihydrogen phosphate monohydrate and 4.76 mM citric acid monohydrate, adjusted to a pH of 5.6 with concentrated semiconductor grade sodium hydroxide before adding 3 mM sodium dodecyl sulfate, 50 μM ethylenediaminetetraacetic acid (EDTA), 10% methanol (v/v), and 15% acetonitrile (v/v) in ''polished'' water [prepared by filtering NANOpure water (Barnstead, Dubuque, IA) first through a C_{18} cartridge (Sep-Pak, Waters, Milford, MA) then through a 0.2 μm nylon membrane (Millipore, Burlington, MA)]. The mobile phase was pumped at a flow rate of 0.2 ml/min, and the column was kept at 29°C. Calibration was done using external standards prepared in solutions of the above preservative. Concentration/area calibrations were linear over the

concentration ranges of DA and 5-HT found in the microdialysates, with $r^2 > 0.999$. For statistical analyses, an independent two-sample *t*-test (one-tailed) was performed based on the percentage value of the extracellular level of DA and 5-HT. The percentage was computed on the basis of the average across two baseline levels of these neurotransmitters. Due to the spurious measurement readings and inadvertent hardware problem, a small fraction of data ($n = 4$) was omitted during data analysis. To account for the omitted data, a method of pairwise deletion (El-Masri and Fox-Wasylyshyn, 2005) was applied.

III. RESULTS

The acoustic intensity at the focus was 175 mW/cm^2 (I_{spta}). This intensity value corresponded to 3.5 W/cm^2 in terms of spatial peak pulse-average intensity (I_{sppa}). The $^{Max}P_n$ at this parameter was measured to be 0.35 MPa, with a corresponding MI of 0.2. One animal in each group died during the experiment. The animals exhibiting spurious fluctuations of extracellular DA and 5-HT levels exceeding three standard deviations from their baseline intervals were excluded from further analysis (DA—two animals in each group; 5-HT—two animals in Group 1 and one animal in Group 2). The temperature of the animals was maintained between 37 and 38°C throughout the experiment. The average recovery rate of DA was 17.7 ± 0.6% ($n = 17$). Direct sonication on the microdialysis probe yielded recovery rates of 19.9 ± 0.7%, which can introduce significant confounds to the sampled level of dialysates (thus justifying the avoidance of sampling from the sonication pathway). The individual levels of the sampled neurotransmitters were normalized to the baseline level for each rat and are represented as percentage change throughout the experiments. The averaged absolute baseline levels of DA were 657.8 ± 49.2 pg/ml for Group 1 and 934.4 ± 124.9 pg/ml for Group 2. Those of 5-HT were 44.8 ± 3.7 pg/ml for Group 1 and 46.9 ± 11.6 pg/ml for Group 2.

A. Measurement of Extracellular Level of Dopamine. During the baseline and sonication periods, there were no significant differences between the two groups in the extracellular levels of DA (B1: $t(9) = -0.56$, n.s.; B2: $t(9) = 0.56$, n.s.; FUS: $t(11) = 1.12$, n.s.). However, after the sonication, we observed significantly increased levels of extracellular DA in the FUS-treated group as compared with those in the control group (cf. Fig. 2A; P1: $t(11) = 1.85$, $p < 0.05$; P2: $t(12) = 2.22$, $p < 0.05$; P3: $t(13) = 1.80$, $p < 0.05$; P4: $t(13) = 2.04$, $p < 0.05$; P5: $t(11) = 2.89$, $p < 0.01$).

B. Measurement of Extracellular Level of Serotonin. Before the period of P4, there were no significant differences between the two groups in the extracellular levels of serotonin (B1: $t(10) = -0.74$, n.s.; B2: $t(14) = 1.17$, n.s.; FUS: $t(15) = 0.30$, n.s.; P1: $t(11) = 0.22$, n.s.; P2: $t(14) = 0.10$, n.s.; P3: $t(14) = -0.78$, n.s.). As shown in Figure 2B, we observed significantly increased levels of extracellular 5-HT in the FUS-treated group when they were compared to those in the control group beginning at approximately 80 min after sonication (P4: $t(11) = -2.10$, $p < 0.05$; P5: $t(13) = -2.17$, $p < 0.05$).

