ELSEVIER

Cell Calcium 37 (2005) 451–457

cell calcium

www.elsevier.com/locate/ceca

Calcium imaging of cortical networks dynamics

Rosa Cossart [a,*], Yuji Ikegaya [b], Rafael Yuste [b]

[a] *INMED, INSERM U29, Parc Scientifique de Luminy, BP.13, 13273 Marseille, Cedex 9, France*
[b] *1002 Fairchild, Columbia University, 1212 Amsterdam Ave., New York, NY 10027, USA*

Received 18 December 2004; accepted 6 January 2005

Abstract

Studies relating spontaneous network activities to cognitive processes and/or brain disorders constitute a recently expanding field of investigation. They are mostly based either on cellular recordings—usually performed in pharmacologically induced oscillations in brain slices—or on multi-cellular recordings using tetrodes or multiple electrodes.

However, these research strategies cannot link the electrical recordings with morphological characterization of the neurons. The progress made in imaging techniques allows for the first time to have simultaneously a dynamic and global characterization of network activity and to determine the single-cell properties of the unitary microcircuits involved in this activity.

Keywords: Calcium imaging; Cortical networks dynamics; Morphological characterization

The cortex can play many songs: different brain states are represented by specific activity patterns of its neuronal networks. These patterns not only encode the response to a given sensory stimulus, different cortical functions, or behaviors but also can be associated to neural development or particular pathologies. A fundamental question, is therefore, to understand the nature of such network dynamics. Neuronal network activities exhibit complex but apparently precise spatio-temporal patterns. Hence it is well known that network activities embrace different temporal melodies, they can be oscillatory as reported during environment exploration or sleep [1–3], involve hypersynchrony as occurring during epileptiform events [4] or local synchrony for example during the performance of working memory tasks [5]. They might even be associated to the precise firing sequence of a given chain of neurons [6]. The activity of a network is determined both by its structure and by the properties of synaptic transmission between the neurons that compose it.

Understanding the nature of network activities implies being able to localize and characterize local generators, to predict the start of a particular brain state, the sequences of activation of various brain regions, of different cell types or precise functional membrane domains and to identify the event that ends them. Eventually, this converges to the determination of the intrinsic and/or synaptic mechanisms responsible for different types of network activity. It is, therefore, essential to characterize the morpho-functional structure of physiological/pathological activity patterns and identify the individual cells and proteins generating them in animal models.

The high heterogeneity of cortical neurons challenges the understanding of the cellular basis of network events and it most likely reflects a division of labor inside a population serving multiple functions. For example, GABAergic interneurons have been shown to control the generation of major forms of network activities but this population is extremely heterogeneous morphologically, physiologically and neurochemically [7]. Network activities are, therefore, songs performed by a chorus composed of different voice tones, male and female singers.

Initial studies on the organizational structure of neuronal circuits were based primarily on anatomical observations. At the beginning of the last century, the pioneering work of Ramón and Cajal [8] described the architecture of the cortex and its organization in layers containing cells with different morphologies. This anatomical work crystalized in the description by Lorente de Nó of more than 150 classes of neocortical neurons (Lorente, 1922) and was certainly the

* Corresponding author.

0143-4160/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ceca.2005.01.013

root for future functional network studies, like a snapshot of the singing chorus. However, to study the role of different populations of neurons in generating network activity, we need to have both a dynamic and global vision of the network but also assess the unitary properties of its key components. To achieve this goal, one needs to access simultaneously three levels of observation: the *network*, the *cellular* and *molecular* levels. Ideally, the type of information that should be measured is the real time voltage fluctuations occurring in the different somato-dendritic domains for identified cell types. This can be achieved at the cellular level using for example single-cell patch-clamp recordings coupled to post hoc morphological identification. To record from different cells in a microcircuit, multiple patch-clamp recordings can be performed simultaneously, but the number of cells thus recorded is limited. Still, the measurement of the synaptic influx impinging onto a cell provides significant information of the network activity since it is by itself the post-synaptic imprint of the events occurring in the circuit the cell is embedded into. For example, the timing of the occurrence of synchronous network events and the description of their synaptic mechanisms can be performed using patch-clamp recordings. Hence, this approach enabled to study the mechanisms leading to the generation of a major type of oscillatory activity, giant depolarizing potentials, that is the fingerprint of maturating networks [9]. Similarly, the first evidence for synfire chains in cortical slices was obtained by large-scale computational analysis of single-cell voltage-clamp recordings [6].

