Layers of rhythms - from cortical anatomy to dynamics

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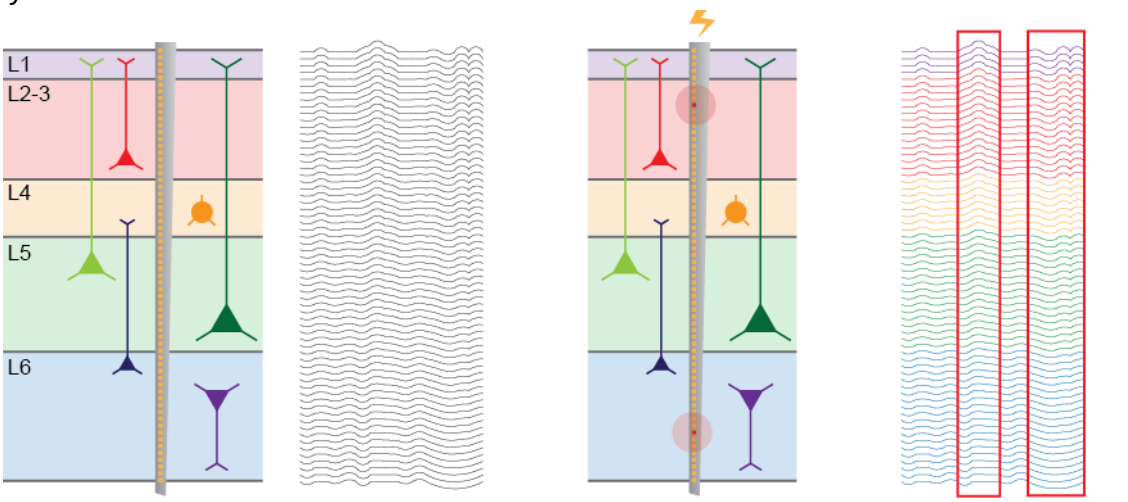
The activity of the cerebral cortex patterns into recurring dynamic motifs. In the present issue of Neuron, Senzai et al. (2019) elucidate how these motifs recruit excitatory and inhibitory neurons across cortical layers and how brain state modulates laminar interactions.

The cerebral cortex is the main center of our cognitive activity. This region subdivides in functional areas performing specific operations in sensory perception, decision making and movement generation. Cortical areas, in turn, consist of a repetition of parallel functional modules called columns. The anatomical structure of cortical columns is well-preserved across areas and mammalian species, and is composed of six layers, each having a distinct connectivity profile and distribution of cell types (Douglas and Martin, 2004). As columns are repeated across cortical areas, it becomes an attractive idea that they carry out a similar computational algorithm. In this view, columnar modules can be inserted anywhere in the cortex and process inputs of a different nature. Thus, describing their canonical operations would yield generic insights on the function of the cortex that could be translated across functional areas and species.

Anatomical features are not the only repeating element of cortical organization: In different cortical areas and species, one finds recurring dynamic motifs of cortical activity characterized by specific spectral signatures (Buzsáki, 2006). These patterns are conspicuously observed in electroencephalograms (EEG) or local field potential (LFP) recordings. Their prevalence depends on behavioral states such as sleep, wakefulness and arousal (McGinley et al., 2015). Thus, if columns, layers and cell types describe the static building blocks of the cortex, then these temporal patterns should constitute the blueprints for their interactions. However, historically, it has been challenging to measure the laminar position of *in vivo* recording electrodes with precision. Consequently, the layers and neurons involved in particular patterns of cortical activity remain mostly unknown and our understanding of their function largely speculative.

In this issue of Neuron, Senzai and coauthors (Senzai et al., 2019) make a great step forward in relating the anatomical and dynamical features of the cortical column. The laboratory of György Buzsáki has pioneered the mapping of neuronal signals in the hippocampus using multi-contact arrays of electrodes called silicon probes (Buzsáki, 2006). Applying a similar methodology, recordings were made from the primary visual cortex (V1) in freely-moving mice (Figure 1). High-density silicon probes were implanted to record LFPs and a large number of neurons at various cortical depths over the course of several days. At the end of the recordings, currents were applied to generate electrolytic lesions. The anatomical location of these lesions was determined to uncover the position of the probe's contact points within the cortical layers. To increase the accuracy of their approach, the authors defined functional boundaries based on the laminar profile of two patterns of cortical activity whose propagation within cortical layers is well-known: (i) Responses to visual stimulation and (ii) transition from DOWN-

to UP-states, i.e. the two alternating cortical states observed during slow wave sleep (Sakata and Harris, 2009). Using this approach, it becomes possible to map specific patterns of activity across cortical layers.



The authors first examined how the activity of neurons in different layers is orchestrated by gamma rhythms (~30-100Hz). Gamma rhythms are considered critical for cortical information transmission and assembly formation, and are deregulated in major psychiatric diseases such as schizophrenia (Buzsáki, 2006). A first main finding of Senzai et al. is that each cortical layer exhibits its own gamma rhythm. LFP signals were analyzed using Independent Component Analysis, a technique that can resolve independent sources out of an array of mixed signals. Surprisingly, a total of six sources of gamma activity were identified, whose respective locations aligned with anatomical boundaries between layers. Gamma components were particularly strong in superficial layers, in agreement with previous studies (Vinck and Bosman, 2016). The authors then examined how cortical neurons were recruited by each component. Neurons were separated into inhibitory interneurons and excitatory cells based on action potential waveforms and optogenetic activation. Gamma entrainment was stronger in inhibitory than in excitatory neurons, consistent with the purported role of interneurons as gamma pacemakers (Buzsáki, 2006). More importantly, the authors find that both excitatory and inhibitory neurons were primarily entrained to the gamma rhythm originating in their own layer. This resulted in eight separable clusters of neurons residing in different layers and characterized by distinct patterns of gamma-locking. These findings challenge the canonical paradigm that the neurons in a given cortical column share the same gamma rhythm. A novel view thus emerges in which a column contains a polyphony of gamma rhythms, forming a multitude of neuronal assemblies across layers.

