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A range of pulses commonly used for human transcranial ultrasound stimulation are clearly audible

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Transcrianial focused ultrasound (TUS) has great potential for use as a non-invasive focal brain stimulation technique. It has been proposed that TUS directly modulates neuronal function and firing [1,2], though the exact mechanism is currently not well understood. Recently, the presence of an auditory confound has been noted when applying TUS in both animal models and humans [3,4]. This prevents participant blinding and causes activation of auditory cortices, confounding the interpretation of electrophysiological or behavioural changes seen with TUS [5–7].

Previous work has attempted to mitigate this auditory artifact. Typical TUS protocols involve delivering the high-frequency ultrasound wave (0.2–0.5MHz) either continuously, or in a lower frequency (0.1–1kHz) pulsing pattern, the rate of which is known as the pulse repetition frequency (PRF). Ramping the stimulation onset and offset over several milliseconds, to remove sudden changes in TUS intensity, has been reported to eliminate auditory activation in mice [3]. Likewise, a concurrent audio mask, applied at the PRF, can reduce the auditory perception of TUS in some human participants [4]. However, it remains unclear whether auditory perception can be fully eliminated in humans and, if so, what are the best methods for achieving this.

Here, we assessed auditory perception of several TUS protocols, and investigated whether detection could be reduced through different combinations of ramping and masking. 16 healthy participants (5 female, mean age 32, range 25–46) with normal hearing, took part in one or more experiments across multiple days. The project was approved by the UCL research ethics committee (Project ID 14071/001).

TUS was delivered using a 2-element spherically focused annular array transducer (H115-2AA, Sonic Concepts) with a nominal outer aperture diameter and radius of curvature of 64mm. The transducer was driven at 270kHz by a 2-channel TPO (Sonic Concepts) with the output power and element phase adjusted to give a focal pressure in water of 700kPa (pulse average intensity of 16 W/cm2) and a focal distance of 43mm. The measured -3dB focal size in water was 5mm (lateral) by 30mm (axial). The average insertion loss of these driving parameters due to the skull was experimentally measured to be −9.8dB [8], giving an approximate focal pressure and pulse average intensity in the brain of 230kPa and 1.7 W/cm2.

The participant was positioned in a chin rest and the transducer, connected to an articulated arm, was manually positioned over the inion and held in place using rubber straps. Acoustic coupling was achieved using a gel coupling pad and ultrasound gel. Real and sham stimulation was delivered randomised and double-blind, and participants were asked to report audition of the ultrasound stimulation on every trial using a mouse click. Results are presented in Fig. 1.

In experiment I, participants ($n = 7$) experienced active stimulation conditions with a 300 ms stimulus, at 50% duty cycle, with either 500Hz (as per [9]) or 250Hz PRF. With the lower frequency PRF, a ramp of 0, 0.5 or 1 ms was applied to the beginning and end of each pulse. The pulse on-time for the ramped conditions was adjusted so that all TUS stimulus conditions had the same time-averaged intensity. Without ramping, all stimulation was clearly detected by all participants. The sound was reported as being tonal in nature, scaling in frequency with the PRF. Adding a ramp of 1 ms prevented perception for some participants, though a few developed the ability to detect this condition through the course of the experiment. Statistical comparison of the 250Hz conditions with 0 ms (Expt I, A2) or 1 ms ramp (Expt I, A4) using paired samples *t*-test found a difference approaching statistical significance ($t(6) = 2.22$, $p = 0.069$). In one participant audition of the 500Hz PRF condition (A1) at lower stimulation intensities was tested: the limit of perception was between 10 and 20% of initial output power.

Experiment II ($n = 7$) also manipulated ramping, this time using a 125Hz PRF and thereby allowing for longer ramp times. Again ramping of 1 ms prevented auditory perception in some participants but no further benefit was gained by increasing the ramp time (paired samples *t*-test of 1 ms ramp (Expt II, A2) and 2 ms ramp (Expt II, A4): $t(6) = 0.281$, $p = 0.788$). If the TUS was still audible with ramping it was reported as substantially quieter, in some cases towards the limit of perception. Some participants reported using non-audible cues (such as perceived vibrations) to identify active conditions.

