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Stimulation of Hippocampal Neurogenesis by Transcranial Focused Ultrasound and Microbubbles in Adult Mice

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ABSTRACT

Transcranial focused ultrasound (FUS) and microbubble contrast agent, applied at parameters known to transiently increase blood-brain barrier permeability, were tested for the potential to stimulate hippocampal neurogenesis. In adult mice, FUS treatment significantly increased the number of proliferating cells and newborn neurons in the dentate gyrus of the dorsal hippocampus. This provides evidence that FUS with microbubbles can stimulate hippocampal neurogenesis, a process involved in learning and memory and affected in neurological disorders, such as Alzheimer's disease.

Introduction

Transcranial MRI-guided focused ultrasound (FUS) can be utilized for non-invasive brain-targeted therapies. For example, high-intensity FUS is being investigated in clinical trials for thermal ablation in essential tremor [1,2]. In non-thermal applications, FUS at low intensities with microbubble contrast agents can be used to increase blood-brain barrier (BBB) permeability and deliver intravenous therapeutics to the brain [3–11].

FUS at low intensities without microbubble contrast agents has demonstrated neuromodulatory properties [12–17] and the potential to increase growth factors [18,19], including brain-derived neurotrophic factor (BDNF) [18], which are known to promote neurogenesis [20–22]. We therefore hypothesized that FUS and microbubbles, applied at parameters typical for increasing BBB permeability, can stimulate neurogenesis [7–9,23].

Adult neurogenesis is a process involving the generation, development and integration of new neurons in the brain. Neurogenesis occurring in the dentate gyrus (DG) of the dorsal hippocampus contributes to learning and memory [24] and can be impaired in neurological conditions, such as Alzheimer's disease [25].

Methods and materials

Animals

Adult C57Bl/6/C3H mice (136–137 days) were given food and water *ad libitum*. Experiments were approved by the Animal Care Committee at Sunnybrook Research Institute and performed in compliance with the Canadian Council on Animal Care and the Animals for Research Act.

MRI-guided FUS treatment

Following anesthesia, mice heads were depilated and tails were fitted with a catheter. Mice were secured in a supine position to

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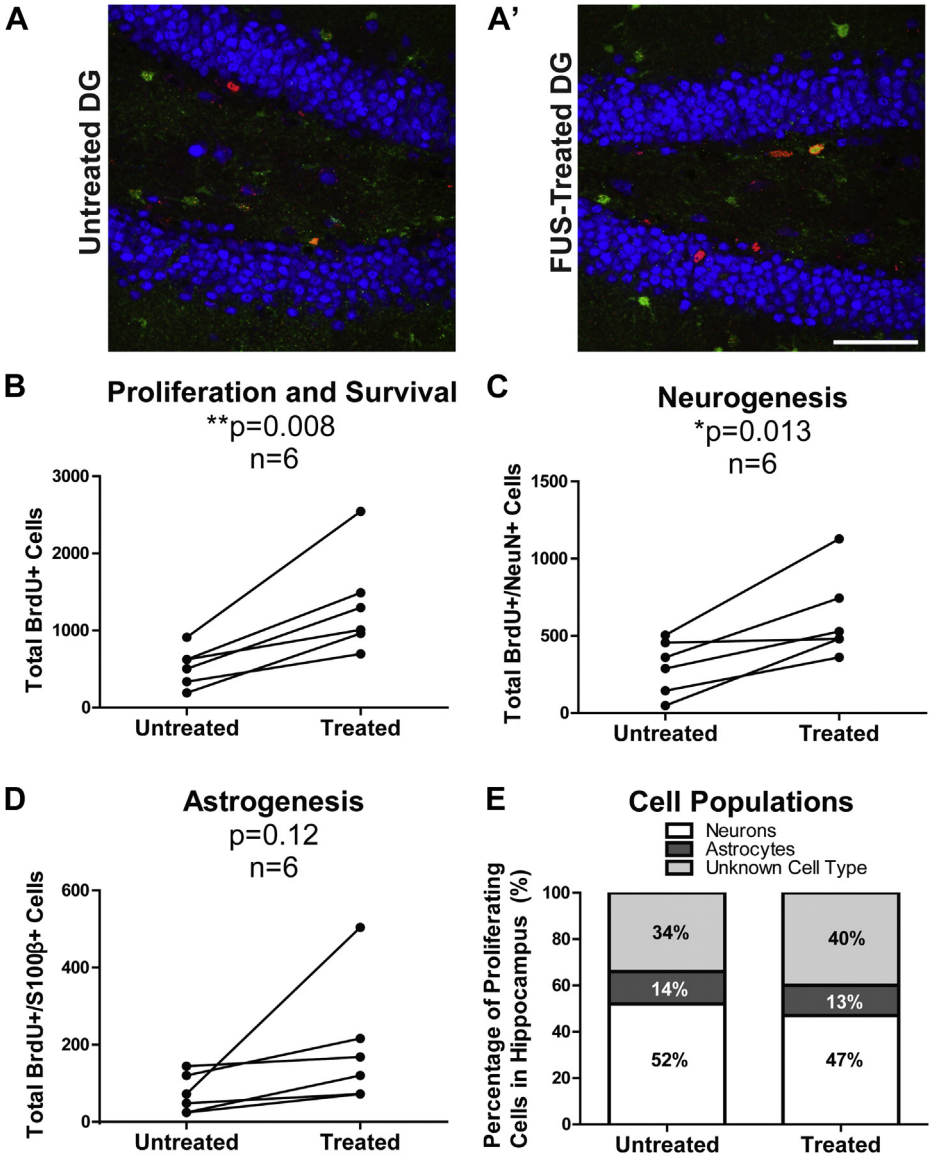

