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Large-scale recording of neuronal ensembles

György Buzsáki

How does the brain orchestrate perceptions, thoughts and actions from the spiking activity of its neurons? Early single-neuron recording research treated spike pattern variability as noise that needed to be averaged out to reveal the brain's representation of invariant input. Another view is that variability of spikes is centrally coordinated and that this brain-generated ensemble pattern in cortical structures is itself a potential source of cognition. Large-scale recordings from neuronal ensembles now offer the opportunity to test these competing theoretical frameworks. Currently, wire and micro-machined silicon electrode arrays can record from large numbers of neurons and monitor local neural circuits at work. Achieving the full potential of massively parallel neuronal recordings, however, will require further development of the neuron–electrode interface, automated and efficient spike-sorting algorithms for effective isolation and identification of single neurons, and new mathematical insights for the analysis of network properties.

Input–output analysis of neuronal networks is complicated because the brain does not simply represent the environment in a different format[1]. Features of the physical world do not inherently convey whether, for a brain, a situation is familiar or novel or whether a stimulus is pleasant or repellent[2]. These attributes are added to the information conveyed by the sensory inputs by a process referred to as cognition[3]. The longer the elapsed time from the onset of an event, the further its influence spreads in the brain, involving an ever-increasing population of co-active neurons. Ensemble activity of neurons therefore reflects the combination of some selected physical features of the world and the brain's interpretation of those features. Even if the stimulus is invariant, brain state is not. The longer the synaptic path length from the periphery, the more activity of single neurons is expected to be determined by the activity of their peers and the less it is determined by the features of the environment. Spike threshold and pattern variability have been traditionally viewed as an indication of the brain's imperfection, a noise that should be averaged out to reveal the brain's true attitude toward the input[4]. Alternatively, we may hypothesize that the 'noise', that is, the mismatch between the physical input and neuronal response, reflects self-organized patterns in the brain, and it is this centrally coordinated activity of cortical neurons that creates cognition[3,5]. Extracting the variant (brain-generated) features, including the temporal relations among neuronal assemblies and assembly members from the invariant features represented by the physical world might provide clues about the brain's perspective on its environment. How should one proceed to test these competing frameworks?

Let us simplify the task and imagine that our goal is to understand the operation and function of an orchestra, without knowing much about the role of strings, woodwinds, brass or percussion instruments and the way they sound. The first available method is to record the total noise generated by the orchestra but without the ability to distinguish the instruments and musicians. The dynamics of the continuous time-variable signal can be analyzed by various mathematical means in the time and frequency domains, but these methods can reveal little about orchestration. This 'temporally integrated field' method is analogous to recording with electroencephalography (EEG) or magnetoencephalography (MEG) in the brain. A second method can take infrared pictures of the orchestra. This will measure the heat generated by the musicians' muscle activity. Given the orderly arrangement of the instruments, the pictures taken during some passages of the melody can identify spots of dominant activity, an approach analogous to functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) snapshots taken from the living brain. Unfortunately, this 'spatial mean field' approach fails to capture the essence of music: temporal dynamics. A third method can sense the sound pressure generated by any one of the instruments and send a pulse to the observer whenever the pressure exceeds a certain threshold, analogous to recording of action potentials (spikes) emitted by single neurons in the brain. By monitoring different but single musical instruments of the same or even different orchestras over many successive performances and pooling the measurements as if they were recorded simultaneously, one can reconstruct some essential feature of the score[6,7]. This independent 'single-cell' approach has yielded significant progress in neuroscience[8]. However, this method would fail when applied to a jazz ensemble where the tune is created by the dynamic interactions among the musicians 'on the fly' and which interactions vary from performance to performance. It also largely fails when applied to central brain circuits where myriad ensembles are at work at multiple temporal and spatial scales.

