Multielectrode recordings: the next steps

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At present, a growing number of laboratories are acquiring the capability of simultaneously monitoring the extracellular activity of over a hundred single neurons in both anaesthetized and awake animals. This paradigm, known as multielectrode recordings, is changing the face of systems neuroscience by allowing, for the first time, the visualization of the function of entire neural circuits at work. Current methods of multielectrode recording employ state of the art technologies; two potential new avenues of research will likely emerge from the further development of these experimental paradigms.

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Introduction: recording from neuronal assemblies

In the 1940s, Donald Hebb [1] originally conceived the hypothesis that neural assemblies form the basic functional unit of operation of the mammalian central nervous system. Following this revolutionary theoretical proposition, the possibility of recording the extracellular electrical activity of populations of single neurons, distributed across multiple structures that define a neural circuit, became one of the 'holy grails' of systems neurophysiology. As a result, since the early 1950s, several neurophysiologists embarked on attempts to design electrophysiological methods capable of testing the principles governing the operation of dynamic distributed neural systems [2,3]. Nevertheless, it was only during the last decade that significant advances in the design and fabrication of both multielectrode probes and custom hardware for multichannel neural signal conditioning allowed a variety of methods for multielectrode recordings to flourish into a feasible experimental approach in modern neuroscience [4–8].

Today, neural ensemble recordings are carried out in a large variety of experimental conditions, which include (but are not limited to) *in vitro* paradigms using cell culture or brain slices [9,10], acute and chronic experiments in anesthetized animals [11], long-term recordings in behaving animals [12,13], and even short-term neurophysiological monitoring in human subjects who require neurosurgical intervention [14]. As a result of these advances, a few laboratories around the world have demonstrated the capability of monitoring the extracellular activity of 50–100 neurons in different species of rodents [4–6,15] and primates [13,16••]. The longevity of neural ensemble recordings has also increased significantly during the last decade. For example, despite a progressive level of degradation, chronic multielectrode recordings in rats can last several months [11,12], and in primates they have been reported to continue for a couple of years [13,17]. Another fundamental achievement was the possibility, for the first time, to simultaneously monitor the activity of neurons distributed across multiple subcortical and cortical components of large neural circuits in behaving animals [6,18].

As more and more laboratories make the transition from the classical single unit recording technique to different methods of neural ensemble recordings, it seems fair to state that the next generation of systems neurophysiologists will be formed almost exclusively of neural ensemble physiologists. This new generation will likely be able to increase by at least one order of magnitude (from hundreds to thousands) the number of single neurons recorded simultaneously (likely using Old World monkeys), sample many more subcortical and cortical brain regions at once, and maintain viable recordings almost indefinitely. Indeed, given preliminary reports of recordings of 300 neurons in Rhesus monkeys, we expect these benchmarks to be achieved within the next five years.

Here, we present a couple of examples illustrating how this expected evolution in neural ensemble physiology will impact the future of neuroscience research. We propose that these advances have the potential not only to drastically broaden the scope of systems neuroscience, but also to determine the emergence of revolutionary experimental approaches to investigate causal relationships between large-scale brain activity and animal behavior.

