Brief transcranial focused ultrasound stimulation causes lasting modifications to the synaptic circuitry of the hippocampus

William W. Watts[1], Benjamin Clennell[1], James K. Jiang[1], Konoha Izaki-Lee[1], Abhishek Binodh[1], Robyn Cuthell[1], Deniz Tonyali[1], Jon Crompton[1], Rosie Taaffe[1], Alanoud Alqahtani[1], Anna Andrieu[1], Igino Rafael Besinga[1], Kate Heesom[1], Tom G. J. Steward[1], Kwangwook Cho[2], Bruce W. Drinkwater[3], Elek Molnar[4] & Daniel J. Whitcomb[1]

[1]Bristol Medical School, Faculty of Health Sciences, University of Bristol, Bristol, UK; [2]Faculty of Engineering, University of Bristol, Bristol, UK; [2]Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, UK-Dementia Research Institute, Maurice Wohl Clinical Neuroscience Institute, King's College London, London, SE5 9NU, UK; [3]Faculty of Engineering, University of Bristol, Bristol, BS8 1TR, UK; [4]School of Physiology, Pharmacology and Neuroscience, Faculty of Health and Life Sciences, University of Bristol, Bristol, BS8 1TD, UK.

University of BRISTOL
Alumni and Friends

Background and Objectives

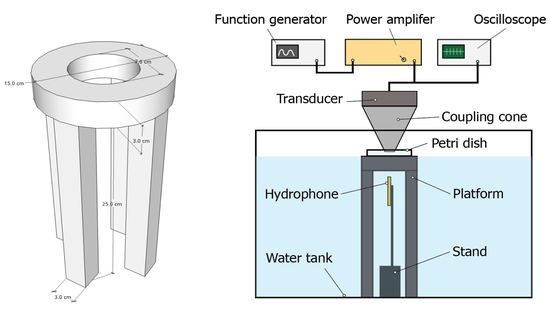
Background: Brief transcranial focused ultrasound stimulation (tFUS) is used in cognitive mapping [1], where it is assumed that the intervention itself does not cause lasting modifications to the underlying networks being targeted. However, how post-stimulation 'offline' effects impact the dynamic function of neural circuits is largely unknown.

Objective: To determine the persistent effects of ultrasound stimulation on hippocampal circuit function.

Methods

We explored this in rats, *in vitro* and *in vivo*. All experiments were approved by the U.K. Home Office as defined by the Animals (Scientific Procedures) Act 1986.

In vitro stimulation

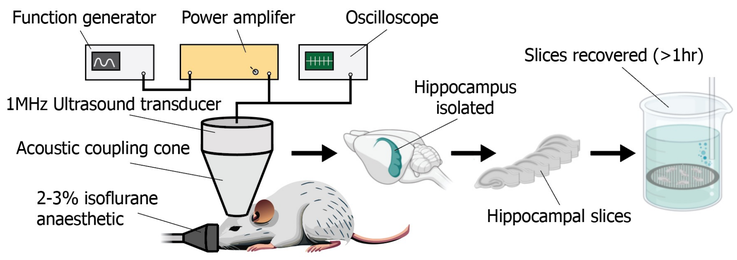


Juvenile P24-30 male & female Han Wistar rats.

Acute hippocampal slices.

Theta-burst focused ultrasound on hippocampal tissue: 5Hz, 40s, 50% duty cycle, I_{sppa} 1.35W/cm^2, 1MHz transducer.

In vivo stimulation



Adult 12-week-old male & female Han Wistar rats.

Stimulate hippocampi *in vivo*, assay *ex vivo.*

Theta-burst transcranial focused ultrasound stimulation on hippocampus: 5Hz, 40s, 50% duty cycle, I_{sppa} 0.44W/cm^2, 1MHz transducer.

Analyse circuits using field electrophysiology:

Following *in vitro* or *in vivo* stimulation and tissue preparation, a recording electrode was placed in the CA1 *stratum radiatum* of the hippocampal slice and CA3-CA1 field responses were evoked by 0.1ms voltage pulses to the Schaffer collateral (SC) pathway. For long-term potentiation (LTP) experiments, LTP was induced by a 100Hz x 1s tetanus and subiculum-CA1 inputs were used as a control.