However, every circuit in the brain is composed of a myriad of functional microcircuits that are at work at multiple temporal and spatial scales. Recording from a single cell is similar to recording from a single voice in a chorus: it might sing with the others, only with voices of similar tone, or even alone. To characterize fully the dynamics of a network, larger scale recordings that describe the temporal relation between different assemblies need to be performed. Several methods are now available to record the song played by the chorus without the ability of distinguishing between the different singers. Functional magnetic resonance imaging, positron emission tomography, field potential analysis, localized multi-site extracellular recording techniques, voltage-sensitive dyes imaging are the principal instruments used to study the intact brain [10].

This review describes our approach to the study of cellular networks. We combine large-scale calcium imaging and single-cell electrophysiological recordings with online statistical analysis to reconstruct the spatio-temporal structure of network activity.

1. Calcium as a reporter of action potentials

Electrophysiological and other mapping techniques have provided important insights into the function of neural circuits and neural populations in many systems. Electrophysiological techniques have an ideal temporal resolution but a single pixel of spatial resolution.

Optical imaging methods offer the possibility of simultaneous measurements from many locations. This is especially important in the study of nervous systems in which many parts of cells or cells or regions are simultaneously active. In addition, optical recording offers the possibility of recording from processes that are too small or fragile for electrode recording.

Optical recordings from neuronal populations enable the simultaneous recording from many neurons in a relatively non-invasive approach. In principle, when using appropriate imaging hardware and probes, the temporal resolution of optical recordings is limited by the time-course of the event being monitored while spatial resolution is limited by the size of the signal and the wavelength of the light used. The use of optical methods for detecting neuronal activity started with the monitoring of intrinsic signals, which is based on small changes in light reflectance of neuronal tissue after its activation due to the fact that neuronal activation produces intrinsic optical signals consisting of light scattering and birefringence or hemodynamic changes. Intrinsic imaging is the most basic type of optical imaging. It relies only on indirect assays of neuronal activity and suffers the disadvantage of a low signal-to noise ratio that does not allow single-cell resolution. Another approach is the use of fast voltage-sensitive dyes. These dyes theoretically represent the ideal indicator since they should translate membrane potential into an optical signal. Thus, by exciting the dye molecule with light energy of a particular wavelength, the voltage of the cell can be determined by monitoring the emitted fluorescence or absorption. Although this temporal resolution is a significant improvement over intrinsic imaging (fast response time of a few microseconds), this approach still presents serious limitations, mostly due to the unspecific binding of these lipophilic dyes to all membranes. For example, the signal to noise ratio is very low since the fluorescence varies at best by 25% per 100 mV change in membrane potential. In extremely optimized conditions of use for these dyes, 50% change/mV has been reported [11]. The most critical limitation is probably that the majority of the information thus acquired is signal from dendrites and axon terminals whereas the information ideally sought after is the depolarization resulting from action potentials.

An alternative optical approach is to use calcium indicators to monitor neuronal activity [12]. An action potential is characterized by the rapid change of the neuron membrane to a strongly positive membrane potential value. Thus, the depolarization produced by an action potential triggers the opening of calcium channels and hence an influx of calcium into the cell body of the active cell. In contrast to sodium and potassium the intracellular calcium concentration is very low and can be thus significantly affected by the calcium influx during an action potential. Furthermore, the slow calcium influx in the soma is a discrete variable that is a monotonic function of the number of action potentials