IV. DISCUSSION

Our findings provide compelling evidence that FUS sonication noninvasively modulates the extracellular level of specific neurotransmitters. The sonication increased the extracellular levels of DA and 5-HT in the FUS-treated group as compared with those in the unsonicated group. We also found the extracellular level of DA decreased to about 80% of its baseline level while 5-HT decreased to approximately 60% of its baseline level in the control animals which did not receive any sonication (cf., Fig. 2). The observed time-dependent extracellular decrease of these neurotransmitters in the control group was in support of previous observations involving urethane-anesthetized rats (Men and Matsui, 1994; Keck et al., 2002) whereby the administration of urethane is known to suppress striatal neuronal activity and basal DA concentrations moderately (Warenycia and McKenzie, 1988; Hamilton et al., 1992; West, 1998) as well as serotonergic neurotransmission (Dringenberg and Vanderwolf, 1995). It is also notable that the direct sonication to the microdialysis probe resulted in a marked increase in the in vitro recovery rate, which potentially confounds the reliability of the level of dialysis. This is likely due to the effects of mechanical agitation on the probe permeability, and therefore, the dialysates of DA and 5-HT were not sampled directly from the sonication path in the present study. In addition, the sufficient distance between the probe and sonication path (~5 mm) did not affect the permeability of the probe's membrane to a considerable extent in the present study. We found the validity of our approach from our recent observation which indicated the extracellular levels of gamma-aminobutyric acid (GABA) decreased under the same experimental condition (unpublished data). If sonication had increased permeability of the probe's membrane, the extracellular levels of GABA would have concurrently increased. Therefore, it is highly unlikely that the current experimental configuration introduced the confounders during the measurement of the neurotransmitters.

Although little is known about the detailed mechanism underlying FUS-mediated neuromodulation, the increased levels of extracellular concentrations of DA and 5-HT can be generally explained by independent or combinatory ramifications from (a) increased rates of synthesis or release of these neurotransmitters, (b) their deficient removal from the synaptic cleft, and (c) their slow degradation during the period of dialysate sampling.

A. FUS-Mediated Increased Level of Extracellular Dopamine. The thalamus is a critical component in DA modulation of the frontal–cortical/basal ganglia/thalamic circuitry that mediates motivational and emotional processes, as well as planning and cognition related to development and expression of goal-directed behaviors (Haber and Calzavara, 2009). In rodents and primates, the medial intralaminar nuclei of the thalamus have dense reciprocal projections with frontal, limbic, and striatal regions (Groenewegen and Berendse, 1994). A large number of these projections are inhibitory, use GABA as a neurotransmitter, and have a high density of DA receptors (Jones, 1998; Haber and Calzavara, 2009). Thus, thalamo–cortico–striatal circuits modulated by DA are under inhibitory control, and suppression of direct reciprocal cortico–striatal projections is likely to have disinhibitory effects on DA availability.

Although the exocytosis of synaptic vesicles is stimulated by ultrasound (Tyler et al., 2008) and synaptic contacts can be disrupted by ultrasound (Borrelli et al., 1981), a direct impact of FUS on the frontal DA neurotransmission does not seem to account for our observations because of the large distance between the sonication target (thalamus) and the dialysate-sampling site (frontal lobe). Instead, we hypothesize that FUS sonication of the thalamus disrupts the normal functioning in GABAergic inhibitory networks of thalamo–cortical–striatal circuitry, which regulates DA neurotransmission in the frontal brain region (Jones et al., 1988). FUS-

mediated deficiency in thalamic inhibitory control may result in disinhibition of frontal DA neurotransmission, leading to an increase of extracellular DA. In further support of this hypothesis, it has been reported that a reduction of GABA-mediated inhibition in the thalamic mediodorsal nucleus leads to an increase in prefrontal DA utilization (Jones et al., 1988). Such a thalamic inhibition hypothesis is in accordance with our previous observations that FUS sonication on the thalamus with the current parameters induced suppressive effects on epileptic electroencephalogram (EEG) activity (Min et al., 2011).

