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Introduction

Venous effect in fMRI

Venous effects in functional magnetic resonance imaging (fMRI) using blood oxygenation level dependent (BOLD) contrast refer to dilution of deoxyhaemoglobin (HbR) in the venous compartment, resulting in higher activation detection sensitivity in larger veins [Duong 2003]. Venous effects are likely to be an important factor compromising our ability to localize sensory or motor activations in the spinal cord, notably due to the relative size of the cord and veins [Zhao 2008], as shown in Figure 1. Therefore, it is crucial to identify vascular compartments where functional responses are recorded.

Intrinsic optical imaging

Intrinsic optical imaging is a means to investigate the dynamics of vascular compartments with high spatio-temporal resolution. It has already been implemented by Lesage *et al.* to quantify variations of HbR, HbO₂ and total blood volume in a model of rats with spinal cord injury [Lesage 2009]. Blood flow was also recorded in the same preparation by means of laser speckle imaging. In this study we report results of fMRI, intrinsic optical imaging (IOI) and laser speckle, providing measurements of HbR and HbO concentration, blood volume (HbT) and blood flow (BF).

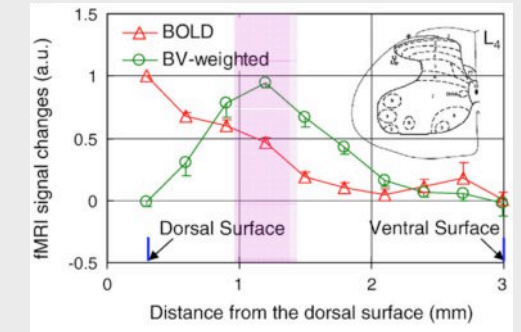


Figure 1. Venous effect in the rat spinal cord. BOLD signal from gradient echo EPI is more sensitive to larger vessels located at the surface of the cord. Adapted from Zhao *et al.*, [Zhao 2008].

FMRI in cats

Methods

Functional MRI studies were conducted on cats (N=9) in a 3T MRI system (Siemens Medical Systems) using standard array coils and a multishot gradient echo echo planar imaging (EPI) sequence. TR/TE/alpha = 2000ms/30ms/45°, matrix = 96x96, voxel size = 2x2x2 mm³, bandwidth = 1630 Hz/pixel. Electrical stimuli were applied in hindlimb nerves following a block design. Runs were repeated ten times to investigate the reproducibility of responses in terms of spatial location and amplitude. More details can be found in [Cohen-Adad 2009].

Results

Focal BOLD responses were detected in seven out of nine cats at the L4-L5 vertebral level, which corresponds to the region to where the stimulated nerves project. T-maps for five cats are shown in Figure 2. Apart from the relatively low number of false positives, the main observation is the poor robustness of the lateralization and the antero-posterior location of peak activations, as summarized in Table 1.