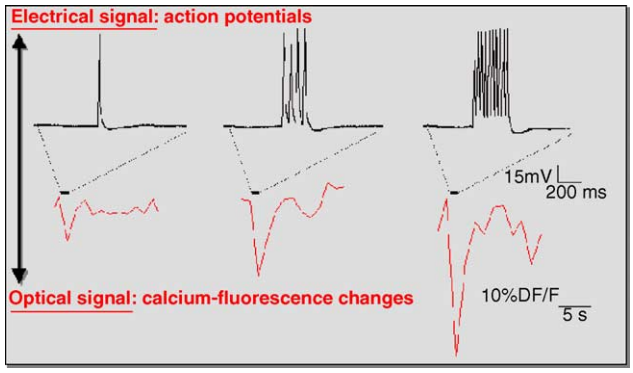

Fig. 1. Correspondence between action potentials and somatic calcium transients. Electrical signals are whole cell current clamp recordings from a layer V pyramidal cell in a slice of mouse V1 (P17) loaded with a membrane permeable calcium indicator (Fura2-AM). The recording pipette included 30 μM of fura pentapotassium salt. Action potentials were triggered by injection of depolarising current pulses. Optical signals are the simultaneously imaged somatic calcium transients in the same imaging conditions as used to image the entire field of view (i.e. 20×, 0.95 NA objective, 512 × 512 resolution, ∼1 frame/s). Note the very different timescales used to represent optical signals as compared to electrical signals.

occurring in the cell [13–15]. It is, therefore, possible to detect whether a neuron fired an action potential or several by monitoring its somatic intracellular calcium concentration (Fig. 1). This calcium influx has onset dynamics typically of about hundreds of milliseconds, and offset dynamics as long as several seconds. It is, therefore, much slower than the high-concentration localized transients produced at the presynaptic terminals near open calcium channels during neurotransmitter release (millisecond time scale). Indeed, calcium accumulations in somata rise in tens of milliseconds and decay monoexponentially in about 1–5 s [15]. Such slow kinetics is a limitation to the detection of high firing rates, although, on the rising phase of the calcium signal, activities reaching 20 Hz can be discriminated [15]. Another limitation to this method could be that other sources than action potential activity can produce a rise in the somatic calcium concentration: (i) calcium spikes, (ii) calcium induced-calcium release from intracellular stores, (iii) subthreshold synaptic events, and (iv) glial calcium oscillations. However, all the calcium events listed above can be easily discriminated from a calcium rise produced by a sodium spike based on their amplitude, their kinetics or their pharmacology. For example, calcium spikes occur in dendrites, which are not detected in large-scale imaging of networks, whereas calcium released from stores or glial events have extremely slow kinetics and subthreshold synaptic potentials produce localized and small calcium signals that are not detectable at the soma [16].

2. Calcium indicators to report sodium action potentials

Optical monitoring of calcium changes simultaneously in many neurons can be achieved either with calcium-sensitive fluorescent indicators or genetically encoded calcium probes [17]. The two main genetic tools are based either on bioluminescent proteins such as aequorin [18] or on the green fluorescent protein (GFP). For example, cameleons are a class of GFP-based genetically designed reporters for calcium, which operate through a conformational change that results in fluorescence resonance energy transfer (FRET) in the presence of calcium ions. One advantage of these genetic probes is that they can be targeted to specific cellular microdomains (mitochondria, ER), which can be difficult to probe otherwise. Still, low signal to noise ration of genetic calcium probes remains a serious limitation to their use for monitoring action potential-induced calcium changes in large neuronal populations.

Acetoxymethyl (AM) fluorescent calcium indicators have the considerable advantage over genetic probes that they show a large amplitude response upon binding Ca^{2+} which enables to investigate changes in intracellular free Ca^{2+} concentrations using fluorescence microscopy. In addition, the development of bulk loading methods enables the non-invasive loading of large populations of neurons in vitro or in vivo [19]. Indeed, the acetoxymethylesters passively diffuse across cell membranes and are cleaved by intracellular esterases once inside the cell to yield cell-impermeant fluorescent indicators. Different indicators are available to detect calcium changes. In general, fluorescent probes are classified according to their excitation and emission characteristics, as well as their chemical and biological properties.