Field potential analysis, imaging of energy production in brain structures and single-cell recording techniques are the principal instruments in the arsenal of contemporary cognitive-behavioral neuroscience for the study of the intact brain. Even their combined, simultaneous application in behaving subjects falls short of the goal of explaining how a coalition of neuronal groups make sense of the world, generate ideas and goals, and create appropriate responses in a changing environment. In the brain, specific behaviors emerge from the interaction of its con-

György Buzsáki is at the Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, 197 University Avenue, Newark, New Jersey 07102, USA.
e-mail: buzsaki@axon.rutgers.edu

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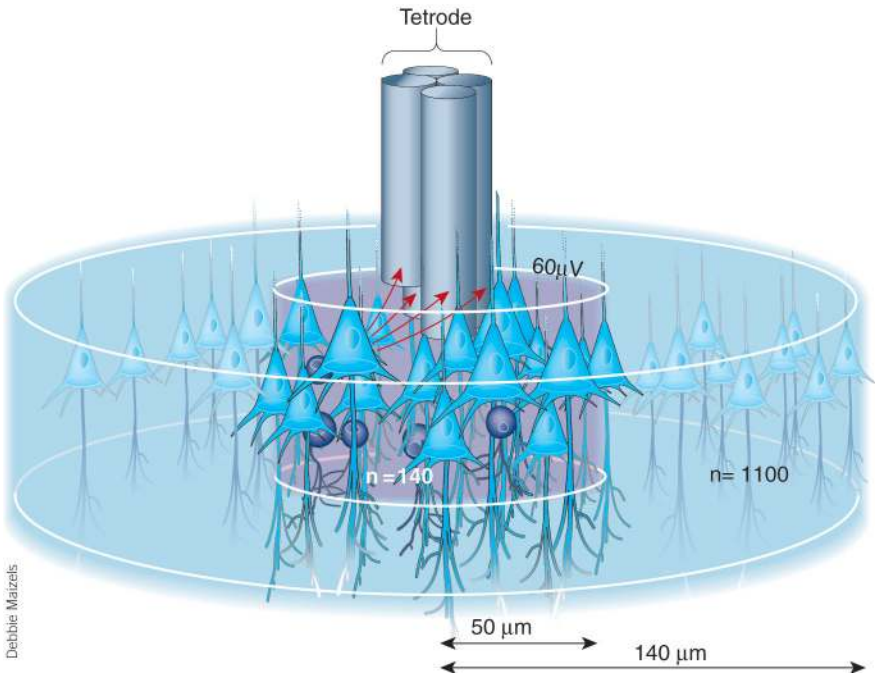

Figure 1 Unit isolation quality varies as a function of distance from the electrode. Multisite electrodes (a wire tetrode, for example) can estimate the position of the recorded neurons by triangulation. Distance of the visible electrode tips from a single pyramidal cell (triangles) is indicated by arrows. The spike amplitude of neurons (>60 μV) within the gray cylinder (50 μm radius), containing ~100 neurons, is large enough for separation by currently available clustering methods. Although the extracellularly recorded spike amplitude decreases rapidly with distance, neurons within a radius of 140 μm, containing ~1,000 neurons in the rat cortex[19,21], can be detected. Improved recording and clustering methods are therefore expected to record from larger number of neurons in the future. (Data are derived from simultaneous extracellular and intracellular recordings from the same pyramidal cells from ref. 19.)

stituents: neurons and neuronal pools. Studying these self-organized processes requires simultaneously monitoring the activity of large numbers of individual neurons in multiple brain areas. Recording from every neuron in the brain is an unreasonable goal. On the other hand, recording from statistically representative samples of identified neurons from several local areas while minimally interfering with brain activity is feasible with currently available and emerging technologies and indeed is a high-priority goal in systems neuroscience. Many other methods, such as pharmacological manipulations, macroscopic and microscopic imaging and molecular biological tools, can aid this task, but in the end all these indirect observations should be translated back into a common currency—the format of neuronal spike trains—to understand the brain's control of behavior.