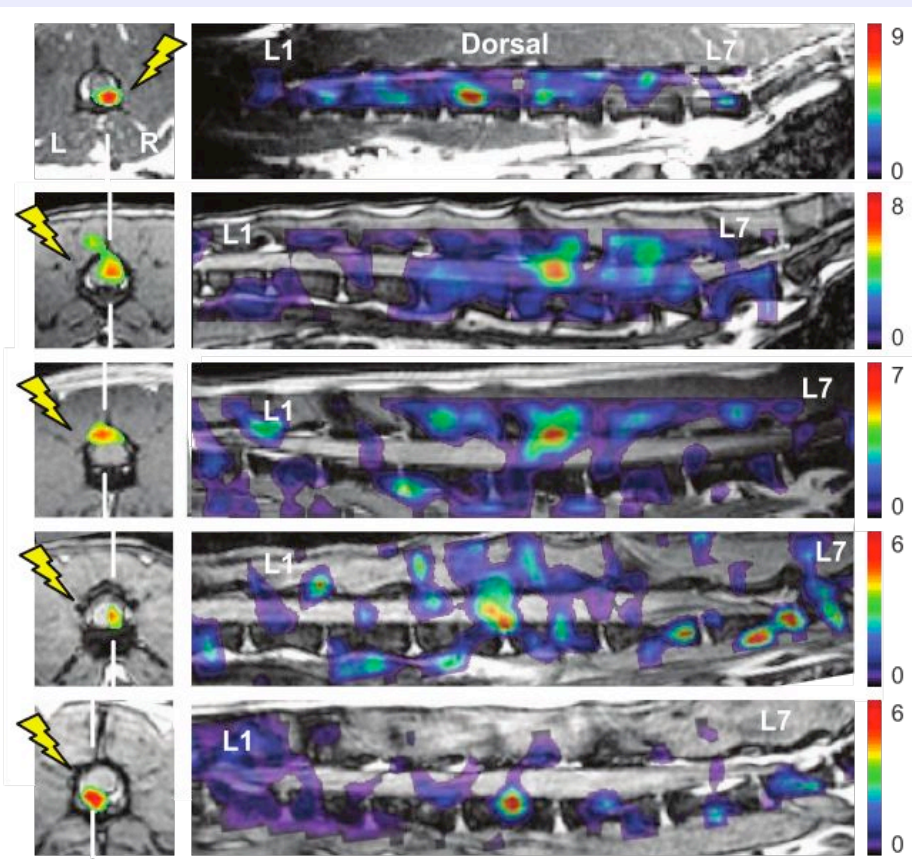


Figure 2. Activation maps in cats #1-5 (same numbering as in Table 1) showing axial (left) and sagittal (right, no threshold) views. Electrical stimulations of hindlimb nerves induced neuronal activation at L4-L5 levels. Activation peaks are seen at the periphery of the cord in three cats (#1, #3, #5).

Cat	Significant activation	R-L location	A-P location	Structure
1	yes	ipsi	ventral	subarach
2	yes	contra	dorsal	spinal cord
3	yes	ipsi	dorsal	subarach
4	yes	contra	ventral	spinal cord
5	yes	ipsi	Ventral	subarach
6	yes	contra	dorsal	spinal cord
7	yes	medial	ventral	spinal cord
8	no	-	-	-
9	no	-	-	-

Table 1. Summary of BOLD response characteristics in terms of lateralization (R-L location), antero-posterior (A-P) location. The compartment where the activation peak lies is indicated in the last column. 'subarach' stands for the subarachnoid space.

FMRI in humans

Methods

A second fMRI study was conducted on humans (N=9) using the same MRI system. Hypercapnic and motor (ball squeezing) tasks were carried out to compare the spatial location of BOLD responses. In both cases, the task was administered using a block-design and lasted nine minutes. Gradient-echo EPI were recorded in axial plane using TR/TE/alpha = 3000ms/30ms/70°. A susceptibility weighted image (SWI) was also acquired to further correlate detected responses with a venogram in the spinal cord. More details of the methods can be found in Posters #247, #628.

Results

In both hypercapnic and motor tasks, significant BOLD responses were detected at the spinal level. Similar to the results found in our study with cats, there was a high inter-subject variability with highest responses observed at the periphery of the cord, but a low intra-subject variability in terms of spatial location of responses (Figure 3). Most hypercapnia-induced responses were located in the subarachnoid space (Figure 4). The venous effect hypothesis is supported by the SWI, showing good agreement between the location of these responses and large veins (Figure 4). A quantitative analysis of mean signal percent change in the parenchyma and subarachnoid space is provided in Figure 5.

