Title: Behavior and arousal state controls neurovascular coupling

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**Abstract**

Hemodynamic signals in the brain are used to infer neural activity, and bilateral correlations in hemodynamic signals have been observed in the absence of any overt stimulus or task. However, recent studies have suggested that the nature and strength hemodynamic signals depend on arousal state. Here, we monitored neural activity and hemodynamic signals in un-anesthetized, head-fixed mice to understand how sleep and wake states impact cerebral hemodynamics. In parallel with electrophysiological recordings, we used intrinsic optical signal imaging to measure bilateral changes in cerebral blood volume (CBV) as well as two-photon laser scanning microscopy (2PLSM) to measure behavior-dependent dilations in individual arterioles. We concurrently monitored body motion, whisker movement, muscle EMG, cortical LFP, and hippocampal LFP to classify the arousal state of the mouse into awake, NREM sleep, or REM sleep. We found that mice regularly fell asleep for a few minutes at a time during imaging. During both NREM and REM sleep, mice showed large increases in CBV relative to the awake state. During NREM sleep, the amplitude of bilateral low-frequency oscillations in CBV increased markedly. Bilateral correlations in neural activity and CBV were highest during NREM sleep, and lowest in the awake state. Our results show that hemodynamic signals in the cortex are strongly modulated by arousal state and emphasize the importance of behavioral monitoring during studies of spontaneous activity. **Add in abstract: During NREM sleep, IOS signals are X times bigger and arteriole dilations are Y times bigger. Same for REM, etc**

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**Introduction**

Sleep is a ubiquitous state across animals (Anafi et al., 2019). In mammals, sleep is driven by an ensemble of brain nuclei that propagate a complex network of brain-wide interactions (Pace-Schott and Hobson, 2002; Sakai, 2019; Saper et al., 2010). In mammals (Cirelli, 2009), broadly sleep is comprised of two stages: non-rapid eye movement (NREM or slow-wave sleep) and rapid eye-movement (REM or paradoxical sleep) (Weber and Dan, 2016). Each of these states is associated with distinct patterns of electrical activity in the brain. During NREM sleep, there are broad-band increases in the local field potential power that are modulated at < 1 Hz (Amzica and Steriade, 1998; Datta and MacLean, 2007; Saper and Fuller, 2017). During REM sleep in the cortex, gamma band power (nominally 40-100Hz) is elevated, and there is a suppression of power at lower frequencies (CITE). In the hippocampus, REM sleep is associated with a marked increase in power in the theta band (nominally 7-10 Hz) (Montgomery et al., 2008; Sullivan et al., 2014).

While the dynamics of neural activity in the cortex and many other brain structures during sleep are well characterized, the cerebrovascular manifestations of sleep are less clear. Pioneering studies using positron-emission tomography (PET) or 133Xenon in humans suggested that cerebral blood flow (CBF) and metabolism is reduced during NREM sleep compared to afternoon waking levels, and increased above said levels during REM sleep (Braun et al., 1997; Townsend et al., 1973), though the temporal and spatial resolutions of these techniques are poor. The degree to which CBF changes during the different sleep states appears dependent upon brain region (Madsen et al., 1991; Maquet and Phillips, 1998), complicating the interpretation of functional connectivity studies looking at correlations between brain regions where a number of subjects may be constantly transitioning between arousal states. However, several fMRI studies have shown significant alterations in hemodynamic signals and functional connectivity mapping during NREM sleep (Boly et al., 2012; Dang-Vu et al., 2008; Fukunaga et al., 2006; Horovitz et al., 2008; Larson-Prior et al., 2009; Mitra et al., 2015) suggesting that the blood oxygen level dependent (BOLD) signal increases during sleep, though changes in the BOLD signal can have many underlying. However, because BOLD signals are generated by a complicated interplay of cerebral metabolism and change in blood flow and volume (Kim and Ogawa, 2012), the vascular basis of these changes and their relation to neural activity are not well understood.

Understanding the vascular basis of the hemodynamic changes during sleep is relevant to many aspects of brain health and function. First, BOLD signal changes during sleep are associated with movement of cerebrospinal fluid (CSF) (Fultz et al., 2019), and the movement of CSF is thought to play an important role in maintaining brain health (Simon and Iliff, 2016; Tarasoff-Conway et al., 2015; Xie et al., 2013). Elucidating the vascular changes associated with these fluid movements would resolve the actual drivers of fluid movement. Secondly, there is accumulating evidence that arousal state transitions drive large hemodynamic changes, both in animals performing tasks (Cardoso et al., 2019), and in humans and animals undergoing resting-state studies (Chang et al., 2016; Liu, 2016; Liu et al., 2018; Tagliazucchi and Laufs, 2014). If the hemodynamic signals during the periods of altered arousal are large or correlated enough, the activity during the sleep states could dominate the functional connectivity signal. Complicating these studies is the issue that head fixed mice do not close their eyes during NREM and REM sleep (Yüzgeç et al., 2018), meaning without careful monitoring or a task, it is possible that many neurovascular studies examining resting state in head-fixed mice may be contaminated with sleep.

Here we measured neural activity, blood volume and arterial dilations from head-fixed mice during the waking state as well as NREM and REM sleep. We found that the dilations and blood volume changes during NREM and REM sleep could be five to ten times larger than those occurring in the awake animal. The correlations between neural activity and hemodynamic signals was greatly increased during NREM sleep, and the functional connectivity between interhemispheric regions of somatosensory cortex also increased.

**Results**

We used intrinsic optical signal (IOS) (Huo et al., 2014; Winder et al., 2017) (14 mice) and 2-photon microscopy (Drew et al., 2011; Shih et al., 2012a) (6 mice) in concert with electrophysiology to measure neural activity (Buzsáki et al., 2012; Harris et al., 2016) from the whisker representation of somatosensory cortex and the CA1 region of the hippocampus in un-anesthetized, head-fixed C57/BL6J mice (Figure 1a). We obtained 361.5 total hours of data from these mice (mean: 23 ± 5.1 hours per mouse from IOS mice, 6.6 ± 2 hours per mouse from 2-photon imaged mice, supplemental Figure X). All experiments were performed during the animal’s light cycle period. We tracked whisking, body movement, and nuchal muscle EMG (CITE) (Datta and MacLean, 2007) (Fig 1X), as spontaneous ‘fidgeting’ behaviors drives the majority of neural activity and hemodynamic signals in the awake mouse (Drew et al., 2019; Musall et al., 2019; Stringer et al., 2019), and these signals can be used to determine the arousal state of the animal. We used an automated algorithm to determine the arousal state from these behavioral measures and hippocampal and cortical LFPs (see Methods), categorizing every five second interval into one of three categories: not asleep, NREM sleep, or REM sleep. Not asleep periods were further characterized into “true” rest and whisking conditions. After mice were habituated to head fixation after a week, data was acquired from each mouse for 3-6 days. All reported values are mean±standard deviation unless otherwise indicated.

