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Mapping of the hemodynamic response in the cerebellum with intrinsic optical imaging: beams vs. patches

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Introduction

Many theories of cerebellar function assume that parallel fibers provide strong excitatory input to Purkinje cells situated along the beam, an assumption supported by physiological studies in which parallel fibers are directly activated electrically. However, studies in which cerebellar circuitry is activated by peripheral somatosensory stimulation fail to demonstrate beam-like patterns of activation. Instead, peripheral stimulation results in patches of activated Purkinje cells that directly overlie activated regions of the granule cell layer. In the present study, we use reflectance optical imaging to contrast direct electrical stimulation of the parallel fibers with peripheral tactile or electrical stimulation of the upper lip *in vivo* in the same animal.

Methods

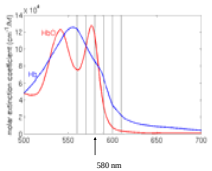
Rats were initially anesthetized with ketamine/pentobarbital. Supplementary ketamine was continuously infused throughout the experiment. An area of skull overlying the cerebellum was removed and the dura matter was transected. A well of dental acrylic was built around the exposure and filled with saline. Animals were free breathing.

The light from a mercury xenon arc lamp was directed through a 10 nm bandpass filter centered at 580 nm coupled to a 12-mm fiber bundle that illuminated the cortex. Images were acquired by a cooled 12-bit CCD camera at 20 Hz.

Electrophysiological recordings from granule cell layer were performed using single metal electrodes (2.5-3.5 MΩ). The signals were filtered at 0.1-500 Hz to record local field potential (LFP) and at 150-5000 Hz to record multiunit spike activity (MUA). The MUA signal was subsequently rectified.

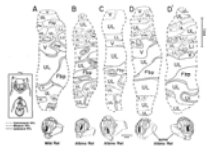
A tactile stimulus of the upper lip was delivered by a computer-controlled piezoelectric stimulator (8 Hz for 2 sec). Electrical stimulation of the upper leap consisted of 2-sec train of 300 μsec, 1-2 mA pulses delivered at 3 Hz. Parallel fibers were stimulated at 3 Hz, using 100 μsec, 500 μA pulses delivered through a single metal electrode touching the cortical surface. 60 stimulus presentations were averaged. No filtering was applied to the optical data.

The hemodynamic response reflects changes in blood oxygenation



Using 580-nm illumination, the main two chromophores that contribute to the optical signal are oxyhemoglobin (HbO, red) and deoxygemoglobin (Hb, blue). Neuronal activity leads to the hemodynamic response, that consists of changes in blood flow, volume and oxygenation. The hemodynamic response is delayed (over 0.5 sec) comparing to neuronal activity, and at the peak is dominated by the inflow of fresh blood to the active area, washout of Hb and increase in HbO. At 580 nm HbO absorbs more than Hb. Therefore, an **increase** in HbO corresponds to a signal **decrease**, or darkening of the image.

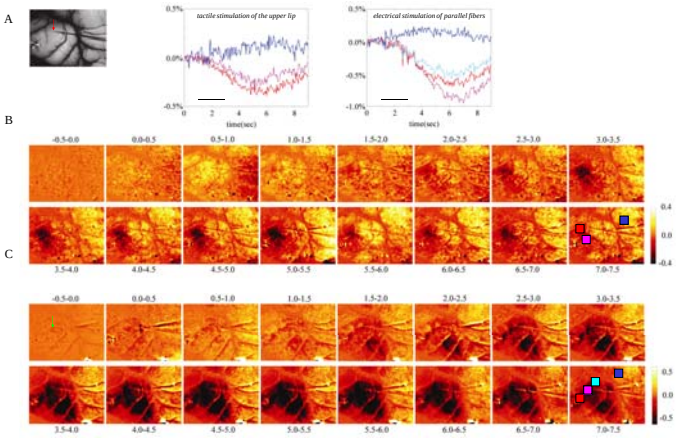
Topographic somatosensory maps in the cerebellum are fractured



Bower and Kassel, 1990

In contrast to the cerebral cortex, tactile maps in the cerebellar cortex consist of topographically discontinuous patches. For example, ipsilateral upper lip (UL) is usually mapped onto 3 discontinuous areas in crus IIA.