Massive parallel recording from multiple single neurons

Action potentials produce large transmembrane potentials in the vicinity of their somata. These output signals can be measured as a voltage difference by placing a conductor, such as the bare tip of an insulated wire, in close proximity to a neuron[9]. If there are many active (spiking) neurons in the vicinity of the tip, the electrode records from all of them (**Fig. 1**). Because neurons of the same class generate identical action potentials (all first violins sound the same), the only way to identify a given neuron from extracellularly recorded spikes is to move the electrode tip closer to its body (<20 μm in cortex) than to any other neuron. To record from another neuron with certainty, yet another electrode is needed. The important advances made by the one electrode/one (few) neuron method[10–14] are highlighted by Chapin (p. 452–455 in this issue)[15]. Because electrical recording from neurons is invasive, monitoring from larger numbers of neurons inevitably increases tissue damage. Furthermore, understanding how the cooperative activity of different classes of neurons gives rise to collective ensemble behavior requires their separation and identification. Because most anatomical wiring is local, the majority of neuronal interactions, and thus computation, occur in a small volume[16]. In the neocortex, the 'small volume' corresponds to hypothetical cortical modules (for example, mini- and macro-columns, barrels, stripes, blobs), with mostly vertically organized layers of principal cells and numerous interneuron types. Thus, improved methods are needed for the simultaneous recording of closely spaced neuronal populations with minimal damage to the hard wiring.

The recent advent of localized, multi-site extracellular recording techniques has dramatically increased the yield of isolated neurons[7,17,18]. With only one recording site, neurons that are the same distance from the tip provide signals of the same magnitude, making the isolation of single cells difficult. The use of two or more recording sites allows for the triangulation of distances because the amplitude of the recorded spike is a function of the distance between the neuron and the electrode (**Fig. 1**)[17–19]. Ideally, the tips are separated in three-dimensional space so that unequivocal triangulation is possible in a volume. This can be accomplished with four spaced wires (~50 μm spread; dubbed 'tetrodes')[18–20]. Wire tetrodes have numerous advantages over sharp-tip single electrodes, including larger yield of units, low-impedance recording tips and mechanical stability. Because the recording tip need not be placed in the immediate vicinity of the neuron, long-term recordings in behaving animals are possible.

Cortical pyramidal cells generate extracellular currents that flow mostly parallel with their somatodendritic axis. Nevertheless, electrodes can 'hear' hippocampal CA1 pyramidal cells as far away as 140 μm lateral to the cell body, although the extracellular spike amplitude decreases rapidly as a function of distance from the neuron[19]. A cylinder with a radius 140 μm contains ~1,000 neurons in the rat cortex[19,21], which is the number of theoretically recordable cells by a single electrode (**Fig. 1**). Yet, in practice, only a small fraction of the neurons can be reliably separated with currently available probes and spike sorting algorithms[5,7,22]. The remaining neurons may be damaged by the blunt end of the closely spaced wires, or may be silent or too small in amplitude. Thus, there is a large gap between the numbers of routinely recorded and theoretically recordable neurons.

An ideal recording electrode has a very small volume, so that tissue injury is minimized. However, a very large number of recording sites is ideal for monitoring many neurons. Obviously, these competing requirements are difficult to satisfy. Micro-Electro-Mechanical System (MEMS)-based recording devices can reduce the technical limitations inherent in wire electrodes because with the same amount of tissue displacement, the number of monitoring sites can be sub-

