Good Vibrations: Resting-State Functional Connectivity Reflects Entrainment of Vasomotion

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In this issue of *Neuron,* Mateo et al. (2017) suggest that hemodynamic measures of resting-state functional connectivity in cortex are reporting the consequences of entrainment of arteriole vasomotion by neuronal activity.

Electrophysiogical and neuroimaging approaches indicate that the brain exists in a state of constant activity, even during periods of rest (Fox and Raichle, 2007). While the temporal landscape of brain activity is broad, with an upper bound in the millisecond timescale of the action potential, most assessments of human brain functional connectivity are based on much slower transitions. Ultra-slow spontaneous activity, occurring on a 1- to 100-s timescale, is present across brain regions and animal species, but its function remains unclear (Drew et al., 2008). Ultra-slow spontaneous brain activity can be detected as regional metabolic changes and observed by functional magnetic resonance imaging (fMRI) as slow fluctuations in blood oxygenation level-dependent (BOLD) signal (Kim and Ogawa, 2012). That these fluctuations exhibit regional temporal correlation across the brain has led to a paradigm of resting-state connectivity approaches whereby functional organization of the brain is inferred from this intrinsic, correlated activity (Fox and Raichle, 2007). In addition to providing insights into the functional organization of the brain, resting-state fMRI has been employed to investigate alterations in intrinsic brain activity in psychiatric and neurological disease contexts. Of particular interest is the default mode network, which has been reported to exhibit alterations in numerous neuropsychiatric contexts. Analyses of these alterations hold promise in identifying novel biomarkers (Zhang and Raichle, 2010).

Central to the interpretation of these exciting investigations is a greater understanding of the basis of resting-state connectivity approaches. Electrophysiological correlates of spontaneous BOLD signal include fluctuations at the low-frequency end of the field potential (<1 Hz), termed the slow cortical potential (He and Raichle, 2009). Spontaneous activity in this frequency range exhibit stereotyped patterns of cortical activation, cortical motifs, consistent with that observed from higher-frequency activity (Chan et al., 2015). In addition, simultaneous electrophysiology and fMRI experiments from the visual system of monkeys have identified slow changes in the band-limited power of local field potential recordings as a correlate of BOLD signal responses (Logothetis, 2008). Yet the question remains: how are slow fluctuations in neuronal activity translated into region-specific correlated alterations in brain tissue oxygenation?

In this issue of *Neuron*, Mateo and colleagues (2017) investigate the physiological link between resting-state changes in neuronal activity and changes in tissue oxygenation that are used to infer functional connectivity in the brain. They implement electrophysiological recordings and an array of optical imaging and optogenetic approaches in mouse cortex to achieve these goals. The authors construct a model of coupled oscillators to elucidate the sequence of events by first identifying three ultra-slow processes in the brain. These processes include the natural oscillatory nature of vascular contractile tone, the slow fluctuation in neuronal activity, and slow temporal dynamics of BOLD signal changes in response to stimulation.

The first question Mateo and colleagues ask is: what is the nature of the relationship between ultraslow fluctuations in neuronal activity and intrinsic vasomotion? In the first set of experiments, the authors establish the relationship between temporal variations in LFP spectral power with vasomotion. Mice implanted with thinned-skull cranial windows were intravenously injected with a bolus solution of fluorophore-conjugated dextran in order to label the lumen of blood vessels. Two-photon laser scanning microscopy was used to monitor fluctuations in the diameter of the fluorescently labeled pial-surface blood vessels in the vibrissa area of parietal cortex. The time series of integrated γ-band power varied with a periodicity of $\sim$0.1 Hz and positively covaried with fluctuations in arteriole diameter. Changes in electrical activity preceded changes in diameter, consistent with the interpretation that neuronal activity drives changes in arteriole diameter. The deterministic relationship was further established by demonstrating neuronal activity was sufficient to entrain vasomotion. This was illustrated in mice expressing Channelrhodopsin-2 in layer-5b pyramidal neurons that were photostimulated at γ-rhythm frequencies. A slow rhythmic envelope in γ-rhythm was incorporated by varying the laser light intensity as a sinusoid centered at 0.1 Hz. Fluctuations in arteriole diameter were phase-locked with the slow envelope of driven γ-rhythms, with electrical activity leading dilation by 1.8 s, consistent with the lead

time measured under natural conditions. Importantly, the reverse relationship was not observed. Optogenetic, rhythmic drive of vascular tone in mice expressing halorhodopsin in arteriole smooth muscle resulted in a decrease in spectral coherence between vessel diameter and the driven envelope of electrical activity. These observations are consistent with the hypothesis that ultra-slow modulation of high-frequency neuronal activity can entrain vasomotion.

Mapping functional organization in the brain by correlated activity reveals a consistent feature of regional symmetry about the midline (Mohajerani et al., 2010). This transhemispheric correlated activity is remarkable given that the distances between mirrored locations can be quite large and span the entire breadth of the brain. If vasomotion is entrained by slow fluctuations in neuronal activity, then one might expect to also see coherent vasomotor fluctuations in corresponding brain regions. This poses a methodologically challenging question, as investigating these types of brain-wide phenomena requires the ability to resolve regional microscopic changes in activity over great distances and with high temporal resolution. An added complexity is acquiring simultaneous electrophysiological measurements to account for ongoing neuronal activity changes. The authors achieve this through the use of ultra-large field-of-view two-photon microscopy in combination with expansive bilateral thinned-skull cranial window preparations, which permits assessment of fluctuations in arteriole diameter in pairs of blood vessels at distances that can span up to 7 mm across cortical hemispheres (Tsai et al., 2015). Pairs of vessels from mirrored locations in parietal cortex in opposite hemispheres exhibited high zero-lag correlation and coherence for changes in diameter that were comparable to values observed for nearby vessels in the same hemisphere. This is striking given that coherence between non-mirrored vessels was shown to decrease at separation distances beyond 600 μm and fall to chance at 1.4 mm. Experiments from I/LnJ mice, which lack a corpus callosum, exhibited significantly reduced but still significant transhemispheric coherence. This observation is consistent with previous work using wide-field, mesoscale imaging of the same mouse strain that illustrated impaired bilateral synchrony of spontaneous slow-wave voltage activity (Mohajerani et al., 2010). Residual coherence that persists in the absence of a callosum may indicate common input from subcortical regions or compensatory adaptations in this mouse strain. These observations are consistent with the notion that the corpus callosum and neuronal projections more generally play an important but non-exclusive role in mediating synchronization of bilateral neural and vasomotor activity.

Finally, Mateo and colleagues established the remaining link in this model of chained oscillators. They relate fluctuations in resting-state arteriole diameter with corresponding changes in oxygenation in surrounding brain tissue using intrinsic optical signal imaging. This was achieved by multi-wavelength monitoring of arteriole diameter via blue-light illumination and changes in oxy- to deoxyhemoglobin by red- and far-red-light illumination. As previously, electrical activity preceded arteriole diameter changes. However, these changes were subsequently followed by increases in parenchymal oxygenation and decreases in deoxygenation, suggesting a model whereby increases in γ-band power result in increases in local tissue oxygenation.

In all, Mateo and colleagues performed a systematic and compelling series of experiments to elegantly identify a crucial missing link in the signaling pathway between ultra-slow neuronal activity and corresponding fluctuations in tissue oxygenation. This study adds nuance to the interpretation of resting-state connectivity revealed by analyses of hemodynamic signals. In light of this new framework, one must now consider that impairment in vasomotor entrainment or dysfunction in the unknown determinants of the spatial scale of coherent arteriole fluctuations may manifest in altered connectivity attributed to neuropsychiatric disease and potentially independently of disordered neuronal activity. Also, while these results clearly establish links between neuronal activity and vasomotion, broader questions remain. By what mechanism is the spectral power of high-frequency LFP temporally varied, and for what purpose? Likewise, by what means does neuronal activity drive vasomotor synchrony? Future studies motivated by these questions will benefit as a result of this foundational work from Mateo and colleagues.

REFERENCES

Chan, A.W., Mohajerani, M.H., LeDue, J.M., Wang, Y.T., and Murphy, T.H. (2015). Nat. Commun. *6*, 7738.

Drew, P.J., Duyn, J.H., Golanov, E., and Kleinfeld, D. (2008). Nat. Neurosci. *11*, 991–993.

Fox, M.D., and Raichle, M.E. (2007). Nat. Rev. Neurosci. *8*, 700–711.

He, B.J., and Raichle, M.E. (2009). Trends Cogn. Sci. *13*, 302–309.

Kim, S.G., and Ogawa, S. (2012). J. Cereb. Blood Flow Metab. *32*, 1188–1206.

Logothetis, N.K. (2008). Nature *453*, 869–878.

Mateo, C., Knutsen, P.M., Tsai, P.S., Shih, A.Y., and Kleinfeld, D. (2017). Neuron *96*, this issue, 936–948.

Mohajerani, M.H., McVea, D.A., Fingas, M., and Murphy, T.H. (2010). J. Neurosci. *30*, 3745–3751.

Tsai, P.S., Mateo, C., Field, J.J., Schaffer, C.B., Anderson, M.E., and Kleinfeld, D. (2015). Opt. Express *23*, 13833–13847.

Zhang, D., and Raichle, M.E. (2010). Nat. Rev. Neurol. *6*, 15–28.