Systems neuroscience: **The slowly sleeping slab and slice**

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The physiological functions of structured spontaneous activity in the brain, such as the slow oscillations characteristic of certain sleep states, remain unclear, but new studies using brain slices or slabs are starting to shed light on the underlying neural mechanisms.

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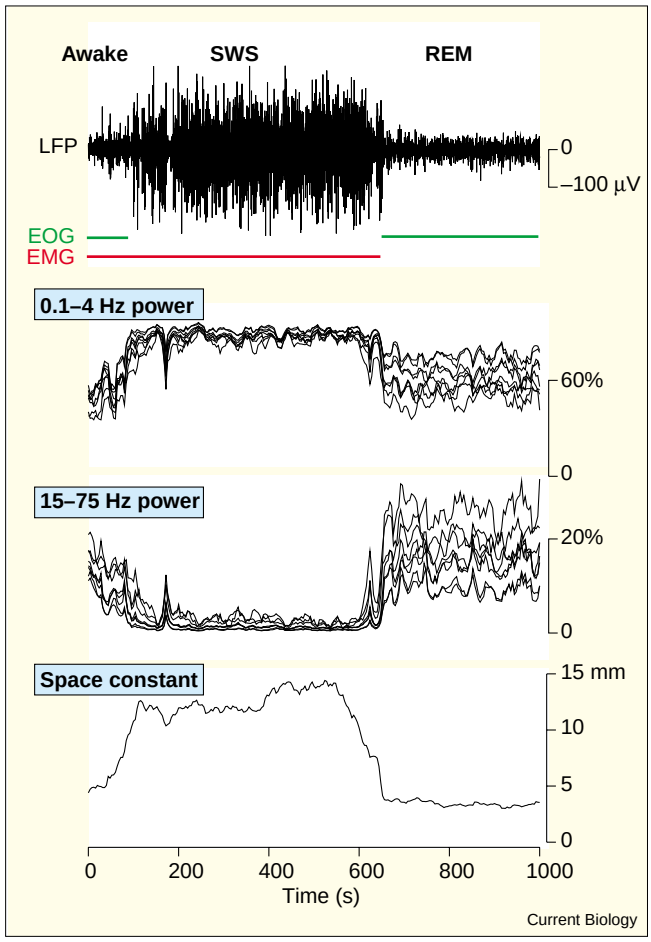
Functional activity in the intact brain can be divided into two main kinds: first, there is the activity that can be related to some external stimulus or cognitive event; and second, there is the activity which has some 'structure' but which is nevertheless apparently spontaneous. Structured spontaneous brain activity has been studied for over a century, starting with the first measurements of electrical potentials in the cranial vault, and includes a wide variety of coherent activities referred to, somewhat inconsistently, as rhythms, oscillations or waves. The functional significance of these spontaneous activities is still not clear, though they have been the subject of much speculation. But recent work is beginning to shed light on the neural mechanisms that generate non-stochastic firing patterns in the brain.

Different spontaneous activity patterns have distinct time courses, with characteristic frequencies that span about three orders of magnitude: for example, there are the gamma and beta rhythms (15–75 Hz), spindle waves (7–14 Hz), theta waves (4–7 Hz), delta waves (about 1–4 Hz) and various slower oscillations (less than 1 Hz). The patterns can occur over a relatively large spatial scale, in that they may be observed in various well-defined anatomical structures, such as the thalamus or hippocampus, all the way up to the entire neocortex. They may occur in combination or in isolation. Different patterns are associated with different behavioural and cognitive states, particularly the various stages of the sleep–wakefulness cycle (Figure 1) [1].