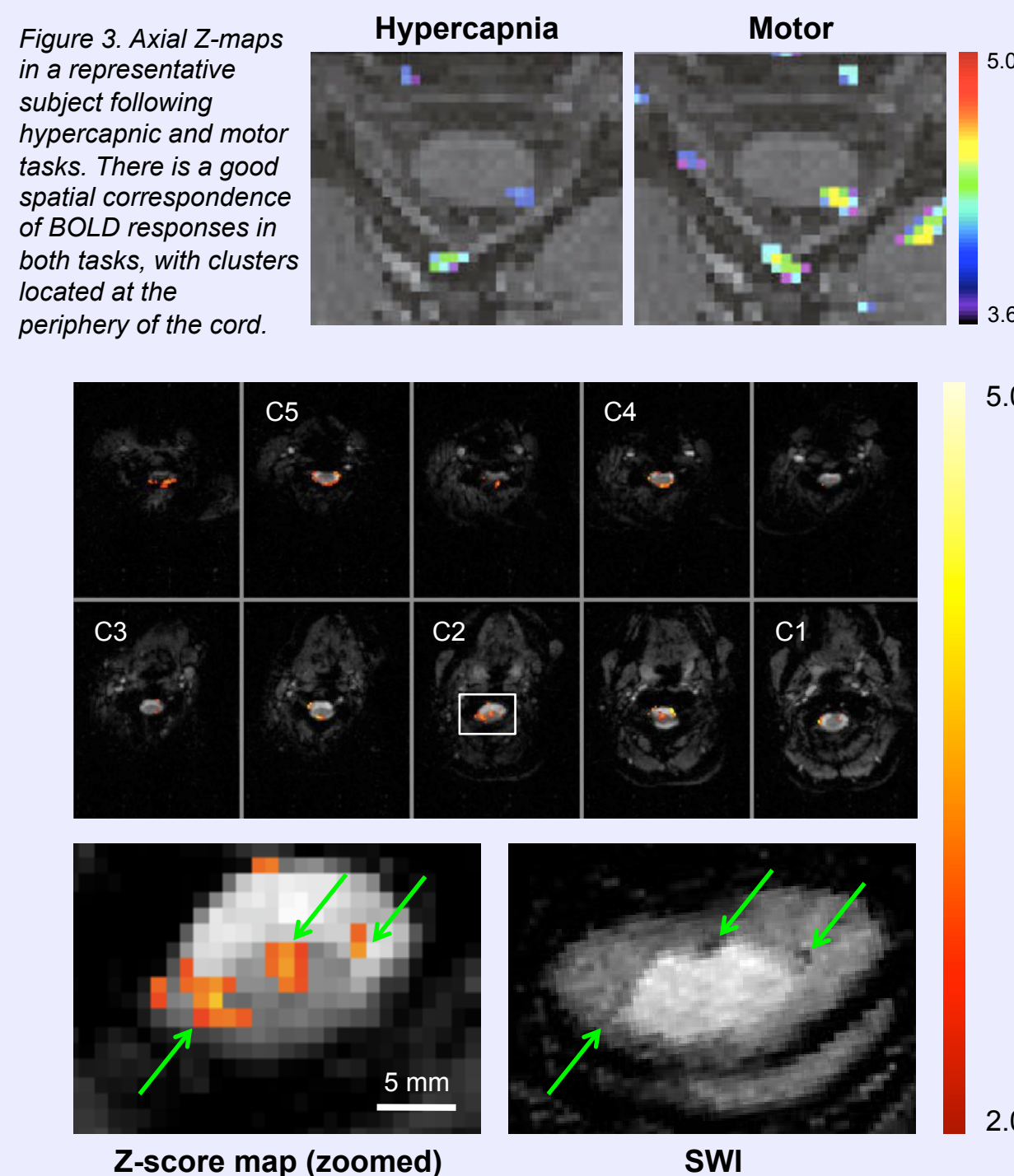


Figure 3. Axial Z-maps in a representative subject following hypercapnic and motor tasks. There is a good spatial correspondence of BOLD responses in both tasks, with clusters located at the periphery of the cord.

Figure 4. Axial Z-maps covering the cervical cord in one subject who experienced hypercapnic challenge. Zoomed panels highlight the spatial correspondence between highest BOLD responses and large veins.

Figure 5. Distribution of percent signal change values in the spinal cord, spinal subarachnoid space (subarach) and brain gray matter (BBS). This histogram clearly shows that, in proportion, lowest signal change (0-2%) occurs in the brain and spinal cord parenchyma, whereas highest change (3-7%) occurs in the subarachnoid space where large veins are located.

Conclusion

In both sensorimotor fMRI studies, significant BOLD responses were detected at the periphery of the cord, with substantial inter-subject variability and lack of lateralization. Additionally to the two fMRI studies presented here, other groups also reported BOLD responses at the periphery of the cord in animals [Zhao 2008, 2009] and humans [Bouwman, 2008 ; Govers, 2007]. The largest BOLD responses may be located at the periphery of the spinal cord due to a venous effect, since in most mammals large draining veins are located along the spinal cord. This hypothesis is further supported by results from optical imaging, where early focal responses within the cord followed by a vascular change spread over several millimetres from the neuronal activation site along large spinal veins have been observed (Figure 6).

Despite difficulties raised above, our results confirm that BOLD responses can be recorded (or measured) in the spinal cord using gradient-echo sequences, with a percent signal change that is comparable to the one observed in the brain (Figure 5). Yet, as for brain fMRI studies, one needs to be careful when trying to infer about the spatial location of neuronal activation based on vascular changes. Appropriate noise modelling is also crucial here due to the high variance in spinal cord time series [Brooks 2008]. For more details on hypercapnia and motor responses, see **Poster #628** and **Poster #247**.

Intrinsic optical imaging

Methods

The optical imaging study was conducted in rats (N=9) anaesthetized with alphachloralose. Following laminectomy, left sciatic nerves were stimulated every 19s during 9min. One stimulus consisted of a 1s pulse train (10 square current pulses of 64 µA). At that intensity, most A-fibres (A_α, A_β) giving rise to non-noxious stimuli were activated. We also expected few noxious C-fibres and A_δ fibres to be activated.

