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Evidence that Neuronal Signaling and not Energy Consumption Controls the Hemodynamic Response

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Abstract

We examined bilateral neuronal and hemodynamic changes, and deoxyglucose uptake in response to a unilateral somatosensory stimulus in rat primary somatosensory cortex (SI). In contrast to the contralateral forepaw area where neuronal activity, blood oxygenation/flow and deoxyglucose uptake increased in unison, we observed blood oxygenation/flow decrease and arteriolar vasoconstriction in presence of increased deoxyglucose uptake in the ipsilateral SI. Voltage-sensitive dyes measurements revealed sequential de- and hyperpolarization bilaterally. Relative to the initial depolarization hyperpolarization was stronger on the ipsilateral side, suggesting stronger recruitment of inhibitory interneurons in ensemble response. Laminar electrophysiological recordings revealed an increase in ipsilateral spiking consistent with the observed increase in deoxyglucose uptake. The vasoconstriction and decrease in blood flow in presence of an increase in both neuronal spiking and deoxyglucose uptake in ipsilateral SI argues against feedback signaling by energy metabolites. Rather, our results are consistent with feed forward neuronal control of vasodilation and vasoconstriction.

Decreases in blood oxygenation and flow are observed in ipsilateral SI

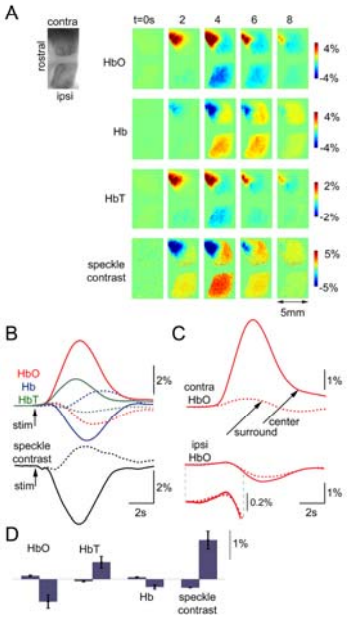

Figure 1. Bilateral hemodynamic optical imaging reveals a decrease in ipsilateral blood oxygenation and flow
(A) HbO, Hb, HbT and speckle contrast images following stimulus onset (t=0). The color scale is expressed as percent signal change relative to pre-stimulus baseline (ΔC/C0). Time (in seconds) relative to stimulus onset is indicated above images. 150 trials were averaged. We assumed baseline concentrations of 60 μM and 40 μM for HbO and Hb, respectively. An image of raw vasculature corresponding to functional frames is shown in upper left corner. Contra – contralateral to the stimulus hemisphere, ipsi – ipsilateral to the stimulus hemisphere.
(B) Top: Signal time-courses extracted from contralateral (solid lines) and ipsilateral (dashed lines) hemisphere for HbO (red), Hb (blue) and HbT (green). Bottom: The same for speckle contrast.
(C) HbO as in (B) broken into the center (within1.5 mm ring around the center of the response, solid red) and the surround (outside the 1.5 mm ring, dashed red). The center was estimated using the earliest HbT response.
(D) Bar graphs of ΔHbO, ΔHb, ΔHbT and Δspeckle contrast quantifying biphasic ipsilateral response. For each measure the 1st bar represents the initial (small) oxygenation/flow increase, and the 2nd bar represents the consecutive (big) oxygenation/flow decrease. Data from 5 subjects were averaged. The error bars indicate standard error across subjects.

Voltage-sensitive dyes (VSD) imaging reveals bilateral sequential de- and hyperpolarization