A recurring theme across cortical areas is that gamma rhythms synchronize activity on relatively small spatial scales, but that slower rhythms synchronize activity on large spatial scales (Buzsáki, 2006). A second important observation of Senzai and coauthors is that a similar trade-off between space and frequency occurs *within* the cortical column. Whereas gamma rhythms were highly localized, neuronal activity was synchronized across all layers in lower frequency bands. Spectral analysis revealed two characteristic low-frequency modes. (i) The first mode corresponded to the transition between DOWN and UP states observed during slow-wave sleep (Sakata and Harris, 2009). UP-states comprise periods of elevated firing, whereas DOWN-states correspond to epochs where the network is silent. Surprisingly, however, Senzai and coauthors discover a group of neurons in deep layers that are specifically active during DOWN-states. (ii) The second mode corresponds to a 3-6 Hz rhythm (Einstein et al., 2017),

which bears close similarities to the alpha rhythm in primates and emerged predominantly during quiescent wakefulness. These two rhythmic modes had similar current-source-density profiles, comprising multiple sources of synaptic currents both in deep and superficial layers. Hence, they may result from a complex pattern of interactions between neurons in deep and superficial layers. Nonetheless, both during UP-DOWN and 3-6 Hz waves, neurons in deep layers tended to lead in phase, suggesting that these low-frequency rhythms orchestrate information flow from deep to superficial layers (Sakata and Harris, 2009).

The dependence of cortical dynamics on arousal and behavioral states suggests that these modulate the directionality of synaptic interactions between cortical neurons. Yet, synaptic interactions are difficult to measure *in vivo* which makes this modulation hard to characterize. The third important contribution of Senzai et al. is to overcome this technical difficulty. Taking advantage of the large number of simultaneously recorded neurons, the authors developed an innovative approach where monosynaptically connected pairs were detected through cross-correlations. This revealed a prominent increase in connectivity from excitatory L2/3 neurons to both excitatory and inhibitory L5 neurons during wakefulness. By contrast, slow-wave sleep was characterized by a strengthening of the recurrent excitatory activity within L5. These findings provide fundamental constraints to our understanding of the link between behavioral states and cortical interactions.

The work of Senzai and coworkers represents a significant step forward in understanding the dynamical blueprints of the cortical column. Like most innovative work however, this study raises a raises a number of questions for future research.

Firstly, studies are needed to refine the general framework developed by Senzai et al focusing on particular behavioral states and motifs. Previous work has indicated the existence of distinct gamma motifs in mouse V1. (i) Narrow-band 55-65 Hz oscillations emerge during elevated states of arousal and locomotion, and propagate from LGN to V1 (Saleem et al., 2017). (ii) Visual stimulation amplifies a broad-band gamma rhythm with energy in the 30-80 Hz range (Perrenoud et al., 2016). (iii) Finally, a low-gamma rhythm with a peak-frequency around 25-30 Hz is induced by large grating stimuli (Veit et al., 2017). This low-gamma rhythm has stimulus dependencies that are comparable to the classic 30-80 Hz narrow-band gamma oscillations found in cat and primate V1 (Vinck and Bosman, 2016). The mapping of these gamma rhythms to the multiple gamma components uncovered by Senzai and coauthors needs to be established.

Secondly, it remains to be seen how the findings of Senzai et al. generalize across cortical areas. Indeed, gamma rhythms depend on brain-state and have been shown to exhibit very specific stimulus-dependencies in the visual cortex of cats and primates (Vinck and Bosman, 2016). It is unclear how these dependencies translate to other sensory modalities or to higher-order areas. Furthermore, slight alterations in laminar structure exist between cortical areas. For example, layer 4 is absent in the motor cortex and in the frontal areas of rodents. Thus, further studies should investigate whether the laminar profile of the dynamic motifs identified in the study of Senzai et al. can be found in other cortical regions.

Thirdly, future experiments should elucidate the mechanisms underlying the state-dependency of layer-specific dynamical motifs. Senzai and coauthors show that the transition from slow-wave sleep to wakefulness induces multiple changes, including: (i) An enhancement of L2/3-to-L5 spike transmission, (ii) an increase in gamma-locking, (iii) a reduction in the amplitude of low-frequency rhythms, and (iv) an increase in L5/6 discharge rates. All of these changes presumably depend on the action of neuromodulators like acetylcholine and thalamocortical interactions (McGinley et al., 2015). However, it is likely that causal relationships also exist between these four variables themselves: During wakefulness, L2/3-to-L5 spike transmission could increase because L5 neurons spend less time in

DOWN-states (low-frequency desynchronization). In addition, L2/3 spikes may have a stronger impact on post-synaptic targets due to enhanced gamma-synchronization (Buzsáki, 2006). A reverse causal flow is also possible: Enhanced L2/3-to-L5 spike transmission could reduce low-frequency synchronization by triggering transitions from DOWN- to UP-states. Experiments are thus needed to disentangle the complex causal relationships between activity patterns in different layers. For example, one could attempt to perturb cortical activity patterns with optogenetic stimulation in mouse lines having layer-specific expression of light-sensitive opsins. The thorough characterization of the way in which specific dynamic motifs involve cortical layers performed by Senzai et al. will provide a highly useful guidance for the design of such experiments.

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