In mice, long ramps prevent auditory activation [3]. In experiment III ($n = 6$) we thus applied a continuous 150 ms stimulation either with or without a 20 ms ramp. Ramping with continuous stimulation did not impact perception (paired samples *t*-test of Expt III, A1 and Expt III, A2: $t(5)$-0.542, $p = 0.611$). This continuous stimulation, regardless of ramping, produced similar levels of audibility as pulsed simulation with ramps (mixed-effects model comparison of Expt I, A2 and Expt III, A2: $t(5) = -1.07$, $p = 0.309$). Continuous stimulation, if heard, was perceived as high-pitched

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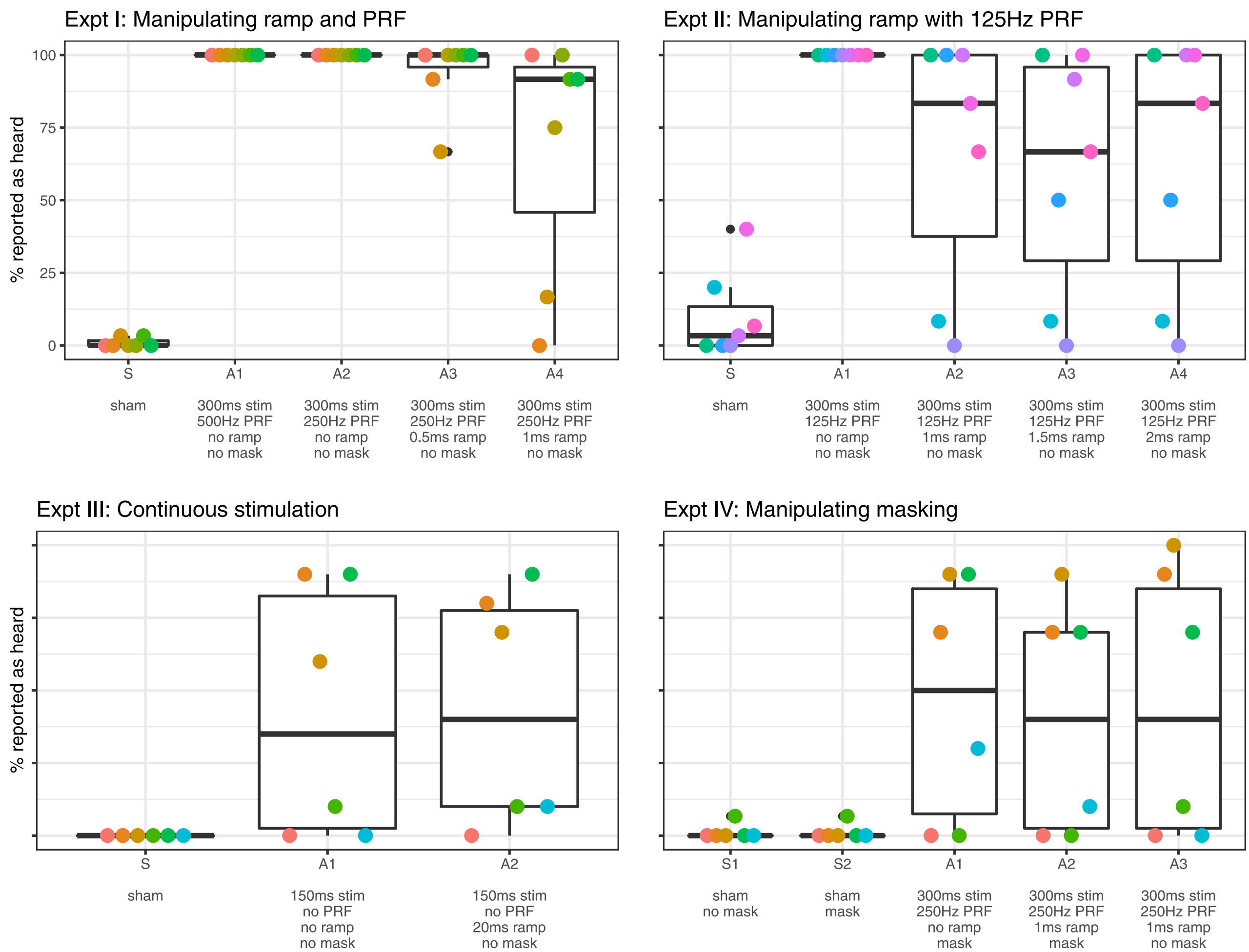

Fig. 1. Percentage of trials reported as being heard by participants across all experiments. Points colour coded by participant identity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

pops or clicks. One participant reported this as very unpleasant. In another participant the limit of perception of the 20 ms ramped condition (A2) was between 5 and 10% of initial output power.

