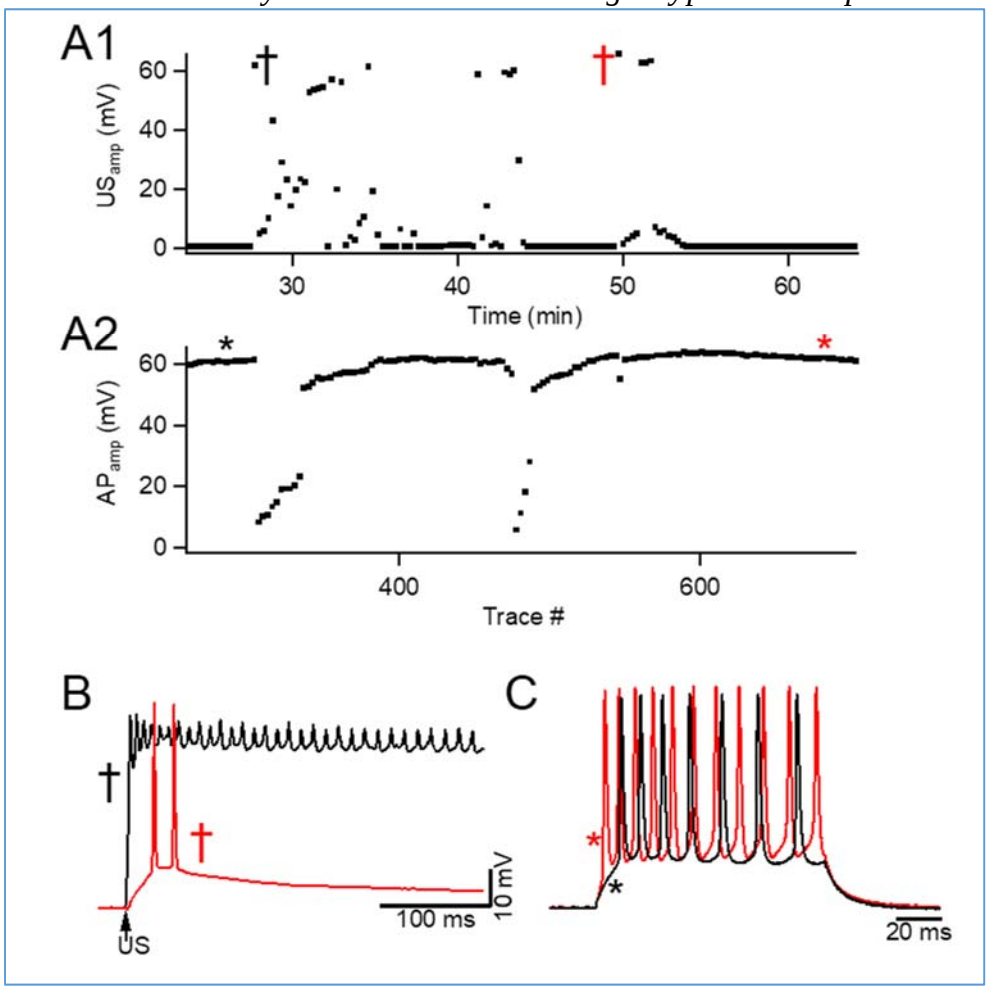
2018-11-28

Supporting data in ENDNOTE for: Focused ultrasound transiently increases membrane conductance in isolated crayfish axon