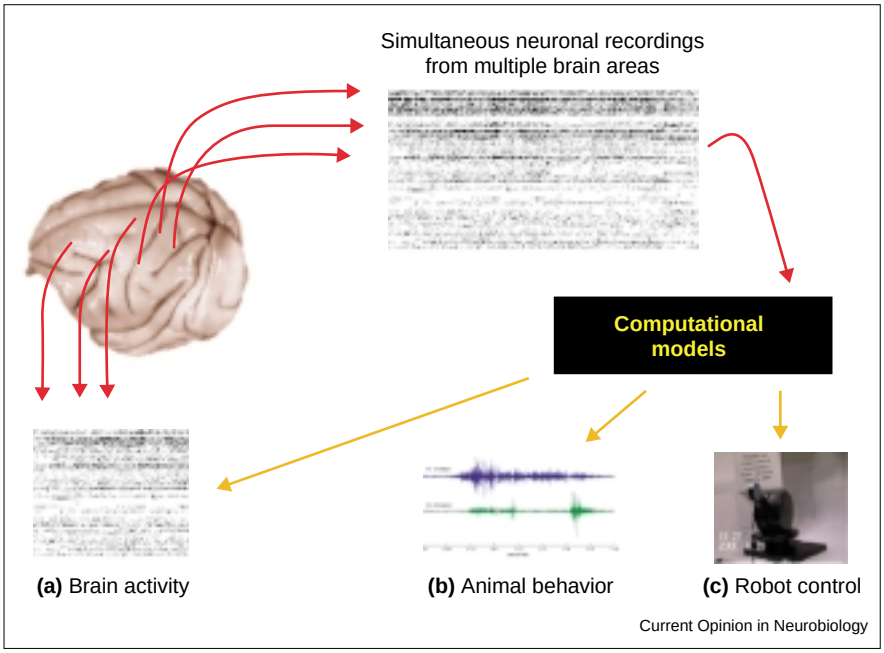
Real-time neurophysiology

The improvement in yield, stability and longevity of multielectrode recordings, combined with the crescent power of computers, has allowed neurophysiologists to implement a new experimental paradigm to investigate the computations performed by complex brain circuits to transform large-scale brain activity into animal behavior. In this paradigm, the electrical activity of populations of single neurons in the brain is sampled, conditioned, digitized and then transformed into the main input of computational models aimed at predicting some aspect of animal behavior. A fundamental tenet of this paradigm is the requirement that the computational models yield their predictions under the constraints of the animal's reaction time (e.g. a few hundred milliseconds). Because of this central aspect, we refer to these paradigms collectively as real-time neurophysiology.

Figure 1 illustrates three potential alternative designs of this new experimental paradigm. The first one (Figure 1a) depicts a computational model used to predict the activity of a particular population of neurons (e.g. a cortical area or

Figure 1

Real-time neurophysiology. Simultaneous neuronal recordings from multiple brain areas are fed to a computational model, which yields real-time predictions of **(a)** the activity of other interconnected brain areas, and **(b)** motor behavior. A similar approach **(c)** allows for the neural control of a robotic device.



a subcortical nucleus) by using a real-time transformation of the activity of other populations of neurons, located in one or multiple brain areas. Long-term simultaneous recordings from samples of neurons that define both the inputs and the outputs of the computational model ensure that this approach can be utilized to validate the model's predictions under a large variety of animal behavioral states. The second example (Figure 1b) depicts how a similar paradigm can be used to test computational models that aim to predict animal behavior from raw brain activity. In this example, a computational model is employed to predict electromyogram activity from the spatiotemporal patterns of neural ensemble activity, simultaneously sampled from multiple cortical motor areas.

The third example (Figure 1c) is the one that best illustrates the potential that real-time neurophysiology has to extrapolate the boundaries of classical systems neuroscience and brain research, and to attract the interest of researchers working in fields such as computer science, biomedical engineering, and robotics. Indeed, this experimental design is now recognized as the central paradigm aimed at testing the notion that, in a not so distant future, a cortical neuroprosthetic device may become a viable clinical alternative to restore motor functions in patients suffering from severe levels of body paralysis. The feasibility of this implementation of real-time neurophysiology has been demonstrated by recent experiments, in which the activity of 50–100 cortical motor neurons served as inputs to computational models designed to control the one-dimensional and three-dimensional movements of robot arms [16••,19,20••]. In these studies, as animals moved their limbs in response to sensory cues, the experimenters were capable of evaluating the efficacy of their models by measuring how well the robot arm reproduced the animal's arm movement. Overall, surprisingly good results were obtained by feeding the activity of these relatively small populations of neurons (50–100) into simple linear models designed to predict the animal's arm movements [16••]. A recent study reproduced this general result, although the level of prediction accuracy was reduced by a much smaller sample (~18) of recorded neurons per animal [21•]. Altogether, these finding suggest that, instead of needing to sample tens of thousands of specific neurons in a given trial to reproduce a complex arm movement, one may obtain very good predictions of complex animal behavior by feeding into simple models only the activity of a few hundred cortical cells at a time.

In a more elaborate rendition of this paradigm, the subject also receives visual feedback information describing the robot arm performance on a video monitor, thus defining a closed control loop between the animal and the robot. Because the experimenter can easily modify the source of the neural activity used as an input to the model, the population coding model employed, and a variety of parameters that define the 'sensory' feedback signals delivered to the subject, it is easy to visualize how this approach could be utilized to address fundamental questions regarding motor coding. In fact, because these recordings remain viable for several months or even years — a period during which animals can learn multiple behavioral tasks — this paradigm will likely play a central role in the future investigation of the neural mechanisms underlying motor learning [12,22].

Although most of the studies to date have focused on predictions of an animal's motor behavior, it is easy to foresee that the general experimental design depicted in Figure 1 could easily be adapted to investigate perceptual or even cognitive functions in freely behaving animals. Once again, the fundamental key in this approach is the imposition of the severe constraints of 'real-life' to the testing of one's models or theories. The crescent introduction of modern advances in microelectronics in neurophysiology will certainly allow the addition of an extra layer of complexity to some of these experiments. Thus, most of the experiments carried out under the general framework illustrated in Figure 1 will likely be carried out in untethered animals in the near future. The introduction of the first generation of low power consumption 'neurochips' for multielectrode neural signal conditioning, as well as the development of multichannel telemetry systems [23], will make several 'dream' experiments feasible in a couple of years. For instance, the idea of using real-time computational models to investigate the neural mechanisms involved in the perception and production of vocalizations in freely ranging monkeys [24] or even in songbirds [25] is likely to be just around the corner.