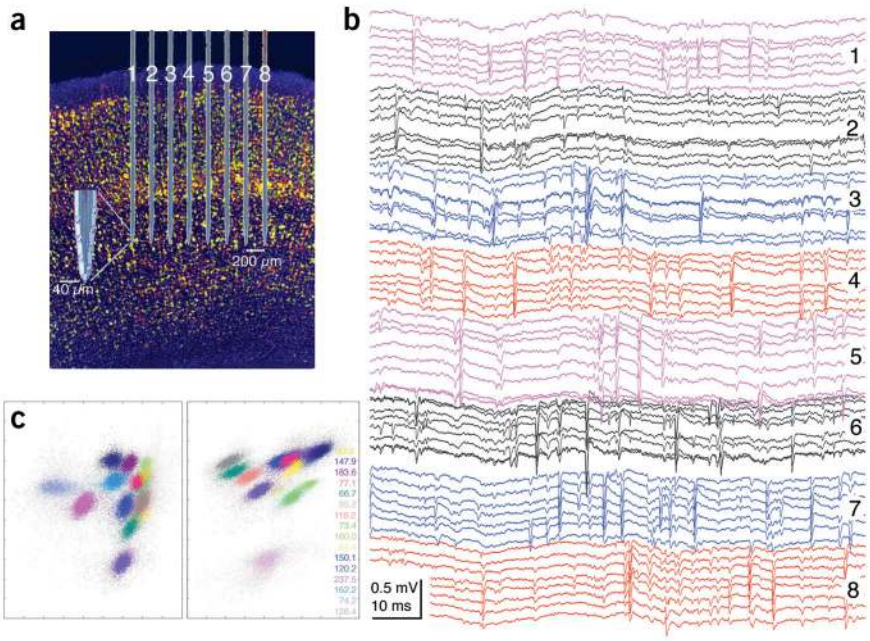

Figure 2 High-density recording of unit activity in the somatosensory cortex of the rat. (**a**) Placement of an eight-shank silicon probe in layer 5. The eight iridium recording sites at the edges of the tip (inset) are connected to the extracranial electronics via 2-μm interconnects[27,28]. (**b**) A short epoch of raw recording, illustrating both field and unit activity (1–5 kHz). Note the presence of spikes on several sites of the same shank (color-coded) and lack of spikes across the different shanks, indicating that electrodes placed ≥200 μm laterally record from different cell populations. (**c**) Two-dimensional views of unit clusters (out of 28 possible views from an eight-site probe) from one shank. Clusters are color-coded. The success of cluster separation is quantified by measuring the Mahalanobis distance from a given cluster center within which as many points belong to other clusters as belong to the specified cluster[35]. The larger values of 'isolation distance' (right) correspond to progressively better neuron isolation. This figure was modified from ref. 28 with permission from the APS (American Physiological Society).

stantially increased[23–25]. Whereas silicon probes have the advantages of tetrode recording principles, they are substantially smaller in size. Furthermore, multiple sites can be arranged over a longer distance, thus allowing for the simultaneous recording of neuronal activity in the various cortical layers[26]. Currently available multi-shank probes can record from as many as a hundred well-separated neurons (**Fig. 2**). Importantly, the geometrically precise distribution of the recording sites also allows for the determination of the spatial relationship of the isolated single neurons[27,28] (**Fig. 3**). This feature is a prerequisite for studying the spatiotemporal representation and transformation of inputs by neuronal ensembles. The principal limitation of increasing the numbers of recording sites is the width of the interconnection between the recording tips and the extracranial connector (**Fig. 3**; 2 μm-wide connections with 2-μm space)[23,25]. It should be noted, though, that industrial production presently uses 0.18 μm line features, and multiple levels of metal and much thinner interconnect lines are expected to become standard in coming years.