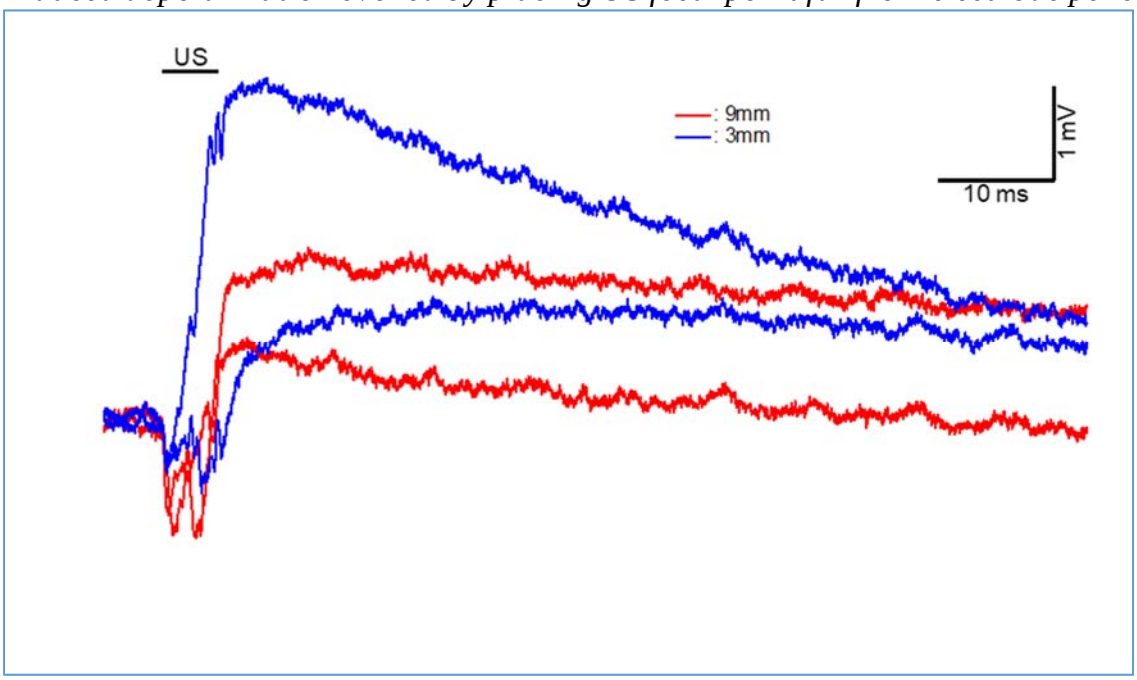
Supporting data:

Membrane excitability remained stable during a typical US experiment.



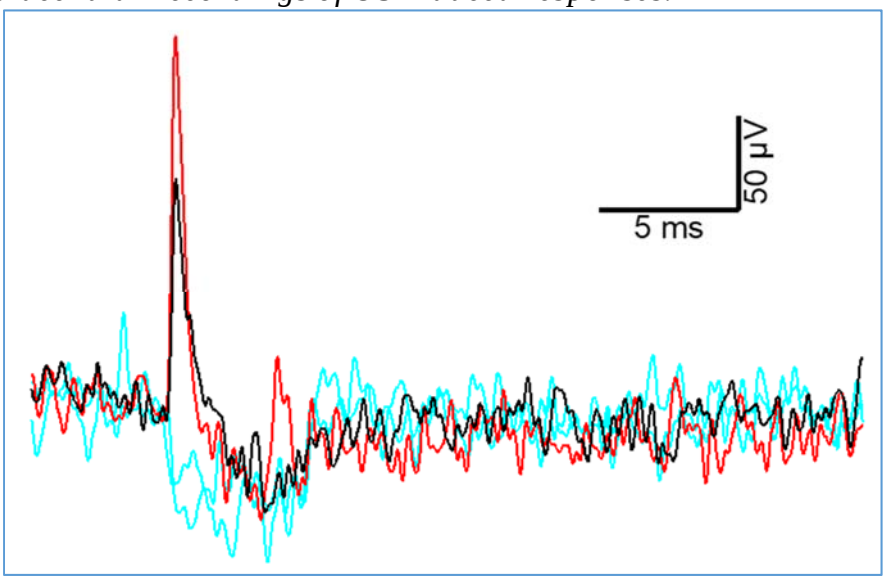
sFig.1: Test of AP stability and axon integrity during US induced responses. (A1) Time-line plot of the amplitudes of US induced depolarizations from an inhibitory axon. The plot also illustrates the stochastic and clustering nature of the responses. The US tone burst, 5 ms at 2.1 MHz and 6.5 mW/cm^2, was delivered at a rate of 0.1 Hz. (A2) Axonal excitability was tested with a suprathreshold current step, inserted between US deliveries. The AP amplitudes measured from resting V_m appeared constant except during the periods with US induced depolarizations and reduced membrane resistance. The X-axis is displayed in terms of trace number but corresponds to the time-line in A. The same current injection amplitude was used during the entire experimental period. (B) Two representative examples of US induced depolarizations. Red and black traces corresponding to the times marked by the daggers of matched colors in A1. The arrow in B indicates the timing of US delivery. (C) Two representative examples of current step induced AP trains, corresponding to the time points "*" in A2. Action potentials were initiated by current injection at the primary branch and recorded at a secondary branching point. There was a slight increase in the number of action potentials over time (red), suggesting a slight increase in R_{in}. There was no channel blocker in this preparation.

US induced depolarization evoked by placing US focal point far from electrode penetration site.