The major parameters that distinguish between different indicators are their affinity for calcium and the wavelength at which they are excited. The high affinity calcium dyes (for example Fura2, Fluo3 or Fluo4) exhibit dissociation constant (K_d) values that are close to typical basal Ca^{2+} levels in neuronal cells (∼100 nM range). The advantage of these dyes is that they produce large and easily detectable signals following an action potential but they also induce a significant buffering of the calcium at rest inside the cell, and therefore, report calcium signals with slower kinetics. It can be argued that such buffering could generate artifactual firing and network activity. However, this does not seem to be the case since the percentage of active neurons or the firing frequencies are not significantly different in loaded cortical slices as compared to control as assessed by cell-attached recordings [21]. Furthermore, network activities first described by field recordings could also be visualized in loaded slices with identical spatio-temporal properties [20,21]. Low affinity dyes (K_d: ∼5–50 μM) should decrease the buffering of intracellular Ca^{2+} and produce a faster response that thus increases temporal resolution. However, signals thus produced are of much smaller amplitude; therefore, they do not enable to reliably resolve single action potentials at the somatic level. These dyes (for example OGB-5N, Mag-Fura, Fura2 FF) are generally used to image calcium transients in single-cell microdomains such as single spines or axonal terminals.

3. Two-photon imaging of calcium events in large neuronal populations

All the dyes described above were originally designed and characterized for single photon excitation. The excitation wavelength can be either in the UV (for example Fura2) or in the visible range (for example Fluo4). In general, longer wavelength excitation is more suitable since it induces less photobleaching of the indicator and less phototoxicity. Longer wavelengths also penetrate deeper in living tissue. All these issues are overcome with the use of pulsed IR femtosecond laser-scanning microscopy to excite the fluorophore, i.e. "two-photon microscopy" [22]. Two-photon microscopy is a relatively new development in the optical imaging of neurons. Calcium imaging of network activity in brain slices can strongly benefit from the use of two-photon excitation of the stained specimen. The most important difference is that, with two-photon microscopy, the label is excited only at the beam focus. Indeed, two infrared photons must collide with the fluorophore simultaneously to excite the molecule to a state virtually identical to that caused by a single visible photon of about half the wavelength. This low-probability event requires a high density of photons, which occurs at the beam focus of a pulsed high-energy laser. The fluorescence is confined to a three dimensional area (focus point of laser beam). There is no out-of-focus fluorescence (hence no need for a confocal aperture, which results in an increase of the signal to noise ratio), so photo-excitation is limited to the plane of focus whereas with conventional confocal microscopy, the whole thickness of the specimen is harmed by every scan. However, the high cost of a two-photon microscope has been one of the limiting factors to the widespread use of this impressive technique. The majority of the cost is attributed to the Ti-Sapphire pulsed laser required in the set-up. It is important to stress that the optical properties of calcium fluorophores might be different for two-photon excitation. For example, the two-photon absorption efficiency of the dye, which is a crucial parameter to obtain a large output fluorescence signal, is generally low (σ: ~20–100 GM). However, since there are no specific dyes designed for two-photon excitation, investigators generally use the same fluorescent probes as used with conventional excitation systems (i.e. Fura2, calcium green or Fluo4).

Two-photon microscopy is a relatively new development in the field of calcium imaging in neurons. This scanning strategy was first used to measure calcium dynamics in dendritic spines from CA1 pyramidal neurons in hippocampal slices, taking an immediate advantage of the high spatial resolution it provides [23]. Five years later, the first study using two-photon excitation to image calcium oscillations in large neuronal networks was performed in slices of newborn rats [20]. In accordance with the properties discussed above, the advantages of using this technique to record network activities in living thick specimen are obvious: (1) low phototoxicity makes it a non-invasive exploratory technique; (2) negligible photobleaching enables long duration recordings (up to 8 h [20]); (3) higher penetration (>1 mm) allows the recording from depths for which the network is preserved from the slicing procedure as well as in vivo [24]; and (4) high spatial resolution enables to resolve individual somata or dendrites even when imaging from multiple neuronal ensembles at low magnification.