Isolation and identification of neurons by extracellular signatures

An indispensable step in spike-train analysis is the isolation of single neurons on the basis of extracellular features. Spike sorting methods fall into two broad classes. The first class attempts to separate spikes on the basis of amplitude and wave form variation[11–15] on the assumption that neighboring neurons generate invariant spike features. This assumption is difficult to justify in most cases[17–19]. The second general approach, triangulation, is based on the tacit assumption that the extracellularly recorded spikes emanate from point sources[17,18] rather than from the complex geometry of neurons. This is obviously a simplistic idea, because every part of the neuronal membrane is capable of generating action potentials[29]. The extent of the somatodendritic back-propagation of the action potential varies as a function of the excitatory and inhibitory inputs impinging on the neuron[30]. Because the extracellular spike is a summation of the integrated signals from both soma and large proximal dendrites[31], the extracellularly recorded spike parameters depend on the extent of spike backpropagation and on other state- and behavior-dependent changes of the membrane potential[32,33] (**Fig. 4**). These changes can affect the esti-

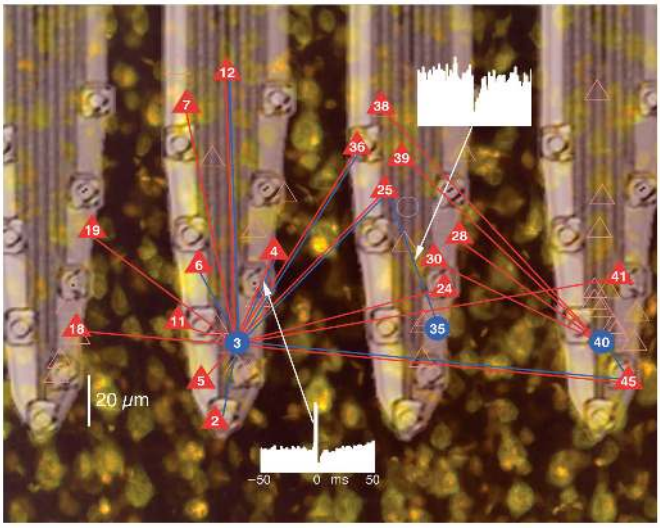

Figure 3 Functional topography within the recorded population in the somatosensory cortex of the rat. Filled symbols, participating pyramidal cells (red triangles) and interneurons (blue circles). Empty symbols, neurons not connected functionally. Red line, monosynaptic excitation; blue line, monosynaptic inhibition. Note that interneurons (*e.g.*, 3 and 40) are activated by large numbers of pyramidal cells[41–43], and an interneuron inhibits several local and distant pyramidal cells[42]. The relative positions of the neurons was determined by calculating the 'center of mass' of spike amplitude recorded from multiple sites. Recording sites are spaced 20 μm vertically. The shanks were 200 μm apart, but for illustration purposes they are placed closer in the figure. Cross-correlograms between an interneuron-pyramidal cell pair (35–25) and reciprocally connected pair (3–4) are shown in white. Large-scale recordings and network analysis offer an opportunity for identifying network and behavior-dependent variation of cell assemblies[3,5,21]. This figure was modified from ref. 28 with permission from the APS (American Physiological Society).

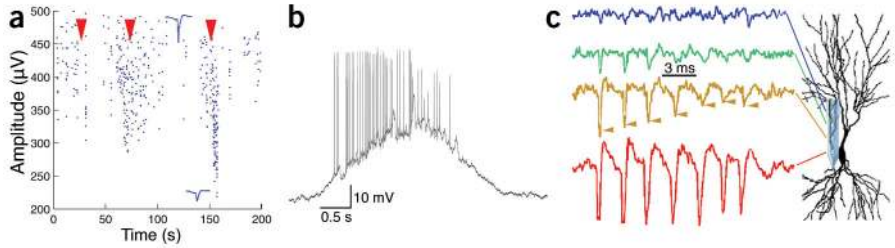

Figure 4 Behavior and network-dependent variability of spike amplitude and waveform is the most important source of unit classification errors. (**a**) Amplitude variability of a well-isolated CA1 pyramidal cell as the rat repeatedly visits the receptive field of the neuron (red arrows). Note large (≥50%) amplitude reduction of the extracellularly recorded spikes within the place field. Insets, spikes outside of place field with low level of activity (top) and in the middle of field with maximum activity (bottom), respectively. (**b**) *In vivo* intracellular recording from the proximal apical dendrite of a CA1 pyramidal cell. Place field-associated synaptic excitation was mimicked by intracellular injection of a 3-second long cosine wave. Note reduction of spike amplitude reduction with increasing level of depolarization and discharge frequency. (**c**) Attenuation of spike backpropagation during a complex spike burst. Note progressive amplitude change at dendritic recording sites (arrowheads). (**a**) Reprinted from ref. 34 with permission from Elsevier. (**b**) Unpublished data from D.A. Henze and G.B. (**c**) Modified from ref. 33.