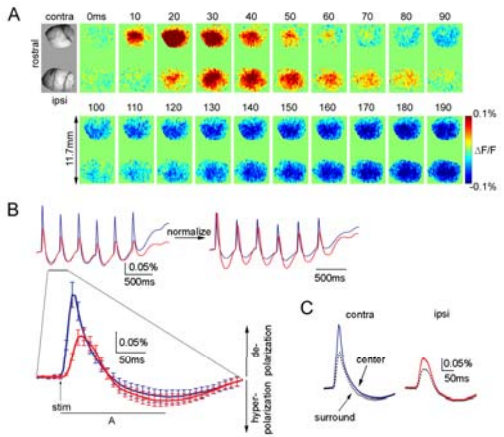

Figure 2. Bilateral VSD imaging shows stronger relative hyperpolarization in ipsilateral hemisphere
(A) Consecutive VSD images following stimulus onset (t=0). The color-scale is expressed as fluorescence percent change relative to the baseline (ΔF/F). 70 trials were averaged within the same subject. Time post-stimulus is indicated above the images. Raw image of corresponding cortical surface is shown on the left. Contra – contralateral to the stimulus hemisphere, ipsi – ipsilateral to the stimulus hemisphere.
(B) Signal time-courses extracted from contralateral (blue) and ipsilateral (red) hemisphere. The response to the first stimulus is expanded below. Error bars represent standard error. Time period corresponding to (A) is outlined below. Data from 8 subjects were averaged.
(C) The same as (B) broken into the center (within1.5 mm ring around the center of the response, solid lines) and the surround (outside the 1.5 mm ring, black dashed lines). The center was estimated using the earliest response (t=10 ms).

Taken together with spectral/speckle imaging results, these data demonstrate that a decrease in blood oxygenation and flow in ipsilateral hemisphere is correlated with stronger recruitment of inhibition that is observed as stronger relative hyperpolarization with VSD imaging.

Laminar electrophysiological recordings demonstrate a bilateral increase in spiking

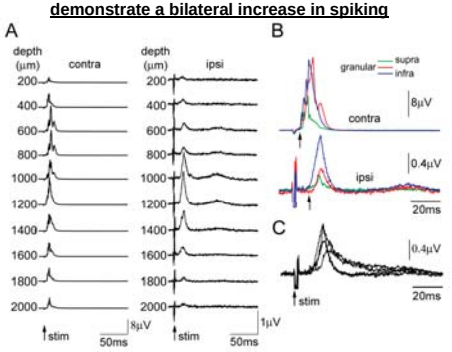

Figure 3. Laminar recordings of multiunit activity measure an increase in ipsilateral spiking
(A) Laminar profile of MUA response in contralateral (left panel) and ipsilateral (right panel) hemisphere. Each trace represents a recording from one single electrode in the array. Corresponding cortical depth is indicated on the left. 500 stimulus trials were averaged. Arrows denote stimulus onset. Note an increase in ipsilateral spiking.
(B) MUA from supragranular, granular and infragranular layers.
(C) Infragranular MUA responses from 4 subjects are superimposed.

These data show that despite the pronounced recruitment of inhibitory interneurons, as demonstrated by distinct hyperpolarization, ipsilateral SI exhibited a small but definite evoked increase in spiking with an implication of an increase in energy consumption.

Ipsilateral increase in neuronal activity is accompanied by an increase in deoxyglucose uptake

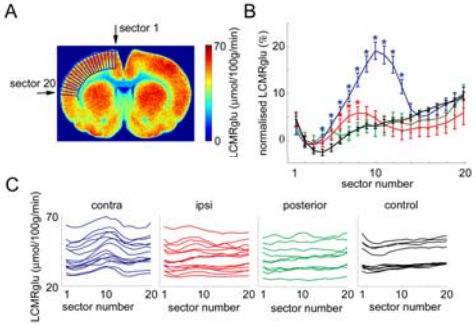

Figure 4. 2DG-autoradiography in response to a unilateral stimulus
(A) Sector map used for quantitative analysis is overlaid on a coronal brain section. The color scale is expressed in units of local cerebral metabolic rate of glucose, LCMRglu (μmol/100g/min).
(B) Cortical glucose utilization profile as a function of sector number. The direction is from close to the medial ridge (sector 1) to the most lateral point of the hemisphere where the horizontal dimension of the brain is the widest (sector 20). Profiles from contralateral (blue) and ipsilateral (red) hemisphere, posterior to the active area (green), and control subjects (no stimulus, black) are superimposed. The profiles extracted from each section have been normalized to the mean section intensity before averaging. Y-axis is expressed as % change relative to mean section intensity. Data points statistically significant from the control ($p<0.05$) are indicated by stars.
(C) Raw (not normalized) profiles extracted from contralateral hemisphere (blue), ipsilateral hemisphere (red), posterior to the active area (green), and control subjects (no stimulus, black). Each line represents one hemisphere. Subjects are superimposed on each plot.

