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Supplemental Information

Spatial and Spectral Mapping and Decomposition of Neural Dynamics and Organization of the Mouse Brain with Multispectral Optoacoustic Tomography

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Supplemental information

Figure S1

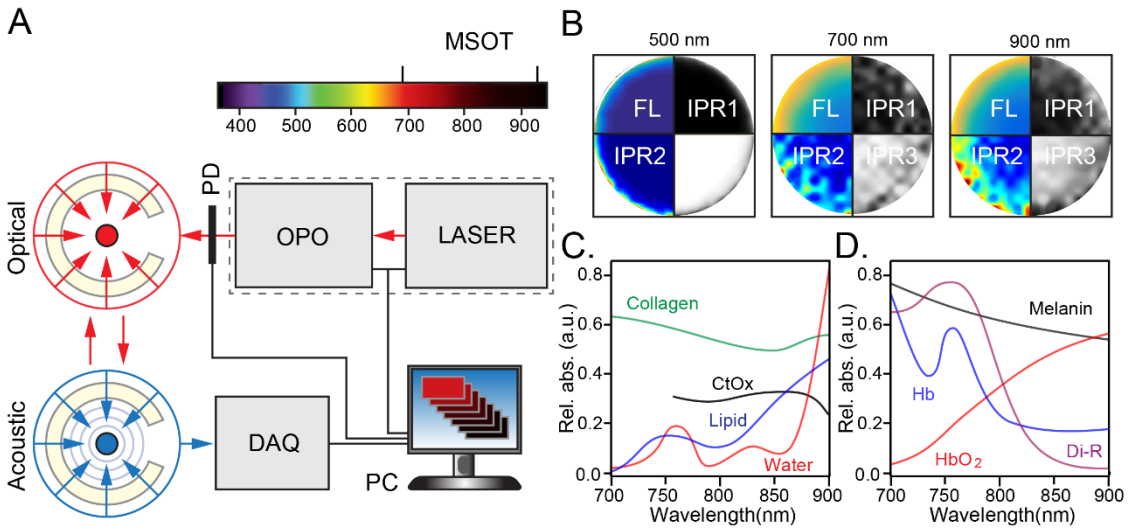

Figure S1: Experimental setup and optimization of imaging parameters for brain imaging with MSOT. Related to Figure 1. (A) Schematic representation of the MSOT: illumination (optical, red) and detection (acoustic, blue) modules; OPO – optical parametric oscillator; PD – photodiode; DAQ – data acquisition system; PC – personal computer. (B) Series of simulation highlighting the effective illumination of circular biological sample at different wavelengths, with a radius of 1 cm. Only oxy- and deoxyhemoglobin absorption values are implemented. FL – optical fluence and spatial maps of the initial pressure rise in three different color maps (IPR1 that is used traditionally, IPR2 that highlights the contrast between different simulated structures and IPR3 that is used throughout the manuscript to demonstrate images). Note much stronger attenuation of the illumination in deeper compartments at 500 nm as compared to 700 nm or 900 nm. (C) NIR absorption spectra of the main endogenous chromophores of the brain. CtOx – cytochrome oxidase; Hb and HbO_2 – deoxy- and oxyhemoglobin.