**Sleep drives larger fluctuations larger than awake behaviors**.

We first examined how arousal state impacts hemodynamic signals using intrinsic optical signal imaging. We measured a combination of the changes in cerebral blood volume in the form of total hemoglobin (∆Hbt), local field potentials (LFP) in the associated brain region’s superficial layers of cortex, and an assortment of behavioral measurements to actively classify arousal-state (Fig. 1a). We measure changes in total hemoglobin through intrinsic optical signal (IOS) imaging, (Huo et al., 2014; Sirotin and Das, 2009; Vazquez et al., 2014), which detects changes in total hemoglobin (∆Hbt) from changes in reflectance, with periods of awake rest lacking whisking be set as the zero baseline (see methods). Decreases in reflectance indicating increases in blood volume, which can be converted into hemoglobin changes (∆Hbt) using the Beer-lamber law (Ma et al., 2016). Periods of These hemodynamic measurements were done through a polished and reinforced thinned-skull window (Fig 1 A) (Drew et al., 2010a; Shih et al., 2012b) encompassing the whisker-related section of the somatosensory cortex (Petersen, 2007). LFP was recorded from the superficial layers of barrel cortex underneath the window to provide a direct measurement of neural activity (Winder et al., 2017). Windows and cortical electrodes were implanted bilaterally to provide neural and hemodynamic measurements from whisker-related portions of somatosensory cortex from both hemispheres. To assist in arousal-state classification, we also recorded the putative CA1 hippocampal LFP, nuchal muscle electromyography (EMG), motion of the whiskers, and body motion, and heart rate.

As a check of the sensory evoked responses, at the beginning of each day’s imaging session, we stimulated the contralateral vibrissae with a brief puff of air drove canonical neural and vascular responses (Fig 1b, Supplemental Fig. X,Y). Consistent with previous work (CITE Winder), gamma band power in the somatosensory cortex (30-100 Hz) increased by ~50%, followed by a 10-10µM increase in HbT (corresponding to a ~2% decrease in reflectance, see Supplemental Fig. X,Y).

In comparison to the sensory evoked-responses, we found much larger changes in Hbt associated with sleep. NREM sleep is characterized by low EMG activity, lack of whisker and body movement, and pronounced power in the low frequency bands of the cortical LFP (Fig 1c) (CITES). REM sleep is characterized by neck muscle atonia, whisker movement, and increased theta-band power in the hippocampus (Fig 1c)(CITES). During the sleep stages Examples of sleep-related changes in blood volume are shown in Fig 1B and supplemental figures XYZ. During NREM sleep, we observe large oscillations in total Hbt, up to 100µM HbT in peak-to-peak amplitude. During REM bouts, there is a prolonged (>30 seconds in duration) increase of Hbt of up to 100 µM or more. Note that as previously observed (Yüzgeç et al., 2018), the eyes of the mouse are open during these sleep states (Fig 1c) . These results show that sleep in headfixed mice is associated with large increase in blood volume, particularly REM sleep.

**Mice regularly enter into NREM and REM sleep during head-fixation.**

We then asked how often headfixed mice sleep and how the probability of sleep varies with time form the start of headfixation. A hypnogram for a single mouse with 5 second resolution over 5 days is shown in Fig 2a. The white lines denote breaks in recording while data is saved to disk. The initial half hour of head fixation, where the mouse stimulated is omitted. It is clear that the mouse has many periods of NREM and REM sleep interspersed with wake. Periods of REM sleep always follows NREM sleep (CITE), and REM periods are usually followed by awakening, Though REM sleep could be followed by NREM periods. Plotting the probability of finding the mouse in each of the state as a function of time in the imaging session (Fig 2B) shows that as time goes by the mouse is more likely to be asleep. As awake mice typically whisk every ~10 seconds, we quantified the probability that the mouse had fallen asleep after a given period of lacking whisking and body movement (‘Rest’) (Fig. 2C) across all animals. We found that during only 50% of ‘resting’ events lasting 10-15 seconds were the mice awake for the whole event, and with longer events showing even lower probability of wakefulness throughout the period (Fig. 2c). This result is reminiscent of studies with humans where the probability of being awake falls rapidly with time during a resting-state fMRI scan (CITE), though as human sleep/wake behavior is much less fragmented than in the mouse this transition time is longer. EMG activity (Fig 2D) was much lower during sleep than wake states, though the amount of whisking (quantified as the variance in the whisker angle) was similar in the awake state and REM sleep. Heart rate was lowest during NREM sleep (Fig. 2 F), though heartrate during REM was comparable to heartrate in awake, nonwhisking mouse, heart rate was slightly elevated by whisking (Fig 2G). These observations underscore the potential prevalence of sleep in ‘resting-state’ data in head-fixed mice and how unimodal measures of whisking or heart rate fail are insufficient to detect these sleep states.

**Sleep drives arteriole dilatations much larger than those seen in the awake brain.**

The intrinsic signal contains contributions from arteries, veins, and capillaries (CITE Huo 2015a and b; Zhang 2019), so to better understand how arterioles changes contribute to the signal, we used two photon microscopy from single vessels (Fig 3 A). We recorded hippocampal and cortical LFP contralateral to the imaging window. Two-photon imaging in awake mice has shown that sensory stimulation and locomotion drive dilations of arteries of approximately 20% (CITE Drew 2011; Huo 2015) and whisking drives dilations of ~5-10 % (Drew/Kleinfeld NEURON review). We observed a similar 5-10% dilation with spontaneous whisking events (Fig 3B). In comparison to the awake state, arteriole diameter during NREM sleep follows a rhythmic dilation/constriction with peaks that can exceed even the longest whisking events (Supp fig X). During REM sleep, the arterioles slowly dilate over tens of seconds, and can reach peak dilations in excess of 50% of the baseline diameter during periods of awake rest. The dilation amplitudes during NREM and REM sleep dwarf those seen in the awake animals.

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**Changes in hemodynamics are consistent with transitions between arousal-states.**

To quantify the dynamics of sleep-related changes in blood volume and arterial diameter, we looked at the dynamics of these signals, as well as LFP and EMG signals, aligned to the arousal state change (Fig. 4).We used transitions between two arousal states where time in each arousal state was at least 30 seconds. Without confounding factors (such as sleep deprivation, illness, certain medication), mammals will typically progress through the Awake-NREM-REM-Awake pattern of the sleep cycle, even if the awake periods between the end of a REM event and the initiation of the next NREM period are very brief. The transition from the awake state into NREM (Fig. 4a) shows an increase in total hemoglobin from the baseline of 0µM Hbt in the awake resting state up to 30 µM over the course of 30 seconds. During this time, the EMG power decreases with similar temporal dynamics. The LFP power in the barrel cortex shows increased power in the delta-band power [1-4 Hz] of around 300%. The transition from NREM into the awake state (Fig. 4b) is largely a temporally reversed version of the awake to NREM transition, where Hbt, LFP and EMG signals all quickly return to baseline values as the animal wakes up. The transition from NREM to REM (Fig. 4c) shows a slow increase in total hemoglobin from 45 µM up to 75 µM, and this increase can take several minutes to peak. The muscles become atonic and the EMG power decreases even more. The LFP in the hippocampus theta-band power [4-10 Hz] increases by around 300%. The transition from REM into the awake state (Fig. 4d) is the largest in terms of total hemoglobin change, as the large blood volume increase seen during REM rapidly reverses (within seconds) as the animal wakes up. Transitions from awake to REM as well as REM to NREM are possible, but are much less common, and did not occur often enough to quantify reliably. At the level of single arterioles, during the transition from NREM to REM will on average go from about 20% dilation up to 40% after a minute (Fig. 4e) and decrease back to baseline upon waking up from REM (Fig. 4f).