B. FUS-Mediated Increased Level of Extracellular Serotonin. As compared with the rapid effects of FUS sonication on DA (cf., Fig. 2A), a significant increase of extracellular 5-HT was observed approximately 80 min after sonication (cf. Fig. 2B). Unlike the presence of direct dopaminergic modulatory influence on the thalamo–cortico–striatal circuitry, 5-HT availability is influenced through longer projections from midbrain nuclei (e.g., raphe nucleus) and feedback onto the raphe nucleus from the dorsal thalamus (Azmitia and Gannon, 1986; McCormick, 1992; Scheibel, 1997; Hornung, 2003). GABAergic projections to the midbrain and serotonin-releasing nuclei serve as an inhibitory reciprocal feedback system that terminates in the forebrain (Yoshida et al., 1984; McCormick, 1992; Scheibel, 1997; Hornung, 2003). It has been proposed that fibers projecting from the reticular nucleus of the thalamus function largely as ''gatelets,'' acting as a gating mechanism for underlying sensory relays and mesencephalic sites (Herkenham, 1986; Sherman and Guillery, 2001; Min, 2010). Thus, interruption of reciprocal connections between the thalamic reticular nucleus and the dorsal raphe nucleus leads to decreased inhibitory gating of midbrain projections to the forebrain and increased forebrain modulation by 5-HT (Azmitia and Gannon, 1986; Scheibel, 1997). Given the lack of direct 5-HT thalamo–cortical projections and inhibitory gating mechanisms of 5-HT projections, we conjecture that a prolonged signal transduction cascade or a remote modulatory effect might be induced by FUS sonication of the thalamus on 5-HT neurotransmission.

Neurotransmission is inactivated by the removal of a specific neurotransmitter from the synaptic cleft, and this removal process involves particular reuptake mechanisms into the presynaptic terminal and specific degradation mechanisms around synaptic clefts. It is one of few presumable explanations for our observations that impaired function of serotonin transporter (SERT), possibly by FUS sonication, may allow 5-HT to remain in the extracellular space for longer periods of time. It is noteworthy that the activity of SERT is modulated by the transmembrane gradient of Na^+ and K^+ (Halbach and Dermietzel, 2006b), and the alterations in transmembrane ion concentrations are induced possibly by FUS sonication. Although the physiological mechanisms caused by FUS sonication are poorly understood, the main effect of FUS is due to a mechanical force that could produce alterations in membrane potential (Gavrilov et al., 1996). It has been consistently reported that ultrasound sonication activates voltage-gated Na^+ and Ca^{2+} channels (Tyler et al., 2008) and that a mechanical force can activate several mechano-sensitive ion channels (Morris and Liske, 1989; Chakfe and Bourque, 2000; Nagasawa et al., 2001), subsequently allowing cation entry (Morris, 1990). Taken together, FUS-mediated mechanical force may temporally modulate the activity of SERT by means of dynamic changes in transmembrane gradients of Na^+ and K^+, thus resulting in the change in the extracellular level of 5-HT. Subsequently, the excess of extracellular 5-HT in the sonicated area may gradually diffuse to the frontal cortex where the microdialysis probe was inserted. This supposition is one possible mechanism to explain the delayed increases of 5-HT availability in frontal cortex post sonication (cf., Fig. 2B).

To validate these hypotheses and to elucidate the detailed mechanism of FUS on the regional modulation of neurotransmitters, further studies are necessary to quantify the activity of neurotransmitter transporters or the extracellular level of their metabolites in relation to sonication; for example, positron emission tomography (PET) could be used with [^{11}C]methyl-L-tryptophan as a radioactive ligand (Diksic et al., 1991; Diksic, 1992) or [^{3}H]5-HT as a selective tracer (Kimelberg, 1986; Anderson et al., 1992). In addition, FUS-mediated neuromodulation of other neurotransmitters such as glutamate, GABA, or acetylcholine could be evaluated using the same techniques. This will show whether or not FUS sonication influences neurotransmission mechanisms differently based on the type of neurotransmitters involved.

The ability to modulate region-specific brain activity, along with the present evidence of modulation of neurotransmission in the brain, demonstrates the potential utility of FUS as a completely new functional mapping technique. So far, there has been no known noninvasive technology to modulate regional brain activity and associated neurotransmitter activity deep inside of the brain. As FUS is noninvasive and has the ability to target spatially specific small brain structures, the FUS can be used not only to probe excitatory function but also to evaluate neuroendocrine function that is associated with deep and small brain regions (e.g., the response to stimulation or inhibition of the deep brain nuclei). It is also important to note that the FUS approach can be used with other imaging modalities, such as magnetic resonance imaging (MRI) or PET, to complement existing anatomical/functional neuroimaging methods.