mation of the neuron's virtual 'point source' location and may place the same neuron at different locations, resulting in omission errors of unit isolation. The spike amplitude variation is most substantial during complex spike burst production, with as much as 80% amplitude reduction[32,34] (**Fig. 4**) because of Na^+ channel inactivation[30]. Improved spike sorting methods therefore analyze not only the amplitude but also wave shape variation of spikes[22,35].

Another problem with the point-source assumption for action potentials is that the somatic origin is not always resolvable with distant recording sites. For example, in the rat neocortex, extracellular spikes can be recorded from the apical shaft of layer-5 pyramidal neurons as far 500 μm from the cell body[26]. As a consequence, a single electrode tip, or a tetrode, placed in layer 4 can equally record from layer-4 cell bodies or apical dendrites of deeper neurons. Such misclassification errors are especially serious in the primate brain where spikes can be recorded from several hundred micrometers away from the cell bodies of large pyramidal cells. These sources of unit sorting errors can be circumvented by recording at multiple sites parallel with the axodendritic axis of the neurons. Importantly, such multiple-site monitoring can be exploited for the study of behavior-dependent intracellular features[19] and for resolving temporally superimposed spikes of different neurons[36].

The amplitude and waveform variability of the extracellularly recorded spike is the major cause of unit isolation errors. Triangulation methods visually analyze two-dimensionally projected datasets at a time. With multiple site-recorded data, successive comparisons of the various possible projections generate cumulative errors of human judgment. Cumulative human errors can be eliminated by automatic clustering methods of high-dimensional data[35–37]. A further difficulty is that no independent criteria are available for the assessment of omission and commission errors of unit isolation. As a result, improvement of spike sorting algorithms[17,18,22,35,36] is not guided by objective measures. In the absence of quantitative criteria for unit isolation quality, inter-laboratory comparison is difficult and makes interpretation controversial[38]. A recent study[19] involving simultaneous intracellular and extracellular recording from the same pyramidal neurons resulted in a new database that allows for the objective assessment of spike classification errors, as well as the development of a semiautomatic clustering algorithm that is superior to manual clustering[35] (software can be downloaded from http://klusters.sourceforge.net or http://klustakwik.sourceforge.net).

After most or all instruments of the orchestra are isolated, the next step is the separation of strings from woodwinds and then oboes from clarinets. This is an important step because brain networks consist of several neuronal classes, each with a specific computation task. Paradoxically, current neurophysiological practice rarely distinguishes among the various neuron classes. Unit classes are typically generated *post hoc*, as they relate to behavior, without reference to the types of neurons that give rise to them. However, the potential conclusion from an experiment reporting that all cortical pyramidal cells did one thing and all interneurons something else is qualitatively different from the conclusion that can be drawn from the information that 80% of all (unclassified) cells behaved differently from the rest. To understand the contribution of different neuron types to network activity, they have to be identified.