Figure S2

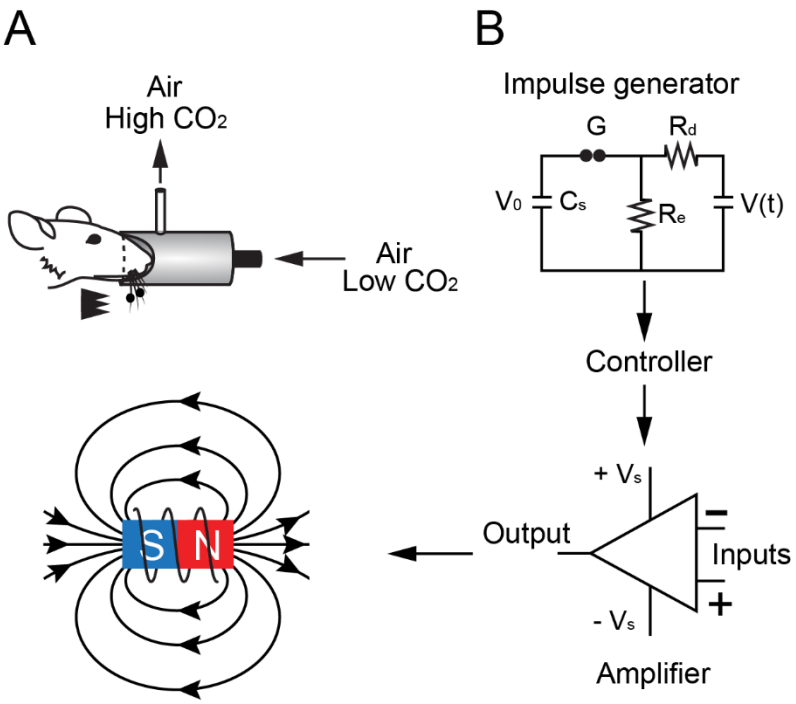

Figure S2: Custom designed breathing mask and device for mechanical whisker stimulation using a push-pull magnet. Related to Figure 1 and Figure 2. (A) Schematic illustration of the custom designed face mask enabling ample air supply with maintained ample space for free deflection of the whiskers decorated with magnetic beads (top) and a coil magnet providing force for movement of the whiskers within the MSOT imaging chamber. (B) A custom designed impulse generator and controller for induction and delivery of stimulation patterns for activation of whisker input in mice.

Figure S3

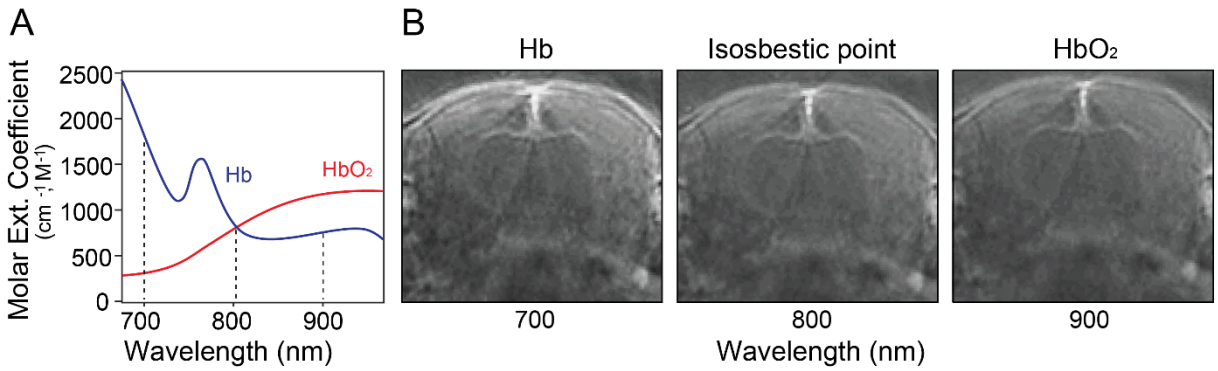

Figure S3: Selection of illumination wavelengths for anatomical imaging and mapping of stimulation induced hemodynamic response and changes of hemoglobin gradients with MSOT in the mouse brain *in vivo*. Related to Figure 1 and Figure 2. (A) Graph of NIR absorption spectra of Hb and HbO_2 with selection of 700 nm and 900 nm wavelengths for deoxy- and oxyhemoglobin readouts, while 805 nm wavelength, corresponding to the isosbestic point of hemoglobin was used as a readout of total blood volume changes. (B) Raw images of mouse brain cross-sections at -1.2 mm Bregma acquired at three different wavelengths.