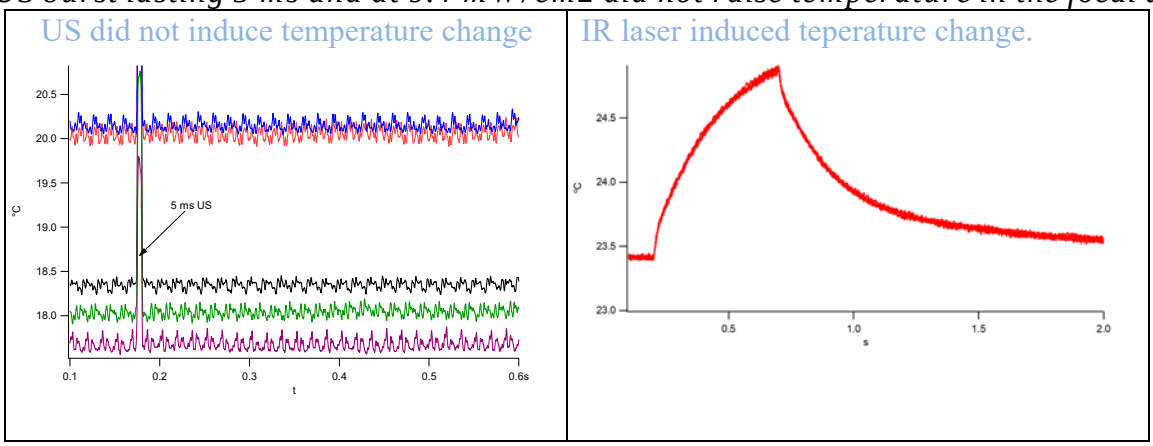
sFig.2: Depolarizations recorded from an axon where US focal points were moved far away from the recording electrode. This experiment was performed to address the possibility that the US induced depolarization had to do with US-microelectrode interaction, rather than due to direct impact of US on axonal membrane. The depolarizations were recorded from the primary branching point. The proximal part of the axon in this preparation had been preserved, ~10mm in length. Depolarizations were recorded when the US transducer was moved horizontally along the length of the axon by 3mm (blue) and 9mm (red), away from the primary branching point. Given the size of the area covered by US focal area, 1.5x1 mm in elliptical major and minor axes, the recording electrode should be completely outside of the focal area after a 9 mm displacement of the US transducer. The presence of the responses under these conditions suggests that the depolarizations are unlikely to be due to US-electrode interaction at the penetration site. Two traces are shown for each location to demonstrate the consistency of evoked responses. US delivery time is indicated with the black bar.

Extracellular recordings of US induced responses.



sFig.3: The purpose of this experiment is to evaluate the possibility that US induced depolarization recorded intracellularly could be due to microelectrode-US interaction mechanically. By using loose-patch extracellular recording, potential damaging effect of US on axonal membrane-sharp_electrode seal could be eliminated. Extracellular recordings, at 5 KHz filtering, did uncover transients coinciding with the onset of US bursts. These events were rare, occurring in 2 out of 55 trials. However, it has been noted in our report that the *dv/dt* maximal values of US-induced depolarization is 30 times smaller than that of action potentials. Since extracellular signal amplitudes are proportional to *dv/dt* of trans-membrane signals, this technique probably only detects the largest US-induced depolarizations (red and black traces represent two examples of US-positive responses, and the blue traces represent US-negative responses.)

US burst lasting 5 ms and at 9.4 mW/cm2 did not raise temperature in the focal area.



sFig.4: We used a 24 gauge thermocouple probe (Physitemp) to evaluate whether typical US bursts used in this report induced temperature change. The manufacturer's specification claimed a 4 ms time constant, which may be optimistic. Nevertheless, the probe should be adequate for evaluating possible mechanisms underlying US-induced depolarization. The issues to be resolved here are: (1) whether there is any US-induced temperature change, (2) if there is a US-induced temperature change, whether this change fluctuates in a way similar to the stochastic occurrences of US-induced depolarization. As a proof of the technology, we first tested the temperature sensitivity and time resolution of the Physitemp probe with an infrared laser (2 mW, 2 μm wavelength and 500 ms in duration). The right panel shows an IR-induced temperature rise, with 5 overlapping traces. In our recording configuration, namely using the same recording dish and saline circulation rate, the temperature rise was invariant from trial to trial for the IR-induced rise. Furthermore, the signal to noise ratio indicated that there should be sub-centigrade resolution. When the same thermocouple probe was placed in the focal area of the US beam, there was no detectable change induced by US (Left panel). (US parameters: 2.1 MHz; 9.4mW/cm^2 and 5 ms duration.) We also changed the bath temperature to verify the sensitivity of the measurements.