The major disadvantage of two-photon microscopy, as of any other scanning-based imaging system is the low temporal resolution. Indeed, conventional systems require several hundred milliseconds to scan a full image field (~512 × 512 pixels). This does not allow a reliable and accurate resolution of single action potentials. This approach, is therefore, more adapted to the spatio-temporal description of network events with slow dynamics and sustained firing such as synchronous oscillations in developing networks [20] or network UP states in the more mature neocortex [6,25]. A technical solution to this limitation is to scan the specimen with multiple foci obtained by the splitting of the laser beam (spinning disk for visible sources or "beam multiplexer" for pulsed femtosecond laser excitation). The scanning speed is improved by a factor of about a hundred and the signal to noise ratio is increased by the use of a camera as the detection system.

Reconstruction of action-potential activity from calcium imaging of stained specimen enables the spatio-temporal description of network activities (Fig. 2). However, the exact physiological event underlying the calcium activity observed as well as the anatomical characterization of the specific microcircuits involved in the generation of the event require the investigator to have an online analysis of the information gathered during the experiment in order to perform pharmacological experiments and electrophysiological recordings of targeted cells. The amount of information acquired in a single movie (change of calcium fluorescence in thousands of cells as a function of time for several minutes) is very important and thus requires computerized signal processing. Also, the theoretical understanding of the network dynamics observed, generally goes far beyond the experimental field and should take significant advantage of computational models.

To investigate the dynamics of spontaneous cortical activity, we have developed a method combining two-photon or spinning disk confocal calcium imaging of large neuronal populations and automated signal processing techniques, to reconstruct and analyze *online* the spontaneous activity of up to 2000 neurons and 100 dendrites in cortical slices (Fig. 2). We combined this large-scale imaging with targeted recordings of individual neurons to investigate the intracellular correlates of network events and morphologically characterize the microcircuits. The next section will describe our approach and the type of network event that can be detected following this strategy.

