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Direct activation of cortical neurons in the primary somatosensory cortex of the rat *in vivo* using focused ultrasound

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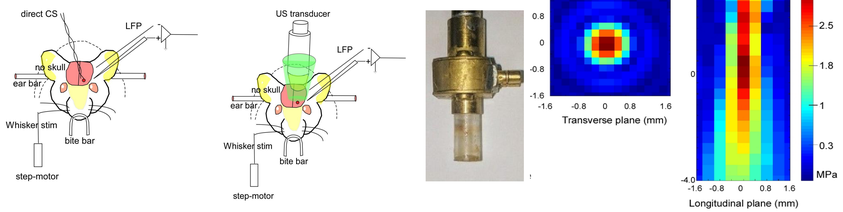
Introduction

There is some controversy as to whether focused ultrasound (US) can directly activate cortical neurons. Recent publications have cautioned that US may not activate these neurons directly, but only indirectly through activation of peripheral neurons (Guo et al 2018; Sato et al 2018).
We addressed this controversy by examining the local field potential (LFP) evoked by focused US applied to the C2 projection area of the neural barrel field against the whisker-activated LFP and direct cortical response (DCR) produced by electrical stimulation of the projection area.

Methods

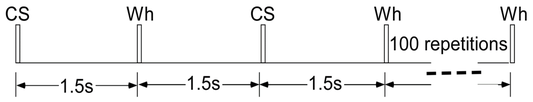
Preparation: Sprague-Dawley 250-300 g was anesthetized with ketamine (80 mg/kg, ip) and xylazine (10 mg/kg ip). Craniotomy was performed to expose the brain with intact dura over the left hemisphere contralateral to the single right whisker (C2) stimulation. The head was stabilized with ear bars in a headholder. The anesthetics in bolus was given as necessary based on breathing pattern and EEG. The LFPs were recorded using a sharp-tip tungsten electrode. Whisker stimulation was done by moving the whisker by a computer-controlled step-motor.

CS-Whisker stim **US-Whisker stim** **Focused US transducer**

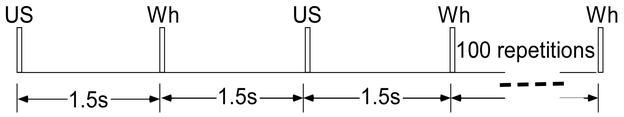


Focused ultrasound stimulation was applied with a transducers developed in house, see picure. A piezoelectric plate generating US at 4 MHz, located within the brass shielding container, is epoxied to a plastic 7mm dia cylinder acting as a sonic waveguide. Attached to the external tip of the guide is a silicon rubber lens focusing the US beam to a with of 0.4 mm and length of 3 mm. The used US peak pressure at the focal point was 3.3 MPa, corresponding to an intensity of 355 W/cm2. The pressure was measure with an Onda needle hydropohone HNC-0200 with a 200 um sensor tip.

Direct cortical stimulation (CS)-Whisker evoked responses.
We stimulated the C2 projection area in the left hemisphere with 1-ms mechanical deflection of the right whisker, alternating with a direct cortical stimulation (CS) (300 µs, 0.4-2 mA)

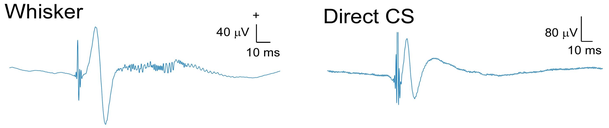


US-Whisker evoked responses.
We stimulated the C2 projection area in the left hemisphere as before. The barrel field centered on the C2 projection area was stimulated with US (4 MHz, 3.3 MPa, each stimulation typically comprises 5-10x0.3 ms pulses spaced 1 ms apart). The two types of stimulation were alternated every 1.5 s for 100 repetitions. The duty cycle for the US excitation using 10 pulses every 3 s is 1/1000.

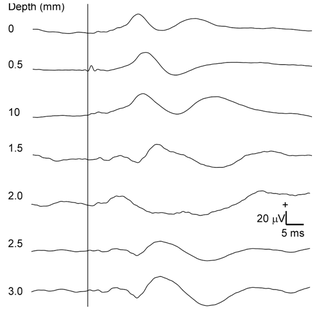


Results

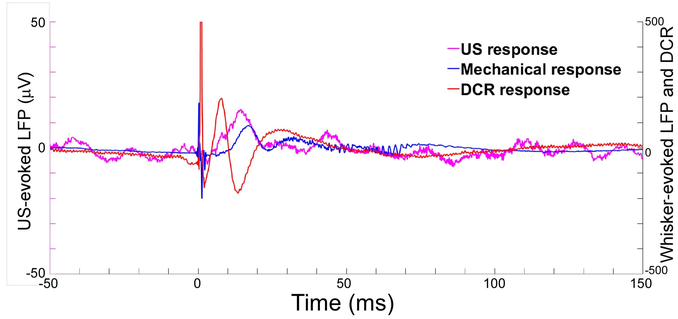
Direct CS-evoked LFP vs Whisker-evoked LFP
Direct cortical stimulation (CS) (300 µs, 0.8 mA) produced an LFP with a onset latency shorter than the whisker-evoked LFP



US-evoked LFPs, Laminar profile.
The laminar depth profile of the US-evoked LFPs were measured along the radial axis of the transducer by placing the electrode every 0.5 mm from the cortical surface. The LFP reversed the polarity at a depth between 1 and 1.5 mm.



US-evoked LFP, whisker-evoked LFP and (DCR), comparison
Onset latency of the US-evoked LFP is intermediate between the DCR and whisker-evoked LFP



Discussion

We have addressed the recent controversy as to whether US can directly activate cortical neurons. We showed that focused US activates cortical neurons in the barrel field of the rat with a laminal profile commensurate with direct stimulation. We also measured the latency of the LFP evoked by US and whisker stimulations using a protocol alternating the stimulations and comparing the evoked LFPs. In the same animals we also compared the DCR vs whisker-evoked LFPs using a similar protocol. This strategy maximized the robustness of analysis. The DCR was evoked with practically no delay as expected since there is no significant conduction or transduction delay. The whisker deflection produced a response with an onset delay of 8-10 ms due to receptor transduction time, conduction time and synaptic delays. The US-evoked responses had latencies of 3-8 ms depending on US parameters, indicating direct activation.
The US pulse trains used are safe for human use according FDA of the USA. The mechanical index was MI=P/(fc)½=1.63, less than the safe value 1.9 for human imaging. Using 10 pulses the value for Ispta is 355 mW/cm2, below the FDA limit of 740 mW/cm2.

Conclusions

Focused US can directly activate cortical neurons in the primary somatosensory cortex of the rat using US amplitudes

References

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