Beyond the Patch Clamp:
Nanotechnologies for Intracellular Recording

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The patch clamp is a fundamental tool for neuroscientists, offering insights that have shaped our understanding of the brain. Advances in nanotechnology suggest that the next generation of recording methods is now within reach. We discuss the complexity and future promise of applying nanoscience to neural recording.

Introduction

Recently there has been a surge of progress in developing nanotechnologies for biological applications (Duan et al., 2013, Rogers, 2014). These nanotechnologies may be described as functional structures or devices with at least one dimension on the scale of 100 nm or less. Critical components involved in producing a "nanotechnology" can include bottom-up synthesis of nanostructures, manipulation and/or assembly of these structures, and nanofabrication methods, which can define and connect structures on a nanoscale and larger, as in, for example, the fabrication of today's computer chips. These developments are reaching a level of sophistication that may impact standard electrophysiological recording methods. Patch clamp allows for intracellular recording and has been key to providing insights into single-cell behavior, with the capability to deconvolve neural microcircuitry (London and Häusser, 2005, Silberberg et al., 2005, Lampl et al., 1999), yet patch pipettes as a general neural interface have changed little over the past several decades and are not without experimental limitations. Here we will consider developments of nanowire electronics and soft polymer probes for intracellular neurological interfaces.

Currently, there are several challenges that limit the experimental design of neural probes. This is in part inherent to the cellular intricacy of the brain, the complexity of neural interactions, and the nonlinearities of the intracellular and network dynamics and is coupled to the need for minimally invasive and long-lasting recordings. Five specific aims and their limitations follow include subthreshold recordings, stimulation, invasiveness, multiple recordings, and stability.

Recording Subthreshold Activity

In many parts of the brain, post-synaptic potentials (PSPs) are summed across multiple neurons before any suprathreshold action potentials are generated. Recording this subthreshold activity is necessary for elucidating synaptic connectivity and microcircuitry (Silberberg et al., 2005). Additionally, neural correlations and functional relationships are widely studied in vivo with extracellular recordings, but this type of analysis must be performed using incomplete suprathreshold point processes. This creates an "iceberg effect" where only the tip of the iceberg, spiking, is used for analysis while the underlying bulk of activity is ignored (Lampl et al., 1999, Steriade et al., 2001, Kruskal et al., 2013). Continuous subthreshold fluctuations give a much more sophisticated and complete picture of how neurons receive and engage with a computation.

Single-Cell Stimulation

Control of stimulation to evoke action potentials with single-cell precision greatly enhances the type of experimental questions one might ask. For example, reverse correlation of PSPs to direct stimulation of presynaptic cells can elucidate microcircuitry (Silberberg et al., 2005). Further, stimulation even on a single-cell scale can evoke a behavioral response (Houweling and Brecht, 2008). As new technologies scale to allow for a multitude of precise stimulations, the ability to control network-level activity may be addressed.

Minimal Invasiveness

New methods are needed to sample without interfering with natural processing in the brain and/or causing excessive damage. Such advances will be of particular importance when translating methods to human subjects and developing brain-machine interfaces.

Multiple Cell-Device Interfaces

The number of cells that may be recorded simultaneously has steadily increased with improvements in new tools. Introducing new types of devices, which have higher spatial resolution, may also help with important questions requiring multiple simultaneous recordings within a single neuron's neurites or microdomains.

Stable Cell-Device Interfaces

For recordings to be useful over longer periods of time, neuronal interfaces must be stable. In particular, for behavioral experiments, having consistent recording sites for neurons across multiple trials is necessary for many sophisticated analyses. Developments in semiconductor nanowire-based devices and device arrays discussed below represent a promising direction for addressing these issues.

Semiconducting Nanowires as Novel Recording Nanodevices

Semiconductor field-effect transistors (FETs) are the basis for most modern electronics and as such have made a substantial impact on amplification and logging of signals from neural probes. These advances in miniaturization have not, however, led to development of fundamentally new types of electrophysiology tools. In general, the conductivity in a semiconductor device is controlled by added dopant atoms that have either one extra or one less valence electron than the host material. When dopants with one extra valence electron/atom are added,