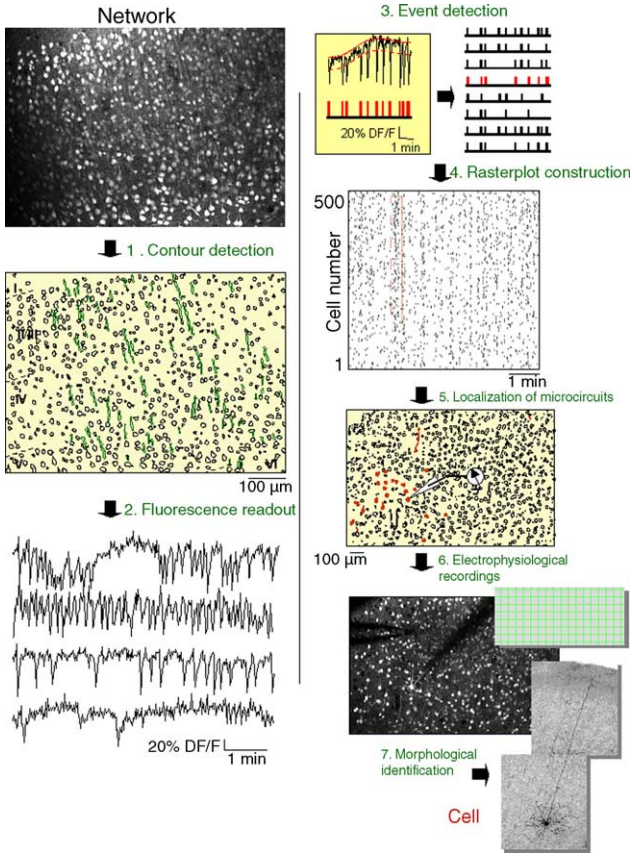

Fig. 2. Two-photon imaging of network dynamics: from the network to the cell. First, the calcium fluorescence changes as a function of time are recorded in a slice loaded with a membrane-permeable calcium indicator using a two-photon microscope for around 5 min. Top left photomicrograph illustrates the two-photon calcium fluorescence image of a V1 slice taken at low-magnification (20×, 0.95 NA, MagFura2-AM loading). Image is an average of 300 consecutive frames. Time resolution: 1.2 s/frame. Then, experiments proceed is as follows: (1) contour detection: contour plot of the thousands of cell bodies and dendrites imaged above are detected by an automatic procedure. (2) Fluorescence readout: representative traces of calcium transients. Time resolution: 1.2 s/frame. (3) Event detection: threshold based event detection, one standard deviation below baseline. (4) Rasterplot construction: representative rasterplot. Each row represents a single cell, and each mark a detected calcium transient. (5) Localization of microcircuits: once an interesting network event is detected, cells involved in it are highlighted in the contour plot (red-filled contours). (6) Electrophysiological recordings: Photomicrograph of the cell thus detected, recorded in whole-cell and filled with biocytin. (7) Morphological identification: photomicrograph of a biocytin-filled neuron.

4. Calcium imaging, online signal analysis and morpho-functional characterization of targeted microcircuits

The cerebral cortex receives input from lower brain regions and is responsible for information processing at multiple levels of abstraction. Therefore, it has traditionally been thought of as a feedforward system that processes input through successive stages to reach an appropriate output. Yet, several observations support the view of cortical circuits as feedback systems, the most striking feature being the fact that

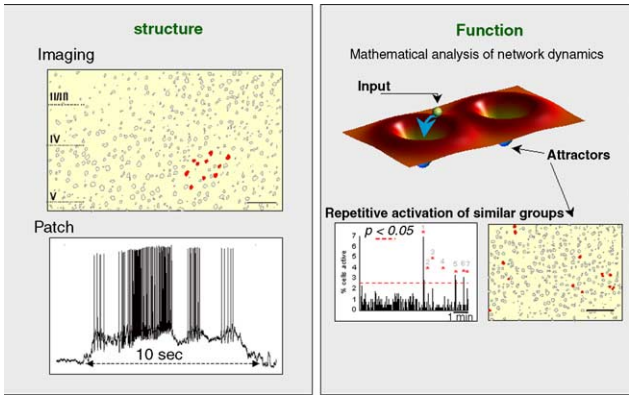

Fig. 3. Structure–function study of network activity in cortical slices. Combining two-photon calcium imaging (representative contour plot showing a group of cells active, red, during a network UP state), patch-clamp recordings of identified interesting elements (current-clamp recording of a cell in a depolarized UP state) and mathematical analysis of network dynamics, we have shown that network UP states were circuit attractors (as schematized in the dynamical system represented by a marble rolling on a surface). Such network events repeatedly involve similar neuronal ensembles (the cells marked in red in the contour plot are active at the seven peaks detected in the adjacent histogram).

they are constantly active even in the absence of sensory input. Such spontaneous firing can be coordinated among specific groups of neurons in a way that probably reflects the underlying functional architecture of the cortex. Using calcium imaging of large neuronal populations we found that, in the absence of any stimulation, groups of cortical neurons simultaneously enter depolarized membrane potential UP states. These 'network UP states' involve small, but highly significant numbers of cells (2–3%). Coactive ensembles of neurons are often organized into clusters, layers, or columns (Fig. 3). Briefly, our procedure was as follows: we first imaged the calcium fluorescence changes of a large field of view (using a low-magnification 20×, high NA objective) for several minutes (5–10 min) with two-photon microscopy. Second, we automatically detected the events and reconstructed the network activity. We then performed a statistical analysis of relevant patterns. This analysis was based on reshuffling techniques. To determine whether a pattern in space or time was significant, we created surrogate arrangements of spike discharges or of active neurons using interval reshuffling or MonteCarlo simulations and determined whether the observed arrangement could be expected by chance or not. Once the cells involved in a significant pattern were localized, we would record from them in order to characterize the physiological events underlying the calcium activity visualized in the network. We found that the intracellular correlate to the calcium activity was a shift of the membrane potential to a depolarized state where it sustained high frequency firing. These periods of sustained firing represented a stable intracellular state since they lasted several seconds, an order of magnitude longer that regular feedforward information flow (Fig. 3). Also, it is worth stressing that during these cellular UP states, not only the action potential firing, but also the calcium concentration