In the hippocampus, several features, such as spike duration, firing rate and pattern, spike waveform and the relationship to field patterns, can be used to separate pyramidal cells from interneurons and some interneuron classes from each other[22,39]. Similar classification

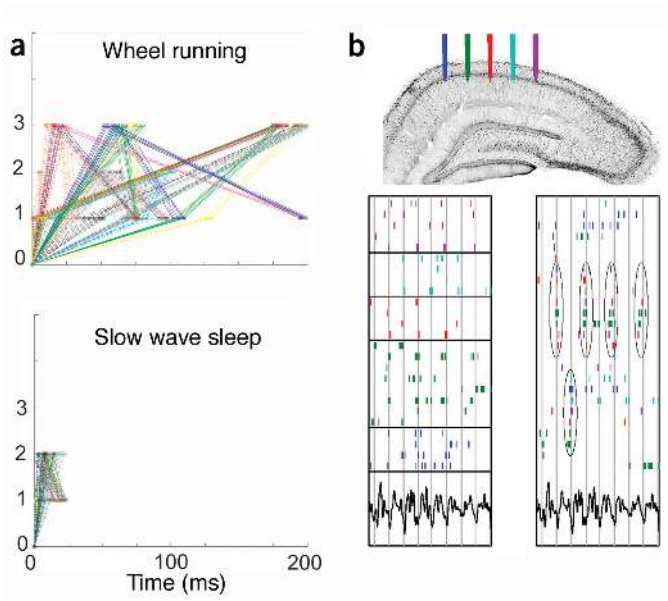

Figure 5 Coordination of assembly patterns in the hippocampus. (**a**) Examples of the spike sequences from a single tetrode (neurons 0 to 3) during wheel running and sleep sessions, with neuron 0 as the sequence initiator. Different colors indicate different patterns. Note time-compressed sequences during sleep. (**b**) Cell assembly activity in a population. Neuronal spikes during a 1-s period of spatial exploration (left) are arranged in order of physical position of the recording silicon probe within the CA1 pyramidal layer (top). Vertical lines: troughs of theta waves (bottom trace). Right, the same spike rasters as seen at the left, but reordered by stochastic search over all possible orderings. Cell assembly organization is now visible, with repeatedly coordinated firing of certain subpopulations (circled). Reprinted from ref. 49 (**a**) and ref. 5 (**b**).

criteria are not yet available in the neocortex[40]. Large-scale, high-density recording of neurons, however, can facilitate the classification process. This is because a small percentage of cell pairs show robust, short-timescale correlations, indicative of monosynaptic connections[21,28,40-42] (**Fig. 3**). Monosynaptic excitation and inhibition can thus identify excitatory and inhibitory neuronal classes, respectively. In turn, the extracellular features of the identified minority can be used as a template for classifying the remaining population. Separation within the excitatory and inhibitory families of neurons is the next critical step, and this task will benefit from a cooperation of various complementary methods, including *in vivo* and *in vitro* juxtacellular and intracellular labeling and molecular biological tools[42–44].

Local field activity

A necessary requirement for understanding the transformation of inputs by a neuron or neuronal assemblies is information about both their input and output. In contrast to recording spike output with high precision, no method is available for monitoring all inputs at the resolution of dendrites and spines of single neurons. Large-scale monitoring of spike patterns of presynaptic cell assemblies to a single neuron is a potential strategy (for example, neuron 3 in **Fig. 3**). An alternative compromise is measuring the extracellular current flow that reflects mainly the linearly summed postsynaptic potentials from local cell groups[45]. Recording the voltage gradients by geometrically arranged sites of silicon probes allows current densities to be calculated for an estimation of the mean input to the neuron group in the recorded volume[45]. High-density recording with silicon probes therefore can be used not only to monitor the spike output from neuronal groups, but also to estimate their summed input. High spatial resolution of extracellular currents together with spiking activity of neurons from which these currents emanate provides a means for assessing input–output relations of neuronal coalitions.