Figure S4

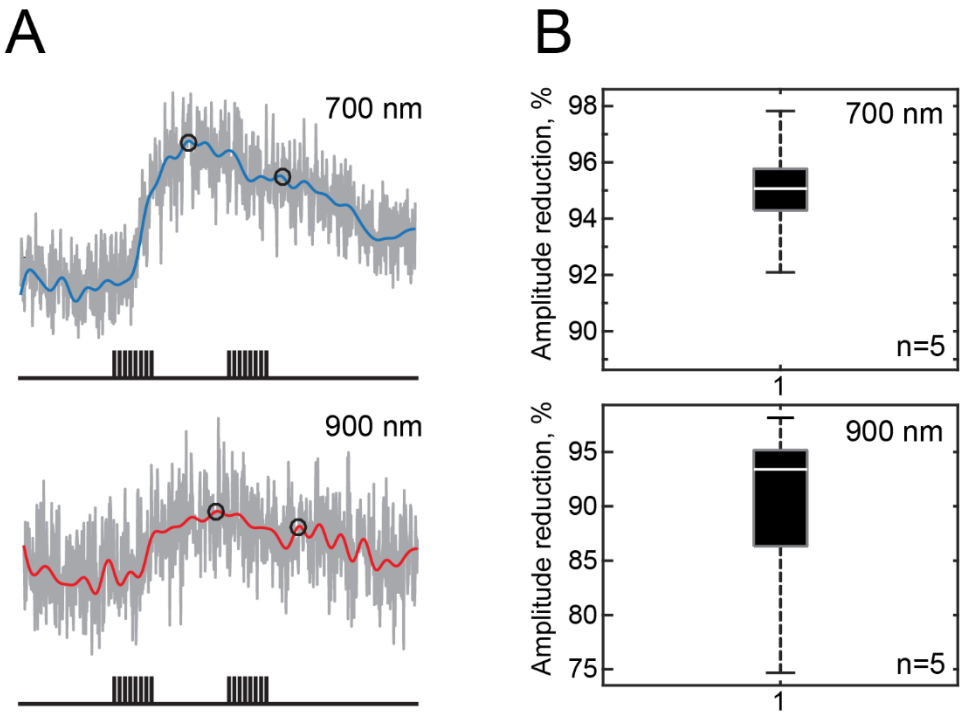

Figure S4: Activity-dependent inhibition of the hemodynamic changes in the somatosensory barrel field induced by two series of repetitive stimulation trains. Related to Figure 1. (A) Representative traces of Hb and HbO_2 signals activated in the somatosensory cortex barrel field induced by two barrages of whisker stimulation (8 × 4Hz) separated by 10 sec interval. Filtered traces have been used for this analysis, with peak amplitude defined manually at the first and second response driven by whisker inputs. (B) Summary graphs illustrating reduction of both Hb and HbO_2 signal in response to two series of stimulation.