These results indicate that ipsilateral increase in spiking is accompanied by small but significant increase in glucose consumption. The finding of the increased ipsilateral glucose consumption is surprising given a decrease in blood flow and oxygenation, and challenges the classical view of tight coupling between blood flow and glucose metabolism.

Arteriolar vasoconstriction underlies ipsilateral decreases in blood flow

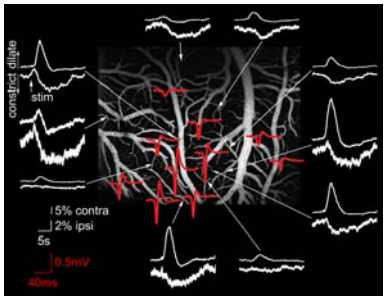

Figure 5. Arteriolar diameter change in response to contra- and ipsilateral stimulus
White traces show percent diameter change relative to the baseline (Δd/d) at different locations indicated by arrows. At every location the upper and lower trace in a pair represents the response to the contra- and ipsilateral stimulus respectively. Dilation is plotted upward, constriction - downward. The center of the neuronal response was mapped on cortical surface using a ball electrode. Surface potential recordings from different locations are shown in red. The strongest amplitude and fastest rise time indicate the center. The traces are overlaid on 2-photon image of vasculature within the exposure. The image was calculated as a maximum intensity projection of an image stack of 0-300 μm in depth. Individual images were acquired every 10 μm. The horizontal dimension is 3.2 mm.

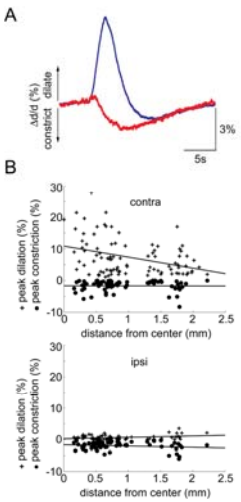

Figure 6. Comparison of arteriolar dilatation and constriction in response to contra- and ipsilateral stimulus
(A) An average of arteriolar diameter changes (Δd/d) in response to contralateral stimulus (blue) and ipsilateral stimulus (red). All measured vessels from all subjects are averaged. Dilation and constriction are plotted upward and downward respectively.
(B) Peak dilation (crosses) and peak constriction (circles) as a function of distance (in mm from the center of evoked neuronal response) in response to contralateral stimulus (top panel) and ipsilateral stimulus (bottom panel). Each dot represents a measurement from a single arteriole. Data from 7 subjects are superimposed.

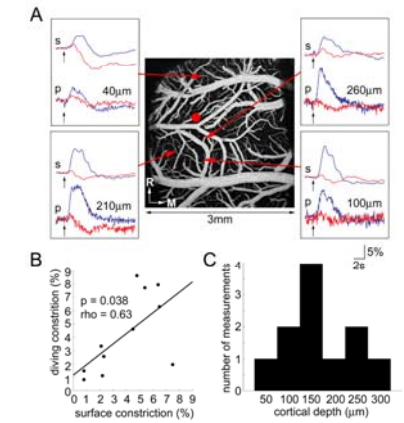

Figure 7. Comparison of surface and diving arterioles
(A) Diameter changes (Δd/d) in response to contralateral stimulus (blue) and ipsilateral stimulus (red) at 4 locations were measured in one subject. At every location the measurement was made from a parent surface arteriole (s, top pair of traces) and a penetrating (diving) arteriole (p, bottom pair of traces). Dilation and constriction are plotted upward and downward respectively. Black arrows indicate stimulus onset. Red arrows point to specific surface arterioles from which the measurements were made. Red circle shows the center of neuronal response. Depth (in μm) is indicated next to each penetrating arteriole. The image of the vasculature was calculated as a maximum intensity projection of an image stack of 0-300 μm in depth. Individual images were acquired every 10 μm.
(B) Peak ipsilateral constriction of diving arterioles as a function of that of a parent surface branch. Amplitude was normalized using dilation in response to the contralateral stimulus. Data from 3 subjects are superimposed.
(C) Depth distribution of the measured diving arterioles.

These data taken together indicate that arteriolar vasoconstriction underlies the decrease in blood oxygenation and flow observed in ipsilateral SI using spectral/speckle imaging (Fig. 1).