1. Enhancement of basal synaptic transmission *in vitro* and *ex vivo*

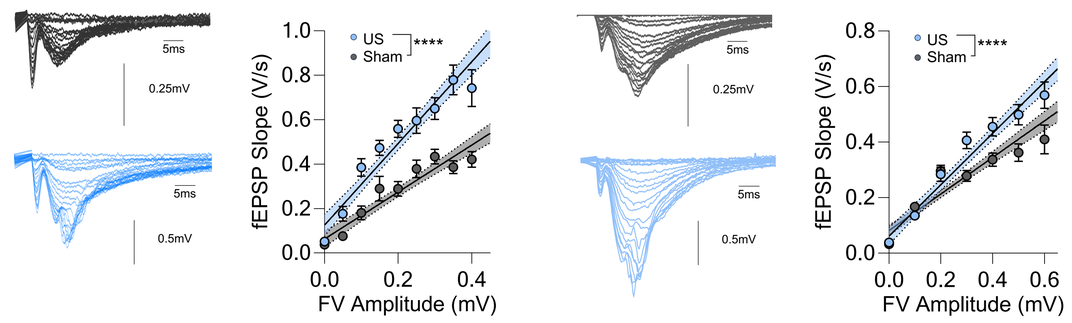


Fig. 1. Effects of theta-burst ultrasound on basal synaptic transmission. A Left, representative voltage traces from CA1 field recordings in response to incremental stimulation (0–20 V) of the SC pathway from sham (grey) and US (blue) treated slices. Right, comparison of FV amplitude and fEPSP slope in corresponding field recordings in sham and US treated slices.). N = 15 animals; 15 slices per condition, paired from each animal. **B** Left, representative voltage traces from CA1 field recordings in response to incremental stimulation (0–20 V) of the SC pathway in hippocampal slices from sham (grey) and US (blue) stimulated brains. Right, comparison of FV amplitude and fEPSP slope in corresponding field recordings in slices from sham and US treated brains. N = 25 slices from 6 sham stimulation animals vs. 19 slices from 6 US stimulation animals. **A,B** fEPSP responses are fit with a linear regression model (shaded area is 95 % CI). Difference between model fits is analysed by Extra Sum of Squares F-test. ****P < 0.0001.

2. Increase in LTP magnitude *in vitro* and *ex vivo*

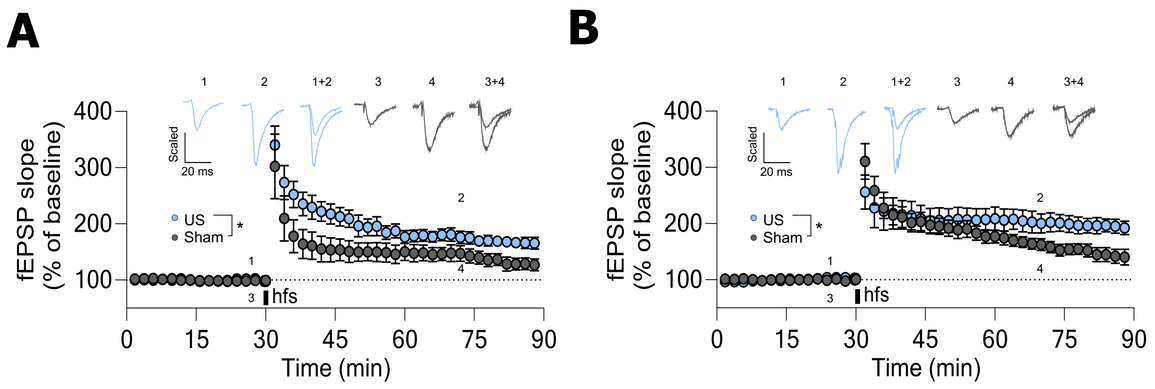


Fig. 2. Effects of theta-burst ultrasound on LTP magnitude. A Change in the fEPSP slope following a 30-min baseline and subsequent delivery of LTP-inducing high-frequency stimulation (hfs) to the SC pathway from sham (grey) and US (blue) treated slices. N = 6 animals; 6 slices per condition, paired from each animal. **B** Change in the fEPSP slope following a 30-min baseline and subsequent delivery of LTP-inducing hfs to the SC pathway from from sham (grey) and US (blue) stimulated brains. N = 6 slices from 6 sham stimulation animals vs. 6 slices from 6 US stimulation animals. **A,B** Trace examples are taken from 20- and 70-min timepoints. Statistical significance reported as difference in LTP magnitude, the mean of responses of the 70-75-min time period, analysed by two-tailed unpaired t-test. *P < 0.05.