Gene expression and the behavior of neural assemblies

The impact of large-scale neuronal assembly recordings in behaving animals will probably have a synergistic effect with converging advances in other areas of biology where breakthroughs are currently taking place, such as in conditional gene manipulation. How does the expression of a particular gene in a specific neural population affect the behavior of neural assemblies distributed throughout the brain? Questions such as this were out of reach to neurobiology a mere decade ago, but can now be addressed by extracting massive amounts of neuronal signal data from unrestrained behaving rodents in which specific genes are temporarily switched on or off.

An increasingly popular way to tackle *in vivo* gene expression is to inject custom-made antisense RNA oligonucleotides into the extracellular space. Upon successful uptake into the cytoplasm — a process that depends on the chemical protection of oligonucleotides against the action of nucleases — antisense strands can block the expression of target genes by hybridization with complementary mRNA transcripts normally produced by the cell [26–29]. Although the antisense approach can elicit changes in gene expression within a few hours of injection, toxicity (due to the use of protective agents such as phosphorothioate) and possible non-specificity of hybridization remain topics of concern [30,31].

An alternative approach uses viruses to deliver DNA constructs of choice to neurons, a strategy that requires virus internalization and integration of the foreign DNA in the genome [32]. When transcribed, these DNA sequences can be used to either silence endogenous genes (via antisense annealing) or to overexpress exogenous, newly introduced ones. Anatomical specificity can be achieved by delivering the viruses to axon terminals, leading to retrograde transport and transgene expression in specific projection neurons [33]. The main disadvantage of the method is temporal: not only does it require several days for recombined phenotypes to be observed, but gene expression changes seem to be persistent for several months post-treatment [34].

Perhaps the most promising approach for achieving site-specific, relatively fast and reversible genetic manipulation is the use of Cre/loxP conditional transgenics, in which discrete genes can be activated or deleted at a defined point in time under the control of tissue-specific inducible promoters [35–38]. The use of viral vectors containing the Cre recombinase gene is a recent strategy for enhanced spatiotemporal control of gene expression changes [34,39].

The successful impairment of synaptic plasticity and memory consolidation by the downregulation of immediate early genes [40,41] and membrane receptors [15,38,42,43,44••] offers a good example of the promises of the methods discussed above. However, the full benefit of these techniques has yet to be taken up by systems neurophysiology, probably because neurophysiologists and molecular biologists prefer different mammalian models, namely rats and mice. Transgenic techniques were only recently extended to rats [45]; conversely, the miniaturization of multielectrode arrays so as to enable the study of the tiny mouse brain is a work in progress. Another important bottleneck is the requirement of very sharp induction kinetics for the study of most neurophysiological processes of interest. For instance, studies of learning and memory consolidation may require switching gene expression on and off in a matter of minutes, rather than hours or days. Still, despite the persistent methodological limitations, the investigation into the interactions between genomic regulation and the dynamics of large-scale neuronal responses is arguably one of the most exciting potential future developments to emerge at the frontier of systems and molecular neuroscience.

In addition to the study of gene function *per se*, the ability to induce the expression of genes of choice in behaving animals will most probably out-compete traditional methods for brain research that rely on 'loss-of-function', such as lesions and chemical inactivation. In this regard, a novel method based on the inducible activation of G-protein-coupled inwardly rectifying potassium channels may finally make it possible to reversibly silence specific neuronal types in the brain within minutes of the induction [46]. This should allow neurophysiologists to gain new insights into complex brain circuits, by independently switching off each of the circuit components for well-defined periods of time.

Conclusions: a new era for neurophysiology

In the past decade, many technological breakthroughs have allowed multielectrode recordings to become

a widely available tool to neurophysiologists. Further development of this approach is likely to lead to a significant broadening of the scope of systems neuroscience in the near future. Here, two potential new experimental paradigms that utilize large-scale multielectrode recordings were reviewed. Both examples illustrate how the combination of large multielectrode recordings with other computational, engineering and molecular tools may revolutionize the way one investigates causal relationships between brain activity and animal behavior. This combination will likely drive the main experimental and theoretical focus of systems neurophysiology to switch from single neurons to neuronal assembles, as originally predicted by Donald Hebb.

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