Supplementary Materials

Getting Started Vignette (MATLAB)

This note provides a minimal example code to load the released functional ultrasound dataset, extract ROI time-series signals, and visualize representative hemodynamic responses. Users can run the script directly after downloading the dataset.

matlab

```
%% Getting Started Example for fUS Dataset
clear, clc, close all

% Load processed dataset
load('mice1-cor1-standard-4g-contralateral.mat');

% Extract signals for the three cortical regions
S1HL_signal = concatenated_signals{1};
M_signal    = concatenated_signals{2};
ACC_signal  = concatenated_signals{3};

% Create time vector (assuming 3.57 Hz frame rate)
fs = 3.57;  % frame rate in Hz
time = (0:length(S1HL_signal)-1) / fs;

% Plot representative fUS signals
figure('Position', [100, 100, 900, 500])
hold on
plot(time, S1HL_signal, 'r', 'LineWidth', 1.5);
plot(time, M_signal,    'b', 'LineWidth', 1.5);
plot(time, ACC_signal,  'g', 'LineWidth', 1.5);
```

```
xlabel('Time (s)');
ylabel('\DeltaI/I_0');
legend('S1HL', 'Motor Cortex (M)', 'ACC', 'Location', 'best');
title('Representative Cortical fUS Signals under 4g Stimulation');
grid on;
hold off;

% Stimulation markers (gray dashed lines)
for i = 1:length(stim_markers)
    xline(stim_markers(i), '--', 'Color', [0.5 0.5 0.5], 'LineWidth', 1);
end
```

Supplementary Figure

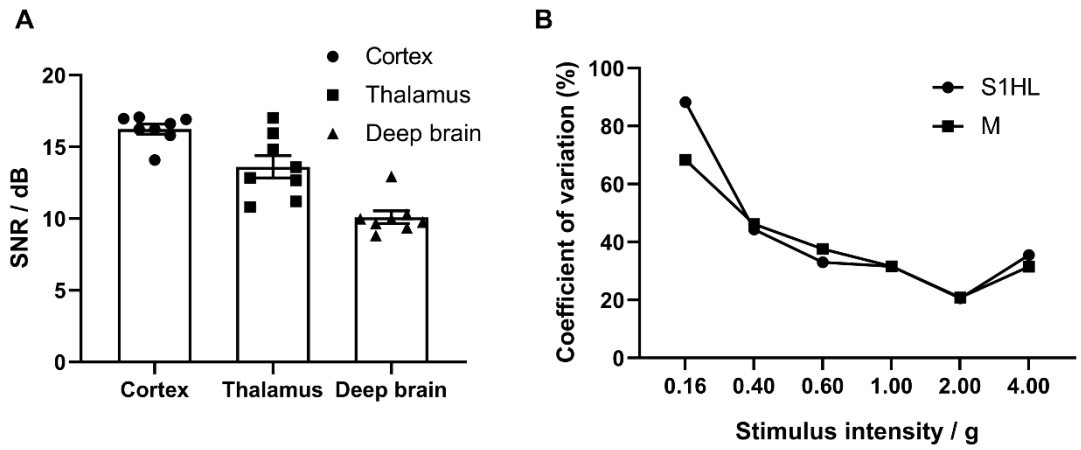

Fig. S1 fUS signal quality metrics. **A** Depth-dependent SNR. Signal-to-noise ratio (SNR) was calculated as 10 × $\log_{10}$(mean ROI signal/mean background signal) using the 50-s pre-stimulus baseline period (n = 8 mice). SNR decreased progressively with imaging depth: cortex (16.12 ± 0.33 dB), thalamus (13.61 ± 0.70 dB), and deep brain (9.97 ± 0.40 dB). Data are shown as mean ± SEM. **B** Between-animal reproducibility. Coefficient of variation (CV) of peak $\Delta I/I_0$ responses in S1HL and motor cortex (M) across eight animals at each stimulus intensity. CV decreased with increasing stimulus intensity, reaching a minimum at 2 g for both regions (S1HL: 20.6%; M: 20.9%), indicating moderate inter-subject consistency for suprathreshold stimuli.

Supplementary Table

Table S1 Complete list of regions of interest (ROIs) with anatomical coordinates and delineation criteria

ROI Name	Hemisphere	Plane	Bregma (mm)	Delineation Criteria
Primary somatosensory cortex, hindlimb region (S1HL)	Contra / Ipsi	Cor1	−0.71	Identified relative to corpus callosum and cingulum based on atlas boundaries
Motor cortex (M)	Contra / Ipsi	Cor1	−0.71	Located anterior and lateral to S1HL
Anterior cingulate cortex (ACC)	Contra / Ipsi	Cor1	−0.71	Midline cortex dorsal to corpus callosum
Caudate putamen (CPU)	Contra / Ipsi	Cor1	−0.71	Large structure lateral to lateral ventricle
Paraventricular thalamus (PVT)	Midline	Cor2	−1.55	Midline region dorsal to third ventricle
Mediodorsal thalamus (MD)	Contra / Ipsi	Cor2	−1.55	Located lateral to PVT based on atlas-defined boundaries
Lateral posterior thalamus (LP)	Contra / Ipsi	Cor2	−1.55	Positioned lateral and posterior to MD
Ventromedial thalamus (VM)	Contra / Ipsi	Cor2	−1.55	Ventral to MD within the thalamic complex
Ventral posterolateral thalamus (VPL)	Contra / Ipsi	Cor2	−1.55	Lateral ventral thalamus
Ventral posteromedial thalamus (VPM)	Contra / Ipsi	Cor2	−1.55	Medial to VPL within the ventral thalamus
Posterior thalamus (PO)	Contra / Ipsi	Cor2	−1.55	Posterior and lateral to VPL
Anterior pretectal nucleus (APT)	Contra / Ipsi	Cor3	−3.07	Pretectal region dorsal to the cerebral aqueduct

ROI Name	Hemisphere	Plane	Bregma (mm)	Delineation Criteria
Midbrain reticular nucleus (MRN)	Contra / Ipsi	Cor3	−3.07	Located lateral and ventral to the cerebral aqueduct
Periaqueductal gray (PAG)	Midline	Cor3	−3.07	Midline structure surrounding the cerebral aqueduct
Substantia nigra, reticular part (SNr)	Contra / Ipsi	Cor3	−3.07	Ventral midbrain, lateral to VTA
Ventral tegmental area (VTA)	Midline	Cor3	−3.07	Midline region ventral to the cerebral aqueduct

Contra, contralateral; Ipsi, ipsilateral. Atlas coordinates and anatomical delineation follow the Paxinos and Franklin Mouse Brain Atlas (4th edition). All fUS images were registered to the atlas space using anatomical landmarks. ROIs were manually delineated for each individual animal on mean power Doppler images based on atlas boundaries and anatomical landmarks. Binary ROI masks were generated separately for each animal while maintaining consistent anatomical definitions across subjects. A total of 16 ROIs were defined across three coronal planes.