The adaptation of the presented FUS technique to a transcranial application is feasible as the frequency used in this study is within the range of optimal transcranial transmission, that is, 440–700 kHz (White, 2006; White et al., 2006). The presented method is also applicable for a single transducer-based system whereby the insonication can be achieved through a portion of the thin temporal bone that is typically located superior to the ear canal. This thin part of the temporal bone in the skull is referred to as a ''sonication window,'' and has been used as one of the typical insonication routes for transcranial Doppler examinations. Transcranial FUS systems, based on arrays of multiple ultrasound transducers, are now commercially available for human applications, with recent applications in thermal ablations of brain tumors (McDannold et al., 2010) as well as in functional neurosurgery (Martin et al., 2009).

In terms of the biological safety of the method, the $^{\mathrm{Max}}P_n$ of the this study (0.35 MPa) is highly unlikely to cause damage to the brain tissue. Cavitation-related brain tissue damage, in the absence of air bubbles, is rare at pressures less than 40 MPa (Dalecki, 2004). The MI of the present study was 0.2, which is also sufficiently within the range of safety guidelines [i.e., 1.9 for all applications except ophthalmic (maximum 0.23); FDA, 2008]. I_{spta} of this study (175 mW/cm^2) is also far less than the upper regulatory limit for nonobstetric ultrasound imaging (720 mW/cm^2; AIUM Clinical Standards Committee, 2004). Although further studies are needed to establish the safety profiles, FUS holds promise as an elegant noninvasive functional mapping technique.

Due to its functional neuromodulatory role, FUS can be applied to fields of neurotherapeutics for the treatment of various neurological and psychiatric diseases. For example, Parkinson's disease is mainly attributed to a loss of DA-containing neurons within the

nigrostriatal DA system (Graybiel et al., 1990; Biju and de la Fuente-Fernandez, 2009). Therefore, we believe that a FUS-mediated increase of DA in the nigrostriatal region can potentially ameliorate the symptoms of Parkinson's disease. In addition, as there is a gradual decline in dopaminergic neurons of the substantia nigra pars compacta during aging, which is accompanied by a reduction of striatal DA levels (Gerhardt and Maloney, 1999; Colebrooke et al., 2006), a FUS-induced increase of DA in such spatially localized brain areas can contribute to alleviation of an aging-related DA reduction. As decreases in DA availability in the forebrain have been shown to lead to motivational and memory-related deficits (Koob, 1996; Robbins and Everitt, 1996; Jay, 2003; Phillips et al., 2008), depressive symptomology involving attention-related bias, avoidance, and decreased approach-related behaviors may benefit from FUS sonication. As for 5-HT, a FUS-mediated modification of 5-HT will provide a potent therapeutic tool to correct aberrant serotonergic neurotransmissions that have been implicated in the pathophysiology of depression, post-traumatic stress disorder, and obsessive–compulsive disorders (OCDs) (Moller et al., 1983; Davis et al., 1997; Kulz et al., 2007). For example, FUS sonication to the frontal cortex of the brain can potentially substitute/complement the traditional pharmacological regimen such as the administration of serotonin reuptake inhibitors (SSRIs) for psychiatric disorders (e.g., depression and OCD).

In spite of these potential advantages in application of FUS sonication, FUS sonication parameters should be finely explored by further studies as regulation of DA and 5-HT beyond the therapeutic efficacy can potentially lead to abnormal neurological/psychiatric symptoms. Indeed, dopaminergic hyperactivity has been observed to be linked to motor abnormalities (Carlsson, 1988; Koob and Swerdlow, 1988), memory deficits (Vago and Kesner, 2008), and several psychotic features, such as hallucinations and manic states (Seeman et al., 2005; Halbach and Dermietzel, 2006a). Similarly, 5-HT-releasing agents are able to induce mood-related disturbances (Wrase et al., 2006; Nemeroff and Owens, 2009) and migraine-like headache (Unge et al., 1983; Johnson et al., 1998).

We observed that the FUS-mediated extracellular levels of DA and 5-HT did not return to those observed from the control group (cf., Fig. 2). Although a systematic investigation of the temporal dynamics associated with sonication has not yet been conducted, it is assumed that recovery to baseline is likely to be observed after a longer time interval, postsonication. The observed increases are possibly due to the over-regulation of these neurotransmitters by a relatively long duration of sonication (e.g., 20 min), which can be rectified with shorter sonication duration. Further systematic refinement of the sonication parameters such as sonication duration and subsequent exploration of selective functional therapeutic efficacy will be needed to disseminate the technique across a wide range of research and clinical fields.

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