New insights from large-scale recording of neuronal populations

Recording from large numbers of neurons will reduce the number of animals, their maintenance costs and the variability inherent in recording data over multiple sessions[46,47]. However, the main goal of large-scale recording of neuronal activity is to reveal information available only from the interaction of the constituents of neuronal ensembles. Brain networks are strongly interconnected, and firing patterns of single neurons are influenced by the activity of their peers, not unlike the way that members of a jam session influence each other. Such emergent qualities can be revealed only by observing statistically representative groups of the population. For example, hippocampal pyramidal cells during rest and sleep produce strongly coherent ensemble bursts believed to be critical in transferring information to the neocortex. Although robust at the population level, no amount of sequential single-cell recording could reveal such cooperative patterns[48]. As in music, the temporal sequence of neuronal spikes conveys information[2,3]. Using large-scale recordings, researchers can follow complex patterns for extended time periods and determine whether their modification by experience will influence self-generated patterns in the absence of environmental inputs. Spike sequences, imposed upon the network by behavioral manipulations, recur spontaneously during subsequent sleep episodes[49–51] (**Fig. 5a**), indicating that neurons organize themselves into preferred cell assemblies, and the seeds of emergence stem from experience-related activity. A postulated signature of the cell assembly is that its participants show a higher probability of spiking together than with members of other assemblies, even in the absence of external inputs[52]. Testing this long-standing hypothesis required recordings from large pools of local neurons and novel mathematical tools. Interactions among hippocampal neurons recorded in parallel revealed the dynamical emergence of assemblies that lasted long enough to have a maximum impact on their targets. The spike time variability of assembly members poorly correlated with environmental inputs but could be predicted from the activity of the simultaneously active neurons[5] (**Fig. 5b**). Such internally driven, self-organized assemblies may reflect mechanisms that give rise to cognitive phenomena[53,54].

This idea is further supported by the lifetime of cell assemblies, which matches the cycle period of gamma oscillation. Using two-dimensional silicon probe mapping covering three connected regions of the hippocampus, it is now possible to establish the coupling of two independent gamma oscillators, their synaptic mechanisms and brain state-dependent coupling strength[41]. In other studies, ensemble recording from anatomically interconnected structures allowed for monitoring the spatio-temporal evolution of input-dependent functional connections[15,55,56]. These global, region-coupling studies now can be analyzed at the level of monosynaptically connected neuron pairs, along with the identification of the participating neurons (**Fig. 3**). The fast-growing field of neuronal assembly control in motor behavior and prosthetic machine coordination is reviewed by Chapin (p. 452–455 in this issue)[15].

Outlook

Progress in large-scale recording of neuronal activity depends on the development of three critical components: the neuron–electrode interface, methods for spike sorting/identification and tools for the analysis and interpretation of parallel spike trains. In addition to increasing the numbers of recording sites on silicon probes, the development of on-chip interface circuitry is another priority. On-chip amplification, filtering and time-division multiplexing[25] will not only dramatically decrease the number of wires between the behaving animal and electronic equipment but may also eliminate the need for large numbers of expensive amplifiers by directly feeding the multiplexed digital signal into a computer processor. Programmed microstimulation through the recording sites[57] and, potentially, real-time signal processing not only will facilitate basic research but also is a prerequisite for efficient, fully implantable neural prosthetic devices[10–13,58].

Current spike sorting procedures are time-consuming and subjective. The importance of these issues is illustrated by the recent surge of novel spike isolation algorithms[22,35,36,59–64]. This area of research can enormously benefit from innovative mathematical tools and neuron-spike modeling studies[31] that will provide error estimates of spike isolation quality and, therefore, allow comparison across laboratories. These initial steps, including physiological classification of units that correspond to anatomically defined neuronal divisions, are indispensable for meaningful analysis of massive data sets recorded in parallel. Large-scale recorded spike trains create new challenges for data management, visualization and analysis[65]. In contrast to the tradition of classic neurophysiology with minimal statistical analysis, the large data bases require extensive 'data mining', a task that may involve several laboratories. Sharing and pooling databases are logical steps of progress, but care should be taken that the necessary standardization procedures will not compromise experimental design and innovations. The stakes are high because high-density recording of neuronal activity may be the engineering tool for getting into the inner works of the brain.

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COMPETING INTERESTS STATEMENT
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