In the final experiment IV ($n = 6$), both active and sham pulses were masked using an intermittent 1 second square wave matching the PRF, synchronised to start approximately 100 ms before the onset of the TUS stimulation. The mask was played over headphones at a fixed level, near the upper limit of tolerability. Masking alone did impact TUS audibility (mixed-effects model comparison of Expt I, A2 and Expt IV, A1: $t(5) = -3.36$, $p = 0.006$), but did not reduce perception over-and-above ramping (paired-samples *t*-test Expt IV, A2 and Expt IV, A3: $t(5) = -0.197$, $p = 0.848$). Many participants reported that the 'sound' of the TUS could be distinguished from the headphone mask due to differences in localisation.

These results show that, without mitigation measures, non-ramped pulse configurations that are commonly used for human TUS experiments (e.g [9]) are likely audible for almost all participants. Here we found that ramping and masking TUS stimulation prevented perception in some participants, but these effects were not additive. Appropriate ramping reduced the unpleasantness and intrusiveness of the TUS 'sound', "turning it from a jackhammer to birdsong" in the words of one participant. Reducing intensity is likely an alternative method for preventing perception, but so far limited studies have found neuronal effects in this power range [10]. While this is by no means an exhaustive study of all TUS parameters, these results should motivate researchers to assess audibility of their own protocols, take measures to improve blinding and control experimentally for auditory confounds, guaranteeing that any measured brain or behavioural effects are due to direct neural modulation by TUS.

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Declaration of competing InterestCOI

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Tyler WJ, Tufail Y, Finsterwald M, Tauchmann ML, Olson EJ, Majestic C. Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. PloS One 2008;3:e3511.
[2] Tufail Y, Matyushov A, Baldwin N, Tauchmann ML, Georges J, Yoshihiro A, et al. Transcranial pulsed ultrasound stimulates intact brain circuits. Neuron 2010;66:681–94.
[3] Mohammadjavadi M, Ye PP, Xia A, Brown J, Popelka G, Pauly KB. Elimination of peripheral auditory pathway activation does not affect motor responses from ultrasound neuromodulation. Brain Stimul 2019;12:901–10.
[4] Braun V, Blackmore J, Cleveland RO, Butler CR. Transcranial ultrasound stimulation in humans is associated with an auditory confound that can be effectively masked. Brain Stimul 2020;13:1527–34.
[5] Park C, Chen M, Kim T. Implication of auditory confounding in interpreting somatosensory and motor responses in low-intensity focused transcranial ultrasound stimulation. J Neurophysiol 2021;125:2356–60. https://doi.org/10.1152/jn.00701.2020.
[6] Guo H, Hamilton II M, Offutt SJ, Gloeckner CD, Li T, Kim Y, et al. Ultrasound produces extensive brain activation via a cochlear pathway. Neuron 2018;98:1020–30.
[7] Sato T, Shapiro MG, Tsao DY. Ultrasonic neuromodulation causes widespread cortical activation via an indirect auditory mechanism. Neuron 2018;98:1031–41.
[8] Gimeno LA, Martin E, Wright O, Treeby BE. Experimental assessment of skull aberration and transmission loss at 270 kHz for focused ultrasound stimulation of the primary visual cortex. In: 2019 IEEE int. Ultrason. Symp. IEEE; 2019. p. 556–9.
[9] Lee W, Kim H-C, Jung Y, Chung YA, Song I-U, Lee J-H, et al. Transcranial focused ultrasound stimulation of human primary visual cortex. Sci Rep 2016;6:1–12.
[10] Yu K, Niu X, Krook-Magnuson E, He B. Intrinsic functional neuron-type selectivity of transcranial focused ultrasound neuromodulation. Nat Commun 2021;12:2519. https://doi.org/10.1038/s41467-021-22743-7.

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