are maintained elevated in the active neuron. In other words, the calcium signal might not only be the fingerprint of sodium spiking and network UP states should be also viewed as maintained high calcium levels in precisely organized neuronal ensembles. Cells active during UP states are also in a particular condition as viewed from their intracellular machinery and it cannot be excluded that this status might regulate a gene expression cascade that could for example reinforce certain synaptic connections or weaken others. Interestingly, similar elevated calcium levels associated to a sustained electrical signal are observed in developing networks, although in this case they tend to involve a majority of neurons [20,26,27]. For example, in the developing neocortex, when functional synaptic connections are sparse, multineuronal calcium domains organized in columns can be observed [27]. In the adult, paroxysmal activities occurring in rewired epileptic circuits are also associated to an increase of intracellular calcium concentration. Synchronous long-lasting calcium events such as described during network UP states might, therefore, be a hallmark of some type of network synchronization.

Furthermore, network UP states are also reminiscent of the elevated firing rates recorded during working memory tasks in the awake animal [5]. Theoretically, working memory can be modeled using circuit attractors [28]. We thus analyzed the dynamics of the network events in order to extract the properties that could be relevant to attractors. We provided experimental evidence for the existence of such attractors in the cortex, which seems to be consistent with the theoretical descriptions, although obviously limited by experimental conditions. In a way, the use of slices and of large-scale calcium imaging allowed us to reveal individual cortical microcircuits, which would otherwise be embedded in a high level of background activity (in vivo) or buried in a circuit of rarely active cells (in vitro). Time resolution was a serious limitation in the study of cortical network dynamics. We thus improved temporal resolution in a more recent study using spinning-disk confocal microscopy and performed large-scale calcium imaging. This confocal microscope uses a multiple pinhole mast (a so-called Nipkow disk) in an intermediate image plane to scan numerous illumination points over the specimen without moving the light source or microscope stage, so it is capable of acquiring images at a rate of up to 300 frames/s (the actual data-acquiring speed is limited by the capability of a CCD camera used). By using this fast-scanning system, we observed that not only network UP states involving the same microcircuits could repeat, but also that the sequence of activation between different UP states was itself stereotyped as if the cortex replayed similar songs [6].

5. Conclusion

We have described our investigation of multicellular network dynamics by the combined use of large-scale calcium imaging, online statistical analysis and targeted electrophysiological recordings. A similar type of experimental and analytical research strategy could be applied to the characterization of other types of songs played by the cortex. For example, it is not known whether an epileptic seizure has specific generators or recruits different cell types in a stereotypical way. Similarly, the morphological and electrophysiological properties of the first active microcircuits in developing cortical structures as well as possible sources for early network oscillations remain to be determined. Finally, large-scale cellular calcium dynamics can be observed in other excitable tissues than the brain. For example calcium waves propagate in cardiac or endocrine tissues. Physiological studies of these systems should benefit from this type of approach.