A digital camera was positioned over the exposed spinal cord at the lumbar level (see picture on the right). Multiple wavelengths (525, 590, 637nm) were used to account for HbR and HbO absorption rate. After IOI recording, experiments were reproduced with laser speckle (780nm) to measure BF as done in [Briers 2001]. Electrophysiological measurements were subsequently conducted to spatially delineate the site of neuronal activity.

Results

HbR and HbO concentrations as well as blood flow exhibited an early focal response within the cord followed by a vascular response spreading over several millimetres from the neuronal activation site along large spinal veins (Figure 6). Electrophysiological measurements confirmed the presence of neuronal activity in regions where early vascular responses occurred in capillaries (Figure 7).

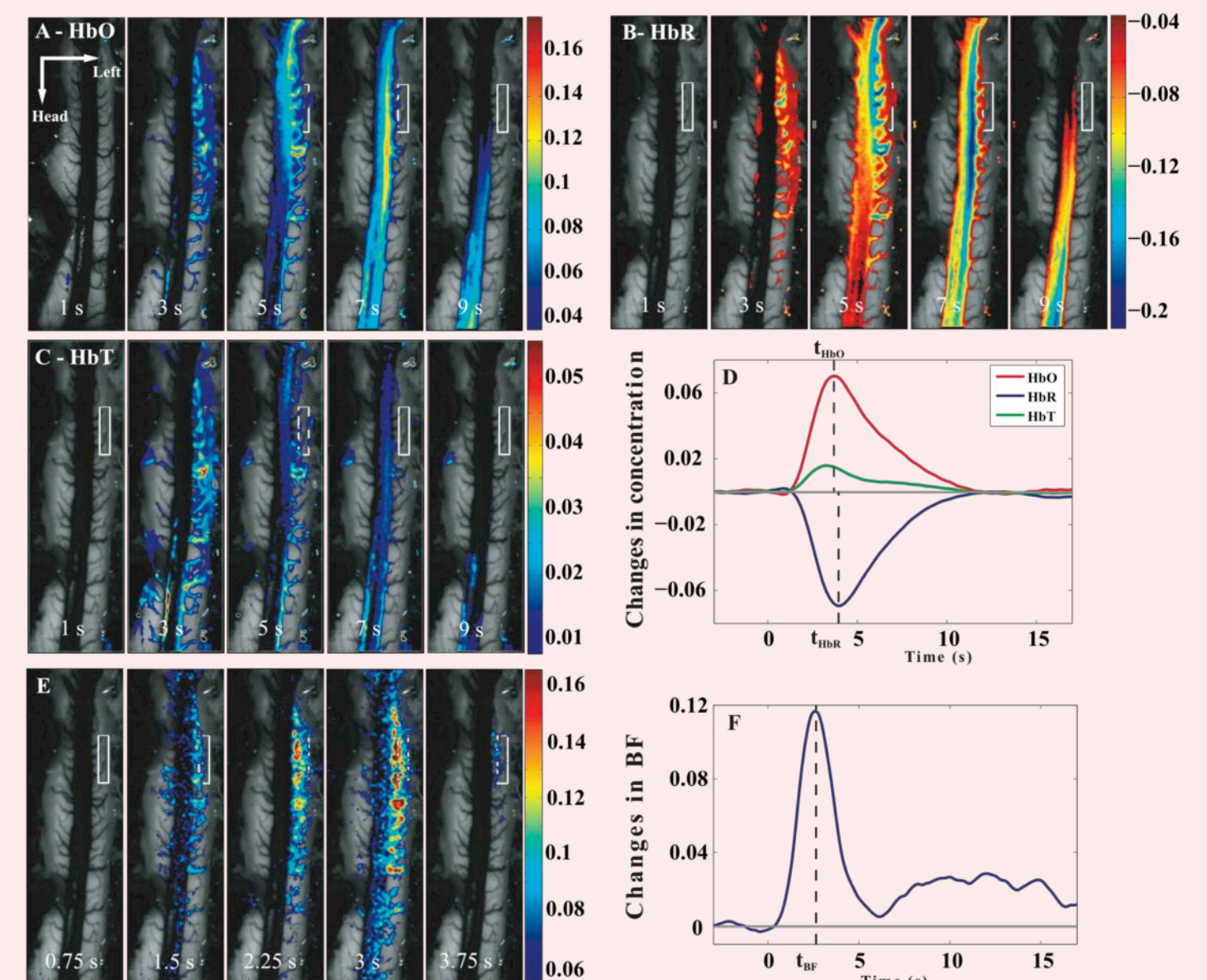


Figure 6. Spatio-temporal distribution of hemodynamic response for HbO (A), HbR (B), BV (C) and BF (E) measurements in one rat. Time course of HbO, HbR and BV (D) and BF (F) responses averaged in ROI for all rats.