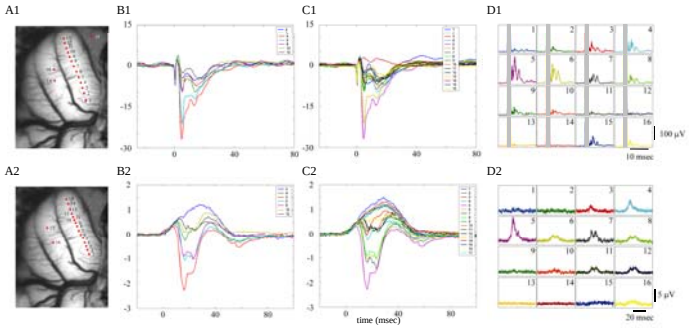
Tactile stimulation produces a patch of hemodynamic activity
Direct stimulation of the parallel fibers produces a beam of hemodynamic activity



A. An image of the cerebellar cortex corresponding to functional images in B and C.

B.-C. Tactile (B) and electrical parallel fiber (C) stimulation. Each image represents an average of 10 frames divided by an average of 20 frames before the stimulus onset. Signal timecourses on top were averaged from regions of interest indicated by color rectangles. The location of a stimulating electrode is marked by an arrow.

Electrical stimulation of the upper lip broadens the fractured maps

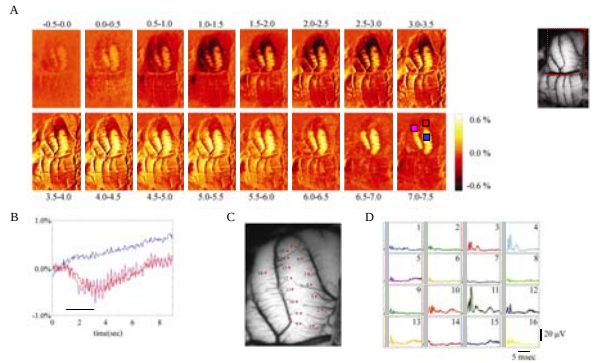


A. An image of the cerebellar cortex. Red dots mark electrode penetration sites. 1 and 2 correspond to electrical and tactile stimulation of the upper lip respectively.

B.-C. Averaged LFP responses recorded from the locations marked in A. B represents a subset of penetration sites.

D. Averaged and rectified MUA responses recorded from the locations marked in A. Gray rectangles indicate the stimulus artifact. Following electrical stimulation there is a continuous response at locations 1-12. Note that both LFP and MUA are plotted on a different scale for electrical vs. tactile stimulus.

The hemodynamic response to electrical stimulation of the upper lip only partially overlaps with neuronal activity

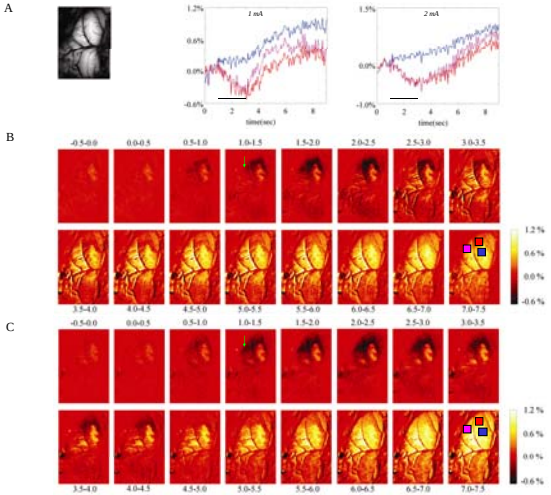


A. Hemodynamic response following electrical stimulation of the upper lip. Each image represents an average of 10 frames divided by an average of 20 frames before the stimulus onset. An image of the cerebellar cortex corresponding to functional images in shown on the right. Dotted red line outlines the region shown in C.

B. Signal timecourse averaged from regions of interest indicated by color rectangles in A.

C. A map of electrode penetration sites (red dots).

D. Averaged and rectified MUA responses recorded at the locations marked in B. Gray rectangles indicate the stimulus artifact. Note that the largest response at location 11 corresponds to the smallest hemodynamic response among the three regions of interest (blue).



A.-C. Another example of electrical stimulation of the upper lip. B and C correspond to stimulus intensity of 1 and 2 mA respectively. Draining vessels are more visible under stronger stimulus intensity (green arrow)

Conclusions

1. Tactile stimulation of the upper lip produces a localized patch of hemodynamic response corresponding to areas of upper lip representation in crus IIA.
2. Direct electrical stimulation of parallel fibers produces a beam of hemodynamic response that runs in the direction of the parallel fibers along the folium.
3. Intrinsic signal optical imaging in the cerebellum provides an evidence that a patch of hemodynamic activity corresponding to peripheral tactile stimulation is obtained in preparations with active parallel fibers.
4. Electrical nerve stimulation is sub optimal for topographic mapping of electrical and hemodynamic responses in the cerebellum (probably due to synchronous and nonselective activation of primary afferents).