**Cortical hemodynamic signals increase during NREM and REM sleep.**

We quantitatively compared the degree to which hemodynamic signals increase during different arousal states. For this quantification, we used awake resting events ≥ 10 sec in duration, awake whisking events 2-5 seconds in duration, NREM sleep events ≥ 30 seconds in duration, and REM sleep events ≥ 60 seconds in duration. All ± indicate s.d. and all statistical values are in comparison to awake rest and corrected for multiple comparisons. The average ∆[HbT] during awake resting data was 0.8 ± 1.5 µM (Fig. 5a) (N = 14 mice, n = 28 hemispheres). It was not zero because of XXXXXX. Awake volitional whisking events between 2 and 5 seconds in duration caused an increase in [HbT] of 8.6 ± 5.6 µM (p < 0.005). This small increase was dwarfed by those that occurred during NREM sleep, 31.5 ± 11.4 µM (p < 0.001) and during REM sleep, 76.4 ± 12.8 µM (p < 0.001). The probability distribution of the mean ∆[HbT] of all individual arousal-state events taken together, (without averaging within animals or hemispheres) is shown in Fig. 5d. There is a clear and pronounces separation between the sleep and awake states, as well as between REM and NREM sleep.

A similar analysis during each arousal-state for the peak arteriole diameter during each arousal event measured with two photon microscopy follows the same trend as the ∆[HbT] data (Fig. 5b). The average peak in vessel diameter ∆D/D (%) during awake resting events (N = 6 mice, n = 29 arterioles) was 7.4 ± 2.6%. Awake volitional whisking events spurred a mean peak diameter increase of 17.0 ± 5.4 % (p < 0.001), with NREM sleep dilating up to 26.4 ± 10.1% (p < 0.001, N = 6 mice, n = 21 arterioles) and REM sleep reaching 50.0 ± 9.1% (p < 0.001, N = 5 mice, n = 13 arterioles). The probability distribution of the peak ∆D/D of all individual arousal-state events taken together without averaging within or between animals is shown in Fig. 5e. Recordings of changes in bulk fluid flow through laser doppler flow velocimetry ∆Q/Q again follow the same trend with flow above the vibrissa barrels increasing in each subsequently described arousal-state (Fig. 5c) (N = 6 mice). Average flow during awake rest was -1.8 ± 1.8% and elevated slightly to 7.1 ± 7.7% (n.s.) during volitional whisking. Flow changes were more apparent during NREM sleep, 16.0 ± 10.5% (p < 0.05), and even more-so during REM sleep, 32.1 ± 17.9% (p < 0.001). The probability distribution of the average ∆Q/Q of all individual arousal-state events taken together without averaging within or between animals is shown in Fig. 5f. These different methods all show a consistent, highly significant trend showing how hemodynamic fluctuations in somatosensory cortex increase substantially during sleep, and far surpassing those seen during common volitional behaviors.

**Flow simulations TBD.**

**Neural and hemodynamic correlations between hemispheres increase during sleep.** Need to discuss how to present data/numbers for coherence/power spectra in these figures. 95% confidence or standard deviation or bootstrapping.

**Neurovascular coupling increases during NREM sleep.** It has previously been shown that neurovascular coupling is similar across awake behavioral states (Winder et al., 2017) and that both spontaneous and sensory-evoked hemodynamics are most strongly correlated with gamma-band power and MUA during awake rest(Schölvinck et al., 2010). To expand upon the neurovascular relationship during different behavioral states, we looked at the relationship between the envelope of neural activity and ∆[HbT] during periods of NREM and REM sleep. Resting correlations between MUA and ∆[HbT] as well as LFP and ∆[HbT] are consistent with previous findings and show a peak correlation of ~0.25 at a lag time of 1-2 seconds. The peak correlations in the frequency-dependent LFP highlight the gamma-band [30-100 Hz]. The peak cross-correlation during NREM sleep of ~0.4 is nearly double that of awake rest, with different spectral characteristics of the correlated frequencies and temporal characteristics of the lag time. In addition to a highly correlated gamma-band, the correlations extend down into the beta-band [13-30 Hz] which extends in lag-time between ± 2 seconds, largely due to the repeated and oscillatory nature of hemodynamic oscillations during sleep. Cross correlations during REM sleep imitate those during awake rest, but with a markedly blurred temporal resolution. They reach a peak cross-correlation of around ~0.2 at 1-2 seconds but remain lightly correlated ~0.05-0.1 through ± 5 seconds.

**Discussion**

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Figure 3b show artery at baseline and peak of whisk

**Main figure legends (See attached PDF)**

**Fig. 1 ﻿| Sleep drives hemodynamic fluctuations larger than awake behaviors. a**, Schematic of IOS experimental setup and key recording sites. **b**, Average response to contralateral whisker stimulation (N = 14 mice, n = 28 hemispheres). Top: average normalized change in LFP power in response to contralateral whisker stimulation. Bottom: average change in total hemoglobin within the ROI. Shaded regions indicate population s.d. **c**, Example data from a continuous 10-minute period. From top to bottom: Nuchal muscle activity through normalized EMG and body motion through a pressure sensor. Whisker motion tracking and heart rate frequency. Changes in total hemoglobin within mirrored 1 mm ROIs over the putative vibrissa barrel cortex. Normalized LFP power from the left hemisphere stereotrode located within the left vibrissa ROI. Normalized LFP power from the right hemisphere stereotrode located within the right vibrissa ROI. Normalized LFP power from the putative CA1 stereotrode from the left hemisphere hippocampus.

**Fig. 2 ﻿| Mice reliably lose consciousness during head-fixed imaging. a**, Individual 5-second bin arousal-state scores from a random forest classification algorithm from the first 3 hours of data collection in a single animal over 6 imaging sessions on different days. **b**, Average probability of an animal being classified in a given arousal-state as a function of time (N = 14 mice for b-g). **c**, Average probability of a quiescent event with no whisker motion or body motion (pressure sensor), a previous metric we used to classify awake rest, of being classified as ‘Awake’ as function of the quiescent event’s duration. **d**, Probability distribution of the mean EMG power during individual arousal-scores (5 sec bins) taken from all animals. **e**, Probability distribution of variance in the whisker angle during individual arousal-scores (5 sec bins) taken from all animals. **f**, Probability distribution of the mean heart rate during individual arousal-scores (5 sec bins) taken from all animals. **g**, Mean heart rate during different behaviors. Circles represent individual mice and diamonds represent population averages ± s.d. (N = 14 mice)

**Fig. 3 ﻿| Sleep drives arteriole dilatations greater than awake behaviors. a**, Schematic of 2PLSM experimental setup and key recording sites. **b**, Average arteriole diameter response to volitional whisker motion events persisting for between 2 and 5 seconds (N = 6 mice, n = 29 arterioles). Shaded regions indicate population s.d. **c**, Example data from a continuous 10-minute period. From top to bottom: Nuchal muscle activity through normalized EMG and body motion through a pressure sensor. Whisker motion tracking. Changes in arteriole diameter of small segment of the middle cerebral artery (MCA) over the right hemisphere putative vibrissa barrel cortex. Normalized LFP power from the left hemisphere stereotrode located within the left vibrissa barrel cortex (contralateral hemisphere to vessel measurement). Normalized LFP power from the putative CA1 stereotrode from the left hemisphere hippocampus.

**Fig. 4 ﻿| Changes in hemodynamics are consistent with transitions between arousal-states. a**, Transition from periods classified as awake into periods classified as NREM. Top: Average change in total hemoglobin within the ROI coplotted with the normalized change in EMG power. Shaded regions indicate population s.d. (N = 14 mice, n = 28 hemispheres). **b**, Transition from periods classified as NREM into periods classified as awake. **c**, Transition from periods classified as NREM into periods classified as REM. **d**, Transitions from periods classified as REM into periods classified as awake. **e**, Mean arteriole diameter during the transition of NREM into REM (N = 5 mice, n = 8 arterioles). **e**, Mean arteriole diameter during the transition from REM into the awake state (N = 5 mice, n = 8 arterioles). Shaded regions indicate population s.d.

**Fig. 5﻿ | Cortical hemodynamic signals increase during NREM and REM sleep. a**, Average change in total hemoglobin within the ROI during the duration of different arousal-states. Circles represent individual hemispheres of each mouse and diamonds represent population averages ± s.d. (N = 14 mice, n = 28 hemispheres). **b**, Average change in peak arteriole diameter during the duration of different arousal-states. (N = 6 mice, n = 29 arterioles for rest/whisk; N = 6 mice, n = 21 arterioles for NREM; N = 5 mice, n = 13 mice for REM). **c**, Average change in laser doppler flow velocimetry during the duration of different arousal-states. (N = 6 mice). **d**, Probability distribution of the change in total hemoglobin from all events taken together from all animals. **e**, Probability distribution of the peak arteriole diameter from all events taken together from all animals. **f**, Probability distribution of the laser doppler flow velocimetry from all events taken together from all animals.

**Fig. 6 ﻿| TBD**

**Fig. 7 ﻿| Neural and hemodynamic correlations between hemispheres increase during sleep. a**, Avearge low-frequency coherence in the changes in total hemoglobin between mirrored regions of vibrissa cortex during different arousal-states (N = 14 mice). **b**, Average power spectral density during different arousal-states (N = 14 mice, n = 28 hemispheres). **c**, Average Pearson’s correlation coefficient between mirrored regions of vibrissa cortex during different arousal-states. Circles represent individual mice and diamonds represent population averages ± s.d. (N = 14 mice). **d-f,** Same as in a-c except for the envelope (≤ 1 Hz) of the gamma-band power [30-100 Hz].

**Fig. 8 ﻿| Neurovascular coupling increases during NREM sleep.** Average cross-correlation between neural activity and changes in total hemoglobin during different arousal-states. MUA power [300-3000 Hz] (top) and the LFP [1-100 Hz] (bottom) were consistently correlated with hemodynamics at varying degrees. **a**, Awake rest. **b**, NREM sleep. **c**, REM sleep. Shaded regions indicate population s.d. (N = 14 mice, n = 28 hemispheres).

**Fig. 9 ﻿| Influence of arousal-state on vascular correlations to ongoing neural activity.** Our awake resting data and stimulus/whisking-evoked data is in line with previously published results showing their contributions to awake neural-hemo correlations. During NREM and REM sleep both low-frequency neural and hemodynamic signals increase far beyond those seen during the awake state. Despite this increase in signal variance from the awake baseline, the connection between ongoing neural activity and subsequent hemodynamic fluctuations is far more correlated during sleep than it is during the awake state.

**Methods**

Processed data is available at **[box link]** analysis code is available at [**github link]**.

**Animal procedures**

All procedures involving the use of animals were conducted in accordance and with approval from the Institutional Animal Care and Use Committee (IACUC) of Pennsylvania State University. All data was acquired from 20 C57BL/6J mice (Jackson Labs, strain 000664) comprised of 11 males and 9 females between the ages of 3 and 8 months of age. Of these 20 animals, 14 were used in IOS experiments and 6 were used in two-photon experiments. Any animals that were excluded from a specific analysis will be noted as such. Mice were given food and water ad libitum and housed on a 12-hr light/dark cycle, remaining individually housed after surgery and throughout the duration of experiments. All experiments were performed during the animal’s light cycle period. Sample sizes are consistent with previous studies(Drew et al., 2011; Huo et al., 2015; Winder et al., 2017). The experimenter was not blind to the conditions of the experiments, data, or analysis.

**Surgery**

*Electrode, EMG, and window implantation procedure for intrinsic optical signal (IOS) imaging experiments.* Mice were anesthetized under Isoflurane (5% induction, 2% maintenance) vaporized in oxygen during all surgical procedures. The incision site on the scalp was sterilized with betadine and 70% ethanol, followed by the resection of the skin and connective tissue. A custom-machined titanium headbar for head-fixation (<https://github.com/DrewLab/Mouse-Head-Fixation>) was adhered atop the occipital bone of the skull with cyanoacrylate glue (32402, Vibra-Tite) posterior to the lambda cranial suture. A self-tapping 3/32” #000 screw (J.I. Morris) was implanted into the center of each frontal bone. A stainless-steel wire ﻿(A-M Systems, #792800) was wrapped around one of the frontal screws to serve as a ground for neural recordings. Two ~4 mm x ~2 mm polished and reinforced thinned-skull windows were bilaterally implanted caudal to the bregma cranial suture above the left and right somatosensory cortices as previously outlined(Drew et al., 2010a; Shih et al., 2012b). The skull is thinned and then polished with 4F and 3F grit. A PFA-coated tungsten stereotrode ﻿(AM systems, #795500) is inserted ~700 μm in the z-axis into the whisker representation of somatosensory cortex (~2 mm caudal, ~3-3.5 mm lateral from bregma) at 45﻿° from the horizontal along the rostrocaudal axis. This is mirrored for left and right hemispheres. A third tungsten stereotrode is implanted ~1500 μm in the z-axis into the CA1 region of the left hippocampus (~2.5 mm caudal, 4-4.5 mm lateral from bregma) at 45° from the vertical along the mediolateral axis. Each electrode is positioned using a ﻿micromanipulator (Sutter Instruments, MP285) through a small craniotomy made at the edge of the thinned area for the barrel electrodes, and slightly caudal the thinned-area for the left hemisphere hippocampal electrode. Each craniotomy was sealed with cyanoacrylate glue, and a #0 coverslip (Electrode Microscopy Sciences, #72198) was placed atop the thinned portion of the window. The skin above the neck is resected and a pair of PFA-coated 7-strand stainless-steel wires (AM systems, 793200) were inserted into each nuchal muscle for EMG recording. The skin is then re-attached back to the edge of the occipital bone (3M, VetBond). Dental cement (Lang Dental, Ortho-Jet) was used to seal the edges of the window and provide structural rigidity to the electrodes, screws, and headbar.

*Electrode, EMG, and window implantation procedure for two-photon laser scanning microscopy (2PLSM) experiments.* As described previously, mice were anesthetized with isoflurane and the surgical site cleaned. A titanium headbolt is implanted in a similar fashion, along with two frontal screws and ground wire. A third self-tapping 3/32” #000 screw is implanted into the left parietal bone. Instead of bilateral polished and reinforced thinned-skull windows, a single ~4 mm x ~ 5mm window above the right hemisphere somatosensory cortex is implanted following thinning and polishing. There are no electrodes under the window, as they can scatter the laser and cause heating of the local tissue. Tungsten stereotrodes are implanted into the left hemisphere barrel cortex and left hemisphere hippocampus in a fashion similar to above. Stainless-steel EMG wires are implanted, and the entire area sealed with dental cement. Following surgery, animals were given 2-3 days to recover before habituation.

**Histology**

Following the conclusion of experiments, animals were heavily anesthetized under 5% isoflurane for several minutes. A transcardial perfusion with heparinized saline was performed for several minutes, followed by 4% ﻿paraformaldehyde. Fiduciary marks were made at the corner of each cranial window. The extracted brains were allowed to sit in a solution of 4% PFA/30% sucrose for several days before being coronally section (~60 μM per section) with a freezing microtome. Sections were stained for the presence of cytochrome oxidase (CO). The whisker barrels are visible in layer IV of the sections and allow the verification of the electrode’s presence in the tissue(Drew and Feldman, 2009; Shirey et al., 2015). The hippocampus is also brightly stained and allows the verification of the hippocampal electrode’s relation to CA1.

Coherence between hemoglobin and HbT for figure 9 summary figure

**Physiological data acquisition**

All data from IOS experiments were acquired with a custom LabVIEW program (2018, National Instruments, Austin TX). Data from 2PLSM experiments were acquired with ﻿Sutter MCS software (Sutter Instruments, Novato, CA) and a different custom LabVIEW program designed to synchronize with the Sutter MCS software. All IOS and 2PLSM experiments were perform in sound-dampening boxes. Both custom LabVIEW programs can be viewed at <https://github.com/DrewLab/LabVIEW-DAQ>

*Habituation.* All animals were gradually acclimated to being head-fixed over the course of three habituation sessions of increasing duration. In the initial session (15-30 min), animals were not exposed to any whisker stimulation and the efficacy of the cortical, hippocampal, and EMG electrodes was determined. Pending clean electrical signals and a tolerance of the head-fixation, animals were habituated for two more sessions of 60 and 120 minutes. During these subsequent sessions, the whiskers were stimulated with directed air puffs. Any animals who showed excessive, visible distress/struggling throughout the habituation were removed from future experiments. Following habituation, IOS animals were run for six imaging sessions lasting of 3-5 hrs, and 2PLSM animals were run for up to six imaging sessions depending on the quality of the thinned-skull window.

*Intrinsic optical signal (IOS) imaging.* Mice (n = 14) were briefly (< 1 min) anesthetized with 5% isoflurane and transferred to the head-fixation apparatus with the body being supported by clear plastic tube. Animals were given 30 minutes to wake up prior to data collection to give the vasculature time to return to baseline. Changes in total blood volume was measured by illuminating each cranial window with two collimated and filtered 530 ﻿± 5 nm LEDs (Thor Labs, FB530-10, M530L3). The 530 nm wavelength is an isosbestic point in which oxy- and deoxy- hemoglobin absorb the light equally. We use the changes in the amount of light reflected from the surface of the brain as a measurement of total hemoglobin concentration. The reflected light is imaged with a Dalsa 1M60 Pantera CCD camera (Phase One, Cambridge MA) positioned above the mouse’s head. The magnification of the lens allows simultaneous collection of data from both the left and right cranial windows. The light entering the camera (green) was filtered using a mounted filter (Edmund Optics, Barrington NJ, #46540) to remove the red light used in whisker tracking. Images for tracking changes in total hemoglobin (256 × 256 pixels, 15 µm per pixel, 12-bit resolution) were acquired at 30 Hz(Huo et al., 2015; Winder et al., 2017).

*Electrophysiology.* Neural activity was recorded simultaneously in both IOS and 2PLSM as the differential potentials between the two leads of either the PFA-coated tungsten microwires ﻿(A-M Systems, #795500)(Huo et al., 2014; Winder et al., 2017)for cortical and hippocampal stereotrodes. EMG activity was identically recorded with PFA-coated 7-strand stainless-steel microwires (AM systems, 793200). Stereotrode tungsten microwires were threaded through polyimide tubing ﻿(A-M Systems, #822200) with an interelectrode spacing of ~100 ﻿µm. The tungsten microwires were crimped to gold pin connectors, with impedances typically between 70 and 120 ﻿kΩ at 1 kHz. EMG stainless-steel microwires were fabricated in a similar fashion, but with an interelectrode spacing of several mm and much lower impedance, typically ~1 to 10 ﻿kΩ at 1 kHz **[double check]**. Each signal was amplified and hardware bandpass filtered between 0.1 Hz and 10 kHz ﻿(World Precision Instruments, DAM80) and then digitized at 20 kHz ﻿(National Instruments, Austin TX, PCIe-6341 for IOS experiments, PCIe-6321 and PCIe-6353 for 2PLSM experiments). The power in each neural band was calculated by digitally bandpass filtering the raw signal ﻿using a fourth-order Butterworth filter. Each result was squared, low-pass filtered below 10 Hz, and resampled from 20 kHz down to 30 Hz. (Matlab function(s): butter, zp2sos, filtfilt, resample).

*Two-photon laser scanning microscopy (2PLSM).* Mice (n = 6) were briefly (< 1 min) anesthetized with 5% isoflurane and retro-orbitally injected with 100 µL of 5% (weight/volume) fluorescein isothiocyanate–dextran (FITC) (Sigma-Aldrich, FD150S-1G) dissolved in sterile saline. Mice were then head-fixed in a similar set-up as during IOS experiments and given 30 min to wake up prior to data collection. Imaging was done on a Sutter Movable Objective Microscope with a Nikon CFI75 LWD 16X W objective. A MaiTai HP Ti:Sapphire laser (﻿Spectra-Physics, Santa Clara, CA) tuned to 800 nm was used to excite the FITC fluorophore infused in the vasculature. Individual pial **(n = #)** and penetrating **(n = #)** arterioles were imaged at a frame rate of 5 Hz in 15-min intervals at a power of 10-20 mW (measured exiting the objective). All arterioles measured were in somatosensory cortex and largely localized in or near the whisker barrel representation.

*Whisker stimulation.* IOS animals were stimulated with brief (0.1 sec) randomized, alternating puffs of air to either the left whisker pad, right whisker pad, or an auditory control for the first ~60 minutes of imaging. The puffs are directed to the distal ends of the whiskers, parallel to the face so as to avoid stimulating other parts of the body/face. Each puff was controlled through solenoid actuator valves ﻿(Sizto Tech Corporation, 2V025 1/4) set to 10 PSI via an air regulator ﻿(Wilkerson, R03-02-000). Each puff occurred at a 1:1:1 ratio and occurred once every 30 seconds.

*Behavioral measurements.* In both IOS and 2PLSM experiments, the right whisker pad was diffusely illuminated by either a 625 nm light ﻿(Edmund Optics, #66-833) during IOS experiments, or with a 780 nm LED (Thor Labs, M780L3) during 2PLSM experiments so as to not influence the photomultiplier tubes sensitive to visible light. In both experimental setups, a Basler ace acA640-120gm ﻿camera (Edmund Optics, Barrington NJ) acquired an image of the whiskers (30 x 350 pixels) at 150 frames per second. The image was narrow enough to only show the whiskers as dark lines on a bright background, with the average whisker angle being estimated using the radon transform(Drew et al., 2010b). More details on whisker tracking can be found at <https://github.com/DrewLab/Whisker-Tracking>. In addition to whisker tracking, animal motion inside the tube was measured using a pressure sensor ﻿(Tekscan, Flexiforce A201, Boston MA) was amplified ﻿(Brownlee Precision, Model 440 for IOS experiments, Stanford Research Systems Model SR560 for 2PLSM experiments) and digitized at 20 kHz by the same acquisition device(s) previously described for the electrophysiology data. For both whisker acceleration and pressure sensor data, a threshold was manually set to establish when the animal behaved. ﻿A basic webcam (Microsoft LifeCam Cinema for IOS experiments, ELP 2.8mm wide angle IR LED Infrared USB camera for 2PLSM experiments) was used to monitor the animal’s well-being during data acquisition via a real-time video stream in the LabVIEW data acquisition program.

*Pupil tracking.* Include and state that it wasn’t used, or ignore altogether?

**Data Analysis**

All data analysis was conducted with code written by K.L.T, R.T.K, K.W.G, and P.J.D (MathWorks, Matlab 2019a or newer) and **Mathematica code (etc)** written by R.T.K.

*Alignment of region of interest (ROI) over whisker barrel cortex in IOS data.* To focus on blood volume changes in the whisker representation of somatosensory cortex, a 1 mmdiameter circle was manually placed over the thinned-skull window’s region of pixels highest correlated to that hemisphere’s gamma-band power during the first fifteen minutes of data of each imaging session (Matlab function(s): butter, zp2sos, filtfilt, xcorr). For each animal (n = 14, hemispheres = 28) this region was typically located in the most caudal, lateral corner of the window consistent with the anatomical location of barrel cortex and implantation sight of the stereotrode, which remained consistent across all days of imaging. The location of the circular ROI and electrode was verified histologically through the alignment of the electrode path and fiduciary marks with respect to the layer IV CO stain. The reflectance in the circular ROI of pixels was averaged together. To correct a slow drift in the CCD camera’s sensitivity to light over several hours, a two-exponent function was fit to the slow drift of a region of interest over the cement. The profile of this exponential function was then used to remove the slow exponential drift of the mean pixel reflectance over time.

*Two-photon laser scanning microscopy imaging processing.* Individual stack frames from 2PLSM were corrected for x-y motion artifacts and aligned through a rigid registration algorithm.(Drew et al., 2011; Gao et al., 2015) Imaging periods with excessive z-plane motion artifacts were excluded from analysis. A rectangular box was manually drawn around a straight, evenly-illuminated segment of the vessel and the pixel extensity was averaged along the long axis; using the pixel intensity to calculate the vessel’s diameter from the full-width at half-maximum(Drew et al., 2011). The diameter of penetrating arterioles was calculated using the thresholded in Radon space (TiRS) algorithm.(Gao and Drew, 2014; Gao et al., 2015)

*Whisker motion quantification.* Images of the mouse’s whiskers were converted into a relative position (angle) by applying the Radon transform (Matlab function(s): radon). The peaks of the sinogram corresponded to the position and the angle of the whiskers in the image. The average whisker angle was extracted as the angle of the sinogram with the largest variance in the position dimension.(Drew et al., 2010b) Any camera frames that were lost dropped during data acquisition were patched at their approximate location using inward linear interpolation between adjacent events. Whisker angle was digitally low-pass filtered ( < 20 Hz) using a second-order Butterworth filter and then resampled down to 30 Hz (Matlab function(s): butter, zp2sos, filtfilt, resample). To identify periods of whisking, whisker acceleration was obtained from the second derivative of the position and binarized by the equation

where is the whisker acceleration at time , and is the empirically-defined acceleration threshold for a whisking event. Ambiguous acceleration events that occurred within 0.33 seconds of each other were linked and considered as a single whisking bout.

*Movement quantification.* Movement data from the pressure sensor was digitally low-pass filtered (< 20 Hz) using a second-order Butterworth filter and then resampled down to 30 Hz (Matlab function(s): butter, zp2sos, filtfilt, resample). To identify movement events, the force sensor data was binarized in a similar fashion to that of the whisker acceleration by setting an empirically-defined threshold.

*Heart rate detection.* During IOS experiments, the heart rate was detected through the time-frequency spectrogram (3.33 sec window, 1 sec step size, [2,3] tapers) of the hemodynamic signal (Chronux toolbox, version 2.12 v03). The heart rate was identified as the frequency with the maximum spectral power in the 5-15 Hz band. This signal was then averaged between the two hemispheres, and digitally low-pass filtered (< 2 Hz) using a third-order Butterworth filter (Matlab function(s): butter, filtfilt).

*Neural data and spectrograms.* Neural signals (cortical and hippocampal) were subdivided into frequency bands as follows: delta [1-4 Hz], theta [4-10 Hz], alpha [10-13 Hz], beta [13-30 Hz], gamma [30-100 Hz], and multi-unit activity (MUA) [300-3000 Hz]. Each neural signal was digitally band-pass filtered from the raw data using a third-order Butterworth filter. The data was then squared and smoothed (< 10 Hz) using a third-order Butterworth filter, and resampled down to 30 Hz (Matlab function(s): butter, zp2sos, filtfilt, resample). Several sets of time-frequency spectrograms with varying characteristics were calculated for each neural signal to be utilized in different analysis (Chronux toolbox, version 2.12 v03, function: mtspecgramc). A 5-second window with 1/5 second step size and [5,9] tapers, a 1-second window with 1/10 second step size and [1,1] tapers, and a 1-second window with 1/30 second step size and [5,9] tapers. Each set had the same pass-band of 1 to 100 Hz to encompass the local field potential (LFP).

*Electromyography (EMG).* Electrical activity from the nuchal (neck) muscles was digitally band-pass filtered (300 Hz – 3 kHz) using a third-order Butterworth filter. The signal was then squared and convolved with a 0.5 second Gaussian kernel, log transformed (base 10), and resampled down to 30 Hz (Matlab function(s) butter, zp2sos, filtfilt, gauswin, log10, conv, resample).

*Laser doppler flow velocimetry (LDF).* Microvascular perfusion data was resampled down to 30 Hz and digitally low-pass filtered (< 1 Hz) using a fourth-order Butterworth filter (Matlab function(s) butter, zp2sos, filtfilt, resample).

*Establishment of awake rest and baseline.* Periods of quiescent rest were identified during each day’s imaging session and were unique for each vessel during 2PLSM experiments. Rest was established as periods with an absence of whisker stimulation, whisker movement, or detectable body movement of 10 seconds or greater for IOS experiments and 5 seconds or greater for 2PLSM experiments (due to having less data per vessel, as well as the absence of the slow venous component in the arteriole signal). In order to establish an accurate awake resting baseline and exclude any drowsy or sleeping data, long periods of wakefulness (typically > 60 sec) were manually identified in each file (if present) based on behavioral and spectral characteristics. Only data from these pre-screen periods of clear wakefulness were used in subsequent baseline calculation and ‘Rest’ behavior comparisons in later analysis. Periods of *true rest* were thus identified from each imaging session and averaged across time, giving a single baseline value per day for the hemodynamic reflectance signal (IOS or 2P), neural signals, EMG, LFD (if present), and neural spectrograms. ﻿The percentage change in each data type was thus identified as

where is the original data, such as diameter for 2PLSM or the spectral power in each frequency band of a time-series spectrogram, and is the baseline value for that data type during the unique imaging day. **Describe reflectance to HbT conversion** **w/ citations**

*Sleep scoring methodology.* All data was subdivided into 5 second bins and classified as ‘Not sleep’, ‘NREM sleep’, or ‘REM sleep’ using a random forest classification model. The model consisted of a ‘bagging’ (bootstrap aggregation) of 128 decision trees where each tree is grown with an independent bootstrapped replica of the input data (Matlab function(s): TreeBagger). 128 trees was chosen as the sufficient where the out-of-bag-error asymptotes as a function of the number of total trees. Data used in the model (within an individual five second bin) consists of: the mean cortical delta-band power [1-4 Hz] obtained from the five-second window time-series spectrogram as the higher value of the two hemispheres, the mean cortical beta-band power [13-30 Hz] obtained from the five-second window time-series spectrogram as the higher value of the two hemispheres, the mean cortical gamma-band power [30-99 Hz] obtained from the five-second window time-series spectrogram as the higher value of the two hemispheres, the mean hippocampal theta-band power [4-10 Hz] obtained from the five-second window time-series spectrogram from the hippocampus, the mean normalized EMG power, the mean heart rate, and the total number of binarized whisking events. For neural data, the five-second spectrogram was chosen as these signals tend to oscillate in time, so a smoothing out of the oscillations assists with individual bins that happen to follow in trough of an oscillation not being misclassified during a long sleeping event. Due to these cortical signals typically being largely bilaterally symmetrical during sleep, the higher of the two values was chosen to emphasize the increases above the baseline value (for 2PLSM experiments, there was only one cortical electrode). To train the random forest classification models, all of the data from the first (session 1) and last (imaging session 6) was manually scored five-seconds at a time as either ‘Not sleep’, ‘NREM sleep’, or ‘REM sleep’ based on the known behavioral and electrophysiology characteristics of the various sleep states. For example, an increase in cortical delta-band power with a low heart rate and little whisker motion is associated with NREM sleep, while an increase in hippocampal theta-band power with a low EMG muscle tone is associated with REM sleep. The decision to classify non-sleeping events as ‘Not sleep’ instead of ‘Awake’ is due to the ambiguity and commonness of awake-NREM transitions, where there is largely no clear separation in the data as the animal transitions between sleep states and is a source of the vast majority of algorithm misclassifications. Half of the manually scored data (1/6 of the total amount) was used to train the random forest classification model, with the other half being used to further validate the model’s accuracy. Since all ‘Awake Rest’ data used in subsequent analysis is from manually scored data, the ‘Not sleep’ data from the random forest classification model will include all awake resting data as well as all of the drowsy and blurry separations between sleep states. For this reason, the ‘Not sleep’ data is not used in any analysis. For data to be classified as either a NREM or REM sleep epoch, it requires 6 consecutive five-second bins (30 seconds) for NREM or 12 consecutive five-second bins (60 seconds). This filter removes the majority of misclassifications and ensures that only very clear NREM and REM sleep events make it into the final data sets that are used in the subsequent analysis. It also provides a minimum length for each event for analysis that require for all data to be the same length (such as cross-correlations, coherence, and power spectrums). To prevent REM events of several minutes in duration from occasionally being broken up into multiple separate events, up to 10 seconds of data in-between ‘REM sleep’ scores were linked after model scoring. Due to the 2PLSM having significantly less data than that of IOS experiments, all of the data was manually scored as there is not enough for the training of an accurate classification model.

*Sleep model accuracy validation.* The out-of-bag error during random forest classification model training provides an initial estimate on the model’s classification accuracy, where *out-of-bag* refers to the mean classification error using training data from the trees that do not contain the data in their bootstrap sample (Matlab function(s): oobError). The out-of-bag error of each model’s training data is then compared to the mean out-of-bag error from 100 randomly shuffled training data sets, which is analogous to random chance. A table of each animal’s out-of-bag error and randomly shuffled out-of-bag error can be found in **supplemental table**. The mean out-of-bag error across all animal models is **x +/- StD.** Each model was then evaluated on a second, unseen data set composed of the alternating 15-minute periods that were manually scored but not used in model training. The model’s scores were then compared to the manual scores combined across all IOS animals are summarized in a confusion matrix **supplemental conf matrix** (Matlab function(s): confusionchart).

*Determination of awake probability.* Probability of an animal being in a given behavioral state as a function of the imaging time was evaluated by concatenating all five second sleep scored decisions with brief NaN time-padding in-between 15-minute periods where the acquisition computer was saving the data. The probability of an animal being in a given behavioral state was then averaged across each IOS animal’s data set consisting of 6 imaging sessions of bilateral imaging and fit with a single exponential fit for ‘Not sleep’, ‘NREM sleep’, and ‘REM sleep’. These three exponentials were then averaged across all 14 animals. The reason for the animal’s probability of being awake at ‘time 0’ not being ~100% is because of the 30 minutes of recover from isoflurane given prior to data acquisition. Due to the animals becoming increasingly habituated and comfortable with the imaging set-up over several imaging sessions, it was not uncommon for many to be asleep during the first hour despite whisker stimulation. The probability of an animal being asleep as a function of the duration of rest (read: quiescence) was done by binning the individual ‘resting’ event to the nearest multiple of 5 seconds (i.e 7.5 second resting event falls in the 5-10 seconds bin). The approximate time and duration of each individual event is shifted in time to the corresponding sleep scoring bin(s). If any of the bins contain a score of ‘NREM sleep’ or ‘REM sleep’ then the individual event is considered asleep. The number of ‘awake’ events in each time increment is divided by the total number of events for each animal, fit with a single exponential, and averaged across animals.

*Behavioral transitions*. Transitions from ‘Awake to NREM’, ‘NREM to Awake’, ’NREM to REM’, and ‘REM to Awake’ were taken by averaging all the events within an animal that had six consecutive behavioral scores (30 seconds) of one behavior of interest followed by six consecutive scores of the other. Hemodynamic (HbT), normalized EMG, normalized cortical LFP, and normalized hippocampal LFP data from each behavioral transition was extracted at the corresponding time indices and averaged together within animals. Bilateral hemodynamic data and bilateral cortical LFP data from the cortical hemispheres was averaged together into one value. Cortical and hippocampal LFP spectrograms used were those with parameters of 1-second window, 1/10 second step size, and [5,9] tapers. Hemodynamic (HbT) data was digitally low-pass (< 1 Hz) filtered using a fourth-order Butterworth filter (Matlab function(s): butter, zp2sos, filtfilt). Transitions from each animal were averaged together, error bars for the hemodynamic and EMG are shown as standard deviation from the mean (N = 14).

*Mean HbT during different behaviors.* The mean change in total hemoglobin in each cortical hemisphere during each behavior was taken during all resting events (≥ 10 sec), all brief whisking events (2-5 sec in duration), all NREM sleep events (≥ 30 sec), and all REM sleep events (≥ 60 sec). All resting events and all whisking events occurred at least 5 seconds after a whisker puff, with the mean value of the whisking behavior taken between the initiation of the whisk (time 0) through 5 seconds **(should be 7 sec?)**. All rest and whisking events occurred within the manually-defined ‘Awake’ periods outlined previously in the *Establishment of awake rest and baseline* section to exclude drowsy behavior. All behavioral events were digitally low-pass filtered (< 1 Hz) with a fourth-order Butterworth filter (Matlab function(s): butter, zp2sos, filtfilt) and then averaged within their individual time series. All behavioral events were then averaged within animals before being averaged across animals. Error bars are shown as the standard deviation from the mean (N = 14, n = 28 hemispheres). The histograms showing the probability distribution of mean change in total hemoglobin is for all time series (individual behavioral event) means from all animals, with no averaging between behaviors within or across animals.

*Mean heart rate during different behaviors.* The mean heart rate during each behavior was taken during all resting events (≥ 10 sec), all brief whisking events (0-5 sec in duration **need to fix this in code to be 2-5**), all NREM sleep events (≥ 30 sec), and all REM sleep events (≥ 60 sec). All resting events and all whisking events occurred at least 5 seconds after a whisker puff, with the mean value of the whisking behavior taken between the initiation of the whisk (time 0) through 5 seconds **(should be 7 sec?)**. All rest and whisking events occurred within the manually-defined ‘Awake’ periods outlined previously in the *Establishment of awake rest and baseline* section to exclude drowsy behavior. All behavioral events were averaged within their individual time series. All behavioral events were then averaged within animals before being averaged across animals. Error bars are shown as the standard deviation from the mean (N = 14).

*Mean peak arteriole diameter during different behaviors.* The mean peak vessel diameter in each arteriole during each behavior was taken during all resting events (≥ 10 sec), all brief whisking events (2-5 sec in duration), all NREM sleep events (≥ 30 sec), and all REM sleep events (≥ 60 sec). All resting events and all whisking events occurred at least 5 seconds after a whisker puff, with the max value of the whisking behavior taken between the initiation of the whisk (time 0) through 5 seconds **(should be 7 sec?)**. All rest and whisking events occurred within the manually-defined ‘Awake’ periods outlined previously in the *Establishment of awake rest and baseline* section to exclude drowsy behavior. All behavioral events were digitally low-pass filtered (< 1 Hz) with a fourth-order Butterworth filter (Matlab function(s): butter, zp2sos, filtfilt) and then the max value was taken within their individual time series. All behavioral events were then averaged within individual arterioles before being averaged across all arterioles from all animals combined. Error bars are shown as the standard deviation from the mean (N = 6, n = **TBD – depends on behavior?** arterioles). The histograms showing the probability distribution of peak arteriole diameter is for all time series (individual behavioral event) peaks from all arterioles, with no averaging between behaviors within or across arterioles. **– figure not yet made**

*Mean laser doppler flow velocimetry during different behaviors.* The mean LDF during each behavior was taken during all resting events (≥ 10 sec), all brief whisking events (2-5 sec in duration), all NREM sleep events (≥ 30 sec), and all REM sleep events (≥ 60 sec). All resting events and all whisking events occurred at least 5 seconds after a whisker puff, with the mean value of the whisking behavior taken between the initiation of the whisk (time 0) through 5 seconds **(should be 7 sec?)**. All rest and whisking events occurred within the manually-defined ‘Awake’ periods outlined previously in the *Establishment of awake rest and baseline* section to exclude drowsy behavior. All behavioral events were digitally low-pass filtered (< 1 Hz) with a fourth-order Butterworth filter (Matlab function(s): butter, zp2sos, filtfilt) and then averaged within their individual time series. All behavioral events were then averaged within animals before being averaged across animals. Error bars are shown as the standard deviation from the mean (N = 6).

***Flow simulations (Ravi) – TBD***

*Spectral coherence during different behaviors.* The coherence between bilateral cortical changes in total hemoglobin and bilateral envelopes of each discrete LFP band (delta, theta, alpha, beta, gamma) during each behavior was taken during all resting events (≥ 10 sec), all NREM sleep events (≥ 30 sec), and all REM sleep events (≥ 60 sec). All resting events occurred at least 5 seconds after a whisker puff and occurred within the manually-defined ‘Awake’ periods outlined previously in the *Establishment of awake rest and baseline* section to exclude drowsy behavior. All behavioral events were mean-subtracted and digitally low-pass filtered (< 1 Hz) with a fourth-order Butterworth filter (Matlab function(s): butter, zp2sos, filtfilt) and then truncated to the minimum behavior length so that all events were the same length. Coherence analysis was run for each data type during each behavior (tapers [3,5], pad = 1, Chronux toolbox, version 2.12 v03, function: coherencyc) and averaged across animals. Error bars are shown as the standard deviation from the mean (N = 14).

*Spectral power during different behaviors.* The power spectra of each cortical hemisphere’s changes in total hemoglobin and bilateral envelopes of each discrete LFP band (delta, theta, alpha, beta, gamma) during each behavior was taken during all resting events (≥ 10 sec), all NREM sleep events (≥ 30 sec), and all REM sleep events (≥ 60 sec). All resting events occurred at least 5 seconds after a whisker puff and occurred within the manually-defined ‘Awake’ periods outlined previously in the *Establishment of awake rest and baseline* section to exclude drowsy behavior. All behavioral events were mean-subtracted and digitally low-pass filtered (< 1 Hz) with a fourth-order Butterworth filter (Matlab function(s): butter, zp2sos, filtfilt) and then truncated to the minimum behavior length so that all events were the same length. Power spectrum analysis was run for each data type during each behavior in each cortical hemisphere (tapers [3,5], pad = 1, Chronux toolbox, version 2.12 v03, function: mtspectrumc) and averaged across animals. Error bars are shown as the standard deviation from the mean (N = 14, n= 28 hemispheres).

*Pearson’s correlation coefficients during different behaviors.* The Pearson’s correlation coefficient between bilateral cortical changes in total hemoglobin and bilateral envelopes of each discrete LFP band (delta, theta, alpha, beta, gamma) during each behavior was taken during all resting events (≥ 10 sec), all brief whisking events (2-5 sec in duration), all NREM sleep events (≥ 30 sec), and all REM sleep events (≥ 60 sec). All resting events and all whisking events occurred at least 5 seconds after a whisker puff, with the correlation value of the whisking behavior taken between the initiation of the whisk (time 0) through 5 seconds **(should be 7 sec?)**. All rest and whisking events occurred within the manually-defined ‘Awake’ periods outlined previously in the *Establishment of awake rest and baseline* section to exclude drowsy behavior. All behavioral events for each bilateral data type were mean-subtracted and digitally low-pass filtered (< 1 Hz) with a fourth-order Butterworth filter (Matlab function(s): butter, zp2sos, filtfilt) and then take the Pearson’s correlation coefficient (Matlab function(s): corrcoef) within each time series. All correlation coefficients for each bilateral data type during each behavior were then averaged within animals and then averaged across animals. Error bars are shown as the standard deviation from the mean (N = 14).

*Cross correlation during different behaviors.* Thecross-correlation between multi-unit activity (MUA) and change in total hemoglobin (HbT) in each cortical hemisphere during each behavior was taken during all resting events (≥ 10 sec), all NREM sleep events (≥ 30 sec), and all REM sleep events (≥ 60 sec). All resting events occurred at least 5 seconds after a whisker puff and occurred within the manually-defined ‘Awake’ periods outlined previously in the *Establishment of awake rest and baseline* section to exclude drowsy behavior. All behavioral events MUA and HbT data were mean-subtracted and digitally low-pass filtered (< 1 Hz) with a fourth-order Butterworth filter (Matlab function(s): butter, zp2sos, filtfilt) and then truncated to the minimum behavior length so that all events were the same length. Cross-correlation analysis was run for each behavior (Matlab function(s): xcorr) with a ± 5 second lag time and averaged across behavioral events within each animal hemisphere and then across all animal hemispheres (N = 14, n = 28 hemispheres). The cross-correlation between LFP and HbT was taken as the cross-correlation between an HbT event and each frequency band of the cortical spectrogram with parameters of 1-second window, 1/30 second step size, and [1,1] tapers. The resulting cross-correlation matrices (lag time x frequency) were then averaged across all behavioral events within each animal hemisphere and then across all animal hemispheres (N = 14, n = 28 hemispheres).

﻿*Statistics.* ***TBD***

*Supplemental figure methods?* ***TBD***

**Author Contributions**

**Competing financial information**

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