Figure S5

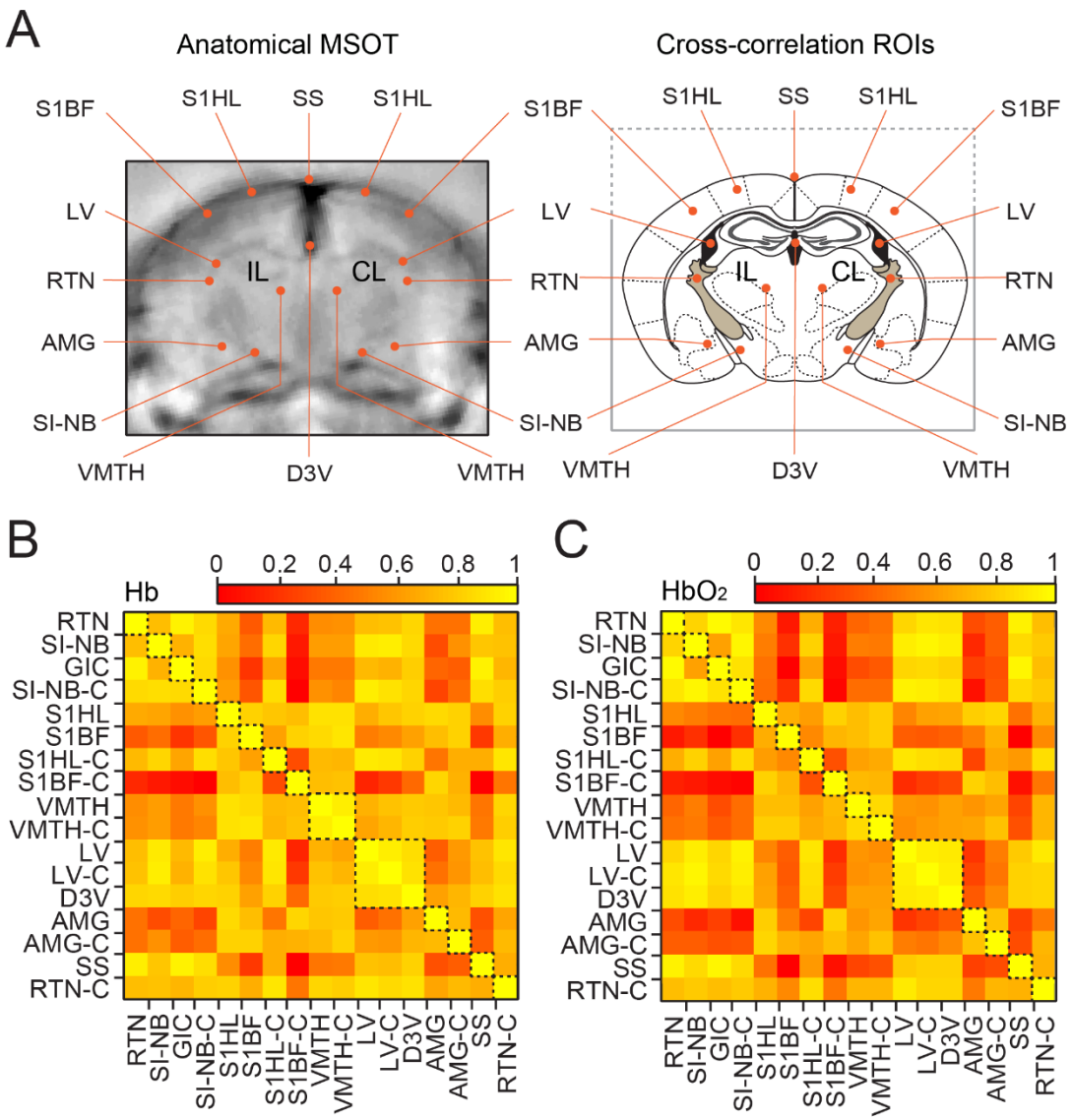

Figure S5: Mapping hemodynamic response in the resting brain of anaesthetized mice with MSOT. Related to Figure 3. (A) Anatomical MSOT cross-section of the brain containing the somatosensory barrel cortex, with regions of interest selected for Hb and HbO_2 analysis. Abbreviations: RTN – reticular thalamic nucleus; SI-NB – substantia innominate nucleus basalis; GIC – granular insular cortex; SI-NB-C – substantia innominate nucleus basalis contralateral; S1HL – primary somatosensory, hind limb; S1BF – primary somatosensory, barrel field; S1HL-C – primary somatosensory, hind limb contralateral; S1BF-C – primary somatosensory, barrel field

contralateral; VMTH – ventromedial thalamic nucleus; VMTH-C – ventromedial thalamic nucleus, contralateral; LV – lateral ventricle; LV-C – lateral ventricle, contralateral; D3V – third dorsal ventricle; AMG – amygdala; AMG-C – amygdala contralateral; SS – sagittal sinus; RTN-C – reticular thalamic nucleus, contralateral. (B, C) Cross-correlation matrices of Hb (700 nm), HbO_2 (900 nm) from seventeen regions of interest.