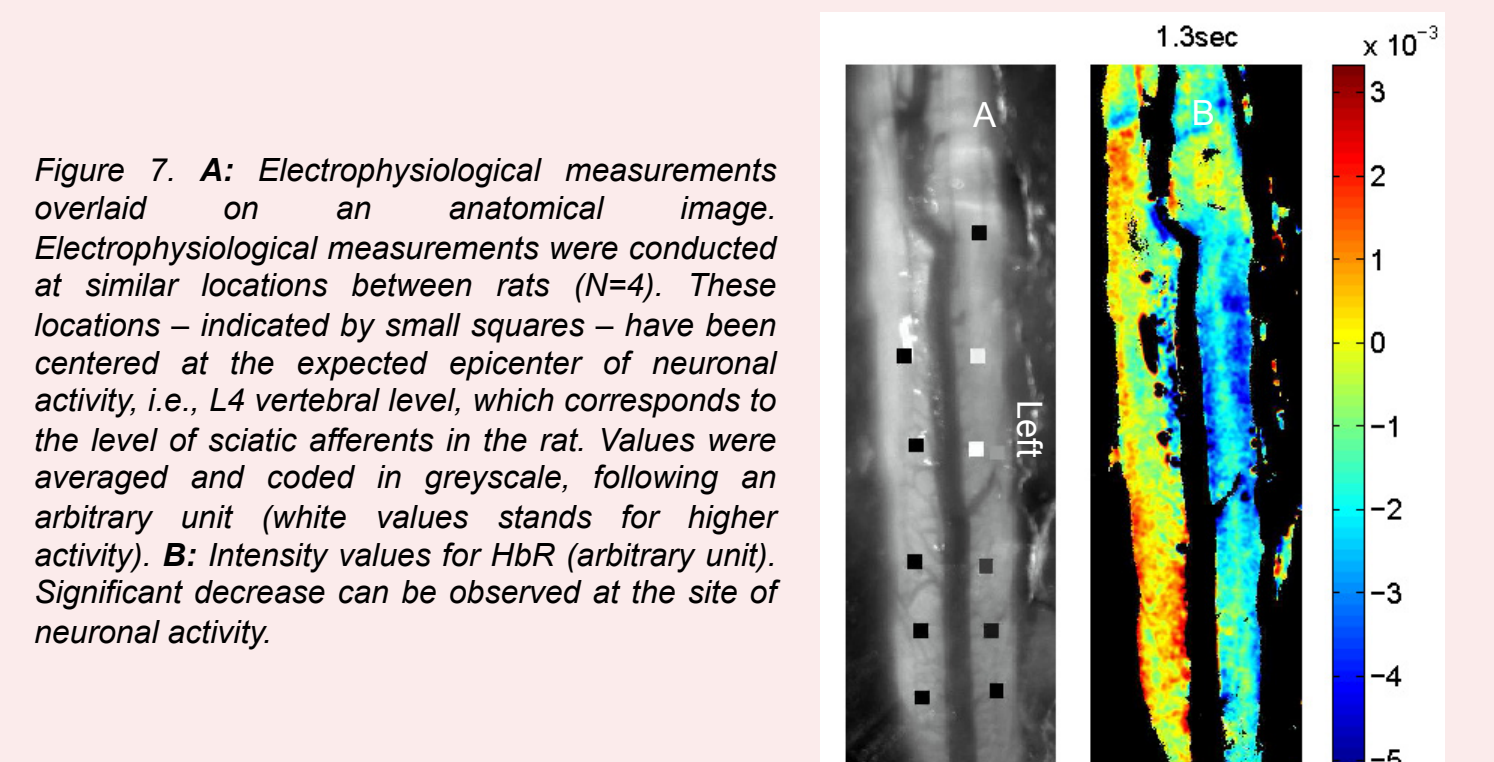


Figure 7. A: Electrophysiological measurements overlaid on an anatomical image. Electrophysiological measurements were conducted at similar locations between rats (N=4). These locations – indicated by small squares – have been centered at the expected epicenter of neuronal activity, i.e., L4 vertebral level, which corresponds to the level of sciatic afferents in the rat. Values were averaged and coded in greyscale, following an arbitrary unit (white values stands for higher activity). B: Intensity values for HbR (arbitrary unit). Significant decrease can be observed at the site of neuronal activity.

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