References

[1] A. Destexhe, M. Rudolph, D. Pare, The high-conductance state of neocortical neurons in vivo, Nat. Rev. Neurosci. 4 (2003) 739–751.
[2] J. Anderson, I. Lampl, I. Reichova, M. Carandini, D. Ferster, Stimulus dependence of two-state fluctuations of membrane potential in cat visual cortex, Nat. Neurosci. 3 (2000) 617–621.
[3] M.V. Sanchez-Vives, D.A. McCormick, Cellular and network mechanisms of rhythmic recurrent activity in neocortex, Nat. Neurosci. 3 (2000) 1027–1034.
[4] T. Badea, J. Goldberg, B. Mao, R. Yuste, Calcium imaging of epileptiform events with single-cell resolution, J. Neurobiol. 48 (2001) 215–227.
[5] P.S. Goldman-Rakic, Cellular basis of working memory, Neuron 14 (1995) 477–485.
[6] Y. Ikegaya, et al., Synfire chains and cortical songs: temporal modules of cortical activity, Science 304 (2004) 559–564.
[7] D.D. Mott, R. Dingledine, Interneuron diversity series: interneuron research—challenges and strategies, Trends Neurosci. 26 (2003) 484–488.
[8] Y. Ramon, S. Cajal, Histologie du systeme nerveux de l'homme et des vertebres, Tome II, Maloine, Paris, 1911.
[9] Y. Ben Ari, Developing networks play a similar melody, Trends Neurosci. 24 (2001) 353–360.
[10] G. Buzsaki, Large-scale recording of neuronal ensembles, Nat. Neurosci. 7 (2004) 446–451.
[11] B. Kuhn, P. Fromherz, W. Denk, Large voltage-sensitive dye signals measured with one and two-photon excitation, Soc. Neurosci. Abstr. (2004/2003).
[12] R. Yuste, L.C. Katz, Control of postsynaptic Ca^{2+} influx in developing neocortex by excitatory and inhibitory neurotransmitters, Neuron 6 (1991) 333–344.
[13] B.Q. Mao, F. Hamzei-Sichani, D. Aronov, R.C. Froemke, R. Yuste, Dynamics of spontaneous activity in neocortical slices, Neuron 32 (2001) 883–898.
[14] D.W. Tank, W.G. Regehr, K.R. Delaney, A quantitative analysis of presynaptic calcium dynamics that contribute to short-term enhancement, J. Neurosci. 15 (1995) 7940–7952.
[15] D. Smetters, A. Majewska, R. Yuste, Detecting action potentials in neuronal populations with calcium imaging, Methods 18 (1999) 215–221.
[16] A. Tashiro, J. Goldberg, R. Yuste, Calcium oscillations in neocortical astrocytes under epileptiform conditions, J. Neurobiol. 50 (2002) 45–55.
[17] M.T. Hasan, et al., Functional fluorescent Ca(2+) indicator proteins in transgenic mice under TET control, PLoS Biol. 2 (2004) 163.
[18] V. Baubet, et al., Chimeric green fluorescent protein-aequorin as bioluminescent Ca^{2+} reporters at the single-cell level, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 7260–7265.

[19] C. Stosiek, O. Garaschuk, K. Holthoff, A. Konnerth, In vivo two-photon calcium imaging of neuronal networks, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 7319–7324.
[20] O. Garaschuk, J. Linn, J. Eilers, A. Konnerth, Large-scale oscillatory calcium waves in the immature cortex, Nat. Neurosci. 3 (2000) 452–459.
[21] X. Leinekugel, I. Medina, I. Khalilov, Y. Ben Ari, R. Khazipov, Ca^{2+} oscillations mediated by the synergistic excitatory actions of GABA(A) and NMDA receptors in the neonatal hippocampus, Neuron 18 (1997) 243–255.
[22] W. Denk, J.H. Strickler, W.W. Webb, Two-photon laser scanning fluorescence microscopy, Science 248 (1990) 73–76.
[23] R. Yuste, W. Denk, Dendritic spines as basic functional units of neuronal integration, Nature 375 (1995) 682–684.
[24] C. Stosiek, O. Garaschuk, K. Holthoff, A. Konnerth, In vivo two-photon calcium imaging of neuronal networks, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 7319–7324.
[25] R. Cossart, D. Aronov, R. Yuste, Attractor dynamics of network UP states in the neocortex, Nature 423 (2003) 283–288.
[26] Y. Ben Ari, Excitatory actions of gaba during development: the nature of the nurture, Nat. Rev. Neurosci. 3 (2002) 728–739.
[27] R. Yuste, A. Peinado, L.C. Katz, Neuronal domains in developing neocortex, Science 257 (1992) 665–669.
[28] J. Hopfield, Neural networks and physical systems with emergent collective computational properties, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 2554–2558.