 Weak correlations between hemodynamic signals and ongoing neural activity during the resting state

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Spontaneous fluctuations in hemodynamic signals in the absence of a task or overt stimulation are used to infer neural activity. We tested this coupling by simultaneously measuring neural activity and changes in cerebral blood volume (CBV) in the somatosensory cortex of awake, head-fixed mice during periods of true rest and during whisker stimulation and volitional whisking. We found that neurovascular coupling was similar across states and that large, spontaneous CBV changes in the absence of sensory input were driven by volitional whisker and body movements. Hemodynamic signals during periods of rest were weakly correlated with neural activity. Spontaneous fluctuations in CBV and vessel diameter persisted when local neural spiking and glutamatergic input were blocked, as well as during blockade of noradrenergic receptors, suggesting a non-neuronal origin for spontaneous CBV fluctuations. Spontaneous hemodynamic signals reflect a combination of behavior, local neural activity, and putatively non-neural processes.

Spontaneous hemodynamic signals are extensively used in resting-state functional MRI (fMRI) studies to infer neural activity not driven by tasks or stimuli1,2. A cornerstone assumption of these studies is that spontaneous hemodynamic signals are coupled to neural activity in the same manner as hemodynamic signals elicited by sensory stimuli. Recent studies have cast doubt on the one-to-one coupling of neural activity to hemodynamic signals during sensory stimulation3–5, making it critical to determine how spontaneous hemodynamic signals are coupled to neural activity. To determine what aspects of neural activity are reported by spontaneous hemodynamic signals during different states, we simultaneously measured neural activity6 and CBV using intrinsic optical imaging7,8 in the vibrissal cortex of awake, head-fixed mice2,9 (Fig. 1a,c and Supplementary Fig. 1a) while monitoring whisker and body movements. Increases in CBV, which show up as decreases in reflectance in intrinsic optical imaging signals, are caused by arterial and capillary dilations7,9,10 and lead to increases in oxygenation, which can be detected with blood oxygen level–dependent (BOLD) fMRI11,12. Although our optical signals originated from the superficial layers, CBV changes have similar dynamics and amplitudes throughout the depth of cortex13, and CBV increases are tightly related to BOLD fMRI signals14.

Results

Behavior drives spontaneous hemodynamic fluctuations

Since humans and animals continuously engage in small bodily motions15,16 and actively sense their environment1, we monitored body movement and whisker position to detect periods of active behavior and rest (Fig. 1f and Supplementary Fig. 1b). We obtained an average of 254 ± 54 min of simultaneous CBV, neural, and behavioral data from each mouse (n = 12 mice; Fig. 1b). We categorized the data into several states: stimulation of the whiskers (contralateral to the window) with brief, gentle puffs of air directed towards the whiskers but not the face1; volitional whisking; and rest (Fig. 1b,f and Online Methods). Periods of time greater than 10s in duration without stimulation in which the animals did not volitionally whisk were defined as true rest. Periods of rest lasting less than 10s were considered transitions between behaviors and were omitted from subsequent analyses. Extended (>2 s) volitional whisker movements were analyzed separately from brief volitional movements since they were generally associated with additional body motion and distinct vascular responses1. We also used auditory stimulation (air puffs aimed away from the body) and stimulation of ipsilateral whiskers, but as these stimuli primarily elicited volitional whisking behavior (Supplementary Fig. 1b), we focus below on rest, volitional whisking, and stimulation of the contralateral whiskers in subsequent analyses.

We first asked whether there were any differences in the amplitude of hemodynamic fluctuations across these different states, as nonstationarities in variance have been postulated to account for most of resting-state connectivity17, though this nonstationarity has been argued to be spurious and driven by head movement and/or sampling variability18. As our signals were unaffected by head motion and as we can parse our data into identified behavioral epochs, we can directly address this issue in our system. The variance of hemodynamic signal fluctuations in our mice was significantly and substantially larger during all of these states than at rest (Fig. 1e). These results show that, in the awake brain, active sensation and volitional movements drive substantial changes in hemodynamic signals, and variability in the amount of these behaviors contributes to the large trial-to-trial variability observed in hemodynamic signals ‘at rest’17,19. While our observation that the variance in the CBV signal differed among behavioral states does not rule out statistical or head-motion-related artifacts in generating or contaminating dynamic connectivity patterns seen in human fMRI18, it shows that the amplitude of spontaneous hemodynamic signals will be affected by behavioral state.

Correlations between spontaneous neural activity and CBV changes

We then examined what aspect of neural activity was correlated with the observed CBV changes. Electrophysiology is considerably more sensitive to low levels of neural activity than calcium imaging, which fails to detect a substantial proportion of the action potentials, particularly at low firing rates20, and which cannot detect local field potential (LFP) oscillations. Consistent with previous measures showing a relatively tight spatial relationship between CBV changes and neural activity21, we observed that the measured reflectance from pixels near the stereotrode recording site was correlated...