Figure 1. Hippocampal cell proliferation and survival, neurogenesis and astrogenesis were analyzed in adult mice 18 days following MRI-guided focused ultrasound (FUS) treatment. Confocal *z*-stacks imaging the subgranular zone and granular cell layer of the dentate gyrus (DG) in the dorsal hippocampus were acquired for both untreated (A) and treated hemispheres (A′) and utilized for analysis. Proliferating cells are labeled with BrdU (red), mature neurons with NeuN (blue) and astrocytes with S100β (green). From the acquired series of images, the total number of BrdU-positive cells were counted, extrapolated for counts in the dorsal hippocampus and compared between untreated and treated hemispheres (B). Co-localization with the mature neuronal marker NeuN (C) and the astrocyte marker S100β (D) were determined, indicative of neurogenesis and astrogenesis, respectively. Overall, the proportion of cell populations in the untreated and treated hemispheres (E) was examined to elucidate FUS effects on the dorsal hippocampus as a whole. Significant differences were defined as $^{*}P < 0.05$ and $^{**}P < 0.01$ ($n = 6$). Scale bar: 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

acquire T1- and T2-weighted scans in a 7.0-T MRI (Bruker). FUS was generated using a custom-manufactured transducer (75 mm diameter, 60 mm radius of curvature) operating at 1.68 MHz, equipped with a wideband receiver [26]. Signals recorded by the receiver were used with an acoustic emissions-based controller program to ensure safe exposures [27]. FUS was delivered in 10 ms bursts at 1 Hz pulse repetition frequency for 120 s, generating average peak pressures of 0.96 ± 0.30 MPa. FUS was targeted to one hippocampus by two foci (0.73 mm lateral beam width, 4.5 mm axial beam width) approximately 1 to 1.5 mm apart, using a 3-axis positioning system similar to [28] to move the transducer accordingly. The contralateral hippocampus remained untreated. At sonication start, mice received 20–40 μL/kg Definity microbubbles (Lantheus) to induce BBB disruption and 200 μL/kg Omniscan (GE) to visualize treated regions with T1-weighted scans. Upon anaesthesia recovery, mice were given 0.05 mg/kg buprenorphine subcutaneously.

Starting 24 h post-treatment until day 4, mice received 50 mg/kg 5-bromo-2′-deoxyuridine (BrdU) intraperitoneally once daily to label cell proliferation.

Immunohistochemistry and analysis

On day 18, mice were deeply anaesthetized and perfused with 0.9% saline and 4% paraformaldehyde. Brains were harvested, post-fixed for 24 h, transferred to 30% sucrose and cut in 40 μm coronal sections.

Systematic series of 1 in 24 sections throughout the hippocampus (1.06–4.04 mm posterior to bregma) were immunostained.