3. Ultrasound-induced 'metaplastic' changes are mediated by Akt

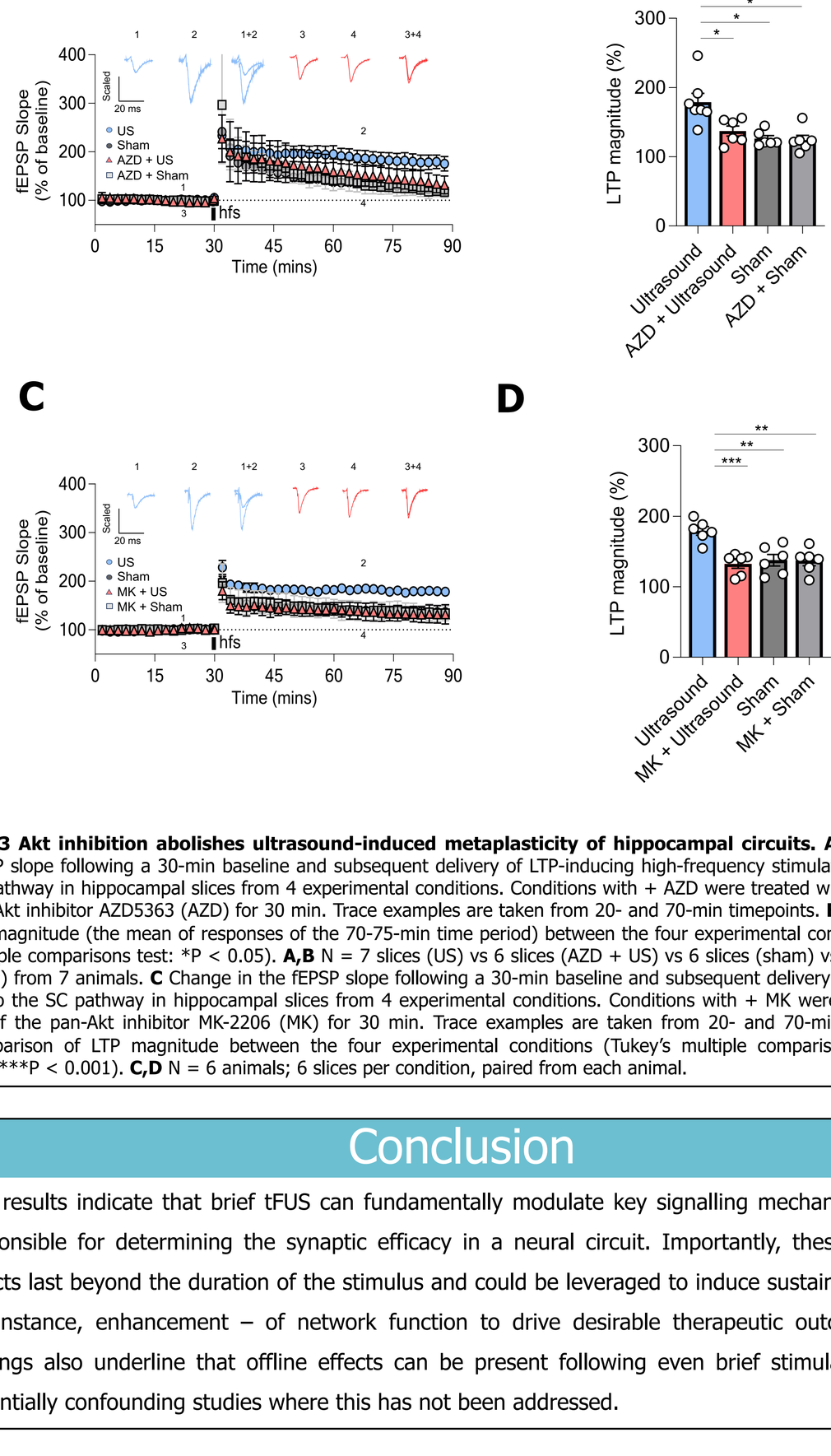


Fig. 3 Akt inhibition abolishes ultrasound-induced metaplasticity of hippocampal circuits. A Change in the fEPSP slope following a 30-min baseline and subsequent delivery of LTP-inducing high-frequency stimulation (hfs) to the SC pathway in hippocampal slices from 4 experimental conditions. Conditions with + AZD were treated with 30 μM of the pan-Akt inhibitor AZD5363 (AZD) for 30 min. Trace examples are taken from 20- and 70-min timepoints. **B** Comparison of LTP magnitude (the mean of responses of the 70-75-min time period) between the four experimental conditions (Tukey's multiple comparisons test: *P < 0.05). **A,B** N = 7 slices (US) vs 6 slices (AZD + US) vs 6 slices (sham) vs 6 slice (AZD + sham) from 7 animals. **C** Change in the fEPSP slope following a 30-min baseline and subsequent delivery of LTP-inducing hfs to the SC pathway in hippocampal slices from 4 experimental conditions. Conditions with + MK were treated with 1 μM of the pan-Akt inhibitor MK-2206 (MK) for 30 min. Trace examples are taken from 20- and 70-min timepoints. **D** Comparison of LTP magnitude between the four experimental conditions (Tukey's multiple comparisons test: *P < 0.01,***P < 0.001). **C,D** N = 6 animals; 6 slices per condition, paired from each animal.

Conclusion

The results indicate that brief tFUS can fundamentally modulate key signalling mechanisms that are responsible for determining the synaptic efficacy in a neural circuit. Importantly, these metaplastic effects last beyond the duration of the stimulus and could be leveraged to induce sustained changes – for instance, enhancement – of network function to drive desirable therapeutic outcomes. These findings also underline that offline effects can be present following even brief stimulation periods, potentially confounding studies where this has not been addressed.

References

1. Bongioanni A, *et al.* (2021) Activation and disruption of a neural mechanism for novel choice in monkeys. *Nature.* **591**:270–4.

- Data included in poster was published (see QR code on bottom right):

Watts *et al.* (2025) Brief transcranial focused ultrasound stimulation causes lasting modifications to the synaptic circuitry of the hippocampus. *Brain Stimulation* **18**:1587-1599.

william.w.watts@bristol.ac.uk