Figure S6

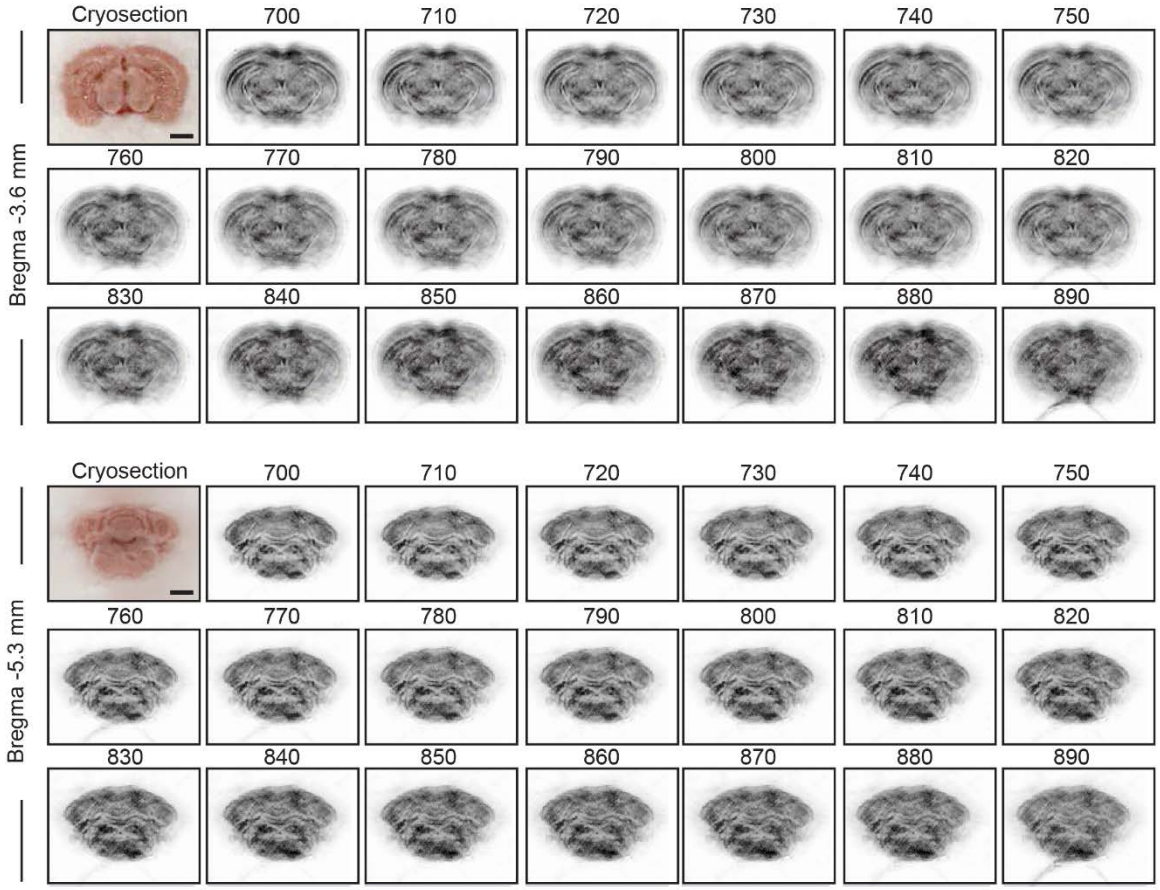

Figure S6: MSOT scans of ex vivo brain acquired at incremental wavelengths between 700 nm and 900 nm at two distinct planes corresponding to the midbrain and hindbrain levels, with corresponding cryo section image. Bregma coordinates on the left. Related to Figure 4 and 5. Note the great visibility of exquisite anatomical details throughout the brain with their wave-length dependent changes.

Mice	Functional MSOT			Structural MSOT				Optimizing Troubleshooting	Total
	No stimulation	Whisker stimulation		*Ex vivo*		*In vivo*			
Settings	Single plane	Single plane	Multiple planes	Perfused	Non-perfused	Tracer	No-tracer		
CD1	0	7	4	6	4	0	0	4	25
Nude	6	0	0	0	0	4	4	3	17

Table S1: Number of animals used for different experiments in this study. Related to STAR Methods.