A major challenge for those studying any functional phenomena in the brain is to bridge observations made on the intact brain with those made on reduced preparations that facilitate the elucidation of underlying mechanisms. For studying activity related to sensory perception or cognition, finding a suitable reduced system is especially difficult — in most cases one must use the intact *in vivo* state (one exception being the isolated intact retina preparation). But many spontaneous activities do survive in a reduced preparation. Two recent studies [2,3] exemplify this approach in the analysis of the neural mechanisms

Figure 1



Distinct global patterns of cortical activity characterize the awake state, slow-wave sleep (SWS) and rapid eye movement (REM) sleep, as shown in the spatio-temporal dynamics of the local field potential (LFP, analogous to the EEG) made in an unanesthetized cat. Slow-wave sleep is notable for its synchronous and slow – seen in the increase in the power between 0.1 and 4 Hz – global activity, while the awake and REM states show much more asynchronous (thus the lower amplitude LFP) and faster activity – seen in the increase in the power between 15 and 75 Hz. The increased synchrony of SWS can also be seen in the associated increase in the 'space constant' measure, derived by correlating the activity between several spatially separated field potential electrodes. The specific slow oscillation investigated by Sanchez-Vives and McCormick [2] and Timofeev *et al.* [3] is an important component of SWS. Note that the awake and REM states show significant eye movements (denoted by EOG), while muscular tonus (EMG) is specifically inhibited during REM (data from [4]).

underlying slow-wave sleep. Both groups studied how reduced systems may capture — or not — a particular spontaneous oscillation seen in the clinical electroencephalogram (EEG), and both are using computational models to help interpret their electrophysiological data.

Several suggestions have been made as to the functional importance of slow-wave sleep. These include both central functions, such as the consolidation of memory [4–6], and peripheral functions such as thermoregulation [7] and the regulation of growth hormone and cortisol production [8]. It is clear that the clinical implications of understanding what controls this part of the sleep cycle at the cellular level are enormous.

In the early 1990s, Steriade and colleagues [9] made extensive intracellular recordings from single cells in the cortex and thalamus of the anesthetized cat. Their results revealed a cellular correlate of the global slow oscillation that is thought to be a model for certain periods of slow-wave sleep. These studies also showed that these patterns are generated in the cortex [10]. Continuing this research, Steriade's group [3] has now carried out a sort of 'back-to-the-future' experiment, involving the surgical isolation of a small section of cortex in the anesthetized cat (preserving the vascular supply but severing most afferent and efferent axonal fibers). This method of isolating portions of cortex *in vivo*, more common some thirty to forty years ago, was in some sense a precursor to the *in vitro* brain slice technique.

In their new experiments, Timofeev *et al.* [3] found that slow oscillations were seen only when the isolated slab included essentially an entire gyrus, encompassing several square centimeters of the cortical surface. When the isolated slab was smaller than this, only occasional spontaneous global depolarizations were seen. This apparent dependence of slow oscillations on the number of cells was analyzed with a two-pronged modelling approach, including a conductance-based Hodgkin–Huxley neural network model and an analytical formulation. Both models predicted that a minimum number of cells would be necessary for sustaining the slow population rhythm.

Sanchez-Vives and McCormick [2], in contrast, observed slow oscillations in slices of ferret visual cortex containing much smaller volumes of brain tissue than the isolated slab preparation of Timofeev *et al.* [3]. Serendipitously, Sanchez-Vives and McCormick [2] found that, when small adjustments were made to the usual recipe for artificial cerebrospinal fluid that maintains the cortical slice in a bath, a spontaneous and global slow rhythm was revealed. Specifically, the rhythm was observed when the Ca^{2+} and Mg^{2+} levels were reduced by about half, and the K^{+} level increased by about 50%. The authors' claim is that these adjustments more closely match the composition of the actual interstitial fluid of the brain; it remains to be seen if these changes are all necessary for rhythm generation.

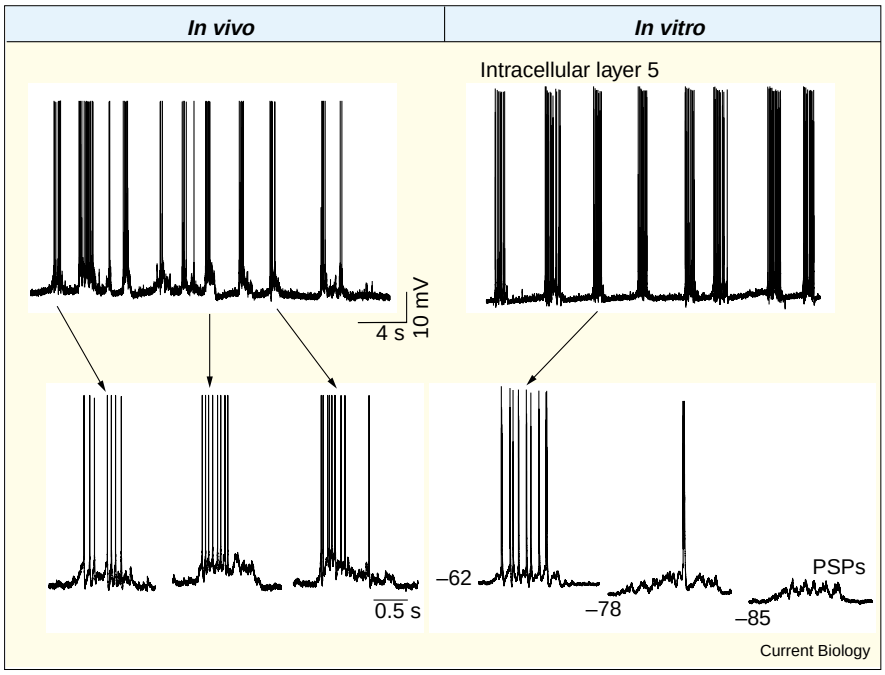
A fundamental issue for evaluating any model system, whether it be a reduced biological preparation or a computational framework, is of course deciding whether the model behaviour reproduces at a fundamental level the original phenomena of the intact system. In other words, if it looks like a duck, walks like a duck and quacks like a duck, is it still a duck? This is a particularly important question for the slow oscillations that have been recorded in slices or slabs of cortex, given the important differences between the results obtained by the two groups [2,3]. Nevertheless, the intracellular recordings seem to capture the *in vivo* phenomena both quantitatively and qualitatively (Figure 2).

McCormick's group has also started a theoretical analysis, with a preliminary network model presented at last year's Society of Neuroscience meeting [11]. It may be argued that ultimate understanding is reached when one is able to model the phenomena in question at the appropriate level. Certainly, the slice preparation facilitates this task, not only by allowing more freedom to pharmacologically dissect the biophysical mechanisms, but also by vastly limiting the anatomical substrate in a very practical way. For example, one of the more notable successes of computational neuroscience has been in modelling the generation of the spindle rhythm at the level of single neuron biophysics [12], based on data collected from the thalamic slice preparation. Thus, the fact that Sanchez-Vives and McCormick [2] hit upon a successful cocktail for observing slow oscillations in brain slices greatly expands the possibilities for characterizing underlying mechanisms.

The finding that the slow oscillations occur at both ends of the spatial scale — in an intact gyrus and in a slice of cortex half a millimeter thick — but not at an intermediate scale presents some challenging questions. One would normally ask what circuitry is necessary for a given phenomena to occur, but perhaps in this case it may be more appropriate to ask what the requirement is for the slow oscillations not to occur. It may be that the cellular properties and connectivity in cortex during a quiescent state, for example without coordinated thalamic input, are such that slow oscillations are quite stable even for a relatively small number of cells. These oscillations certainly involve inhibitory neurons [9], and in principle it does not take many excitatory neurons for a circuit to start to 'oscillate'. If inhibition is blocked in a slice preparation, paroxysmal depolarizing shifts occur that start to look like epilepsy.

The precise balance of inhibition and excitation is thus certainly critical. It could be that, when a functional circuit includes a certain minimum number of inhibitory cells, corresponding to the small slab in which Timofeev *et al.* [3]

Figure 2



Slow rhythmic firing of cortical neurons recorded *in vivo* in the anesthetized cat (left) and *in vitro* in a slice of ferret visual cortex (right), demonstrating the cellular correlate of slow wave sleep. The slow (top traces) and fast (bottom traces) time scales are the same for both the *in vivo* and *in vitro* records. Note in particular the clear distinctions between the depolarized 'up' state, where firing occurs, and the much quieter 'down' state with no intervening action potentials. In the *in vitro* recording, hyperpolarizing currents were applied (lower right; the 'down' state resting potential in millivolts is given at the left of each trace) to reveal the bursts of post-synaptic potentials (PSPs) underlying each 'up' state. The fact that the duration of the 'up' state is relatively insensitive to manipulation of the membrane potential suggests that the voltage-dependent channels of a given neuron play only a minor role in this timing compared to the synaptic input from the network. Nevertheless, it is quite likely that the overall expression of the neurons' intrinsic properties does play a crucial role in determining the collective network dynamics. (Adapted from [2].)

only rarely observed oscillations, then their non-oscillatory spontaneous activity reaches a level sufficient to suppress the slow global oscillation. Above another, higher, critical number of cells, corresponding to the isolated whole gyrus, there might then be sufficient signalling between inhibitory cells for the slow oscillation to re-emerge. Under such a scenario, one can imagine that the critical thresholds would be susceptible to a variety of modulatory influences acting on single-cell properties, allowing subtle, state-dependent expression of the slow oscillations at a range of spatial scales. And if, indeed, slow oscillations *in situ* can occur semi-locally in a dynamic manner, depending on various extra-cortical inputs, this could have profound implications. For example, if some part of our daily experience is locked into long-term memory during slow-wave sleep, the dynamic, contingent oscillations might influence precisely which memories are consolidated where when we turn in for the night.

This hypothesis may be irrelevant if it turns out that either the slab or the slice preparation introduces essentially pathological conditions for the involved neurons. For example, it remains difficult to know precisely what the composition of the artificial cerebrospinal fluid should be to avoid generating artefactual cellular properties (it is surprisingly difficult to measure accurately the ionic composition of the interstitial fluid *in situ*). Similarly, it is hard to imagine that the inevitable disruption of the vascular supply to the isolated cortex in the slab preparation does not introduce some sort of artefact. Of course these sorts of questions apply to the study of any complex system — how much does the act of measuring fundamentally alter the quantity being measured. It is in untangling this conundrum that detailed modelling may provide a real contribution, by allowing explicit Gedanken experiments that, even if they cannot prove anything, at least can strengthen the experiment-based intuitions and suggest new protocols.

The neural basis of slow waves is undoubtedly tied to the intrinsic firing properties of cortical neurons, as much as to the connectivity of the cells and to dynamic synaptic properties of inhibition and excitation. While the classification of these properties (for example, see [13]) into stereotypes has been extremely useful in organizing the myriad cellular types, it has become increasingly clear that a given cell may, under different physiological conditions, exhibit qualitatively different firing behaviours. In the case of slow waves, whether a neuron's basic response is a burst of action potentials or more regular firing seems to be relevant, because these are qualitiatively different dynamical properties. Furthermore, brainstem stimulation during the slow wave has two effects: not only is the slow rhythm abolished, but bursting cells are transformed into regular firing ones. Attempts to untangle this story are hampered by our continued lack of a tenable theoretical description of intrinsically bursting cells in the cortex and hippocampus. For example, two main classes of models of bursting cells, which rely on either calcium currents or specific dendro-somatic morphologies, are clearly inconsistent with the available data [14]. In any event, the

global phenomenology of slow waves may provide important clues as to the biophysical mechanisms underlying bursting in single cortical neurons, and similarly this knowledge at the single-cell level may then help complete our understanding of the neuromodulatory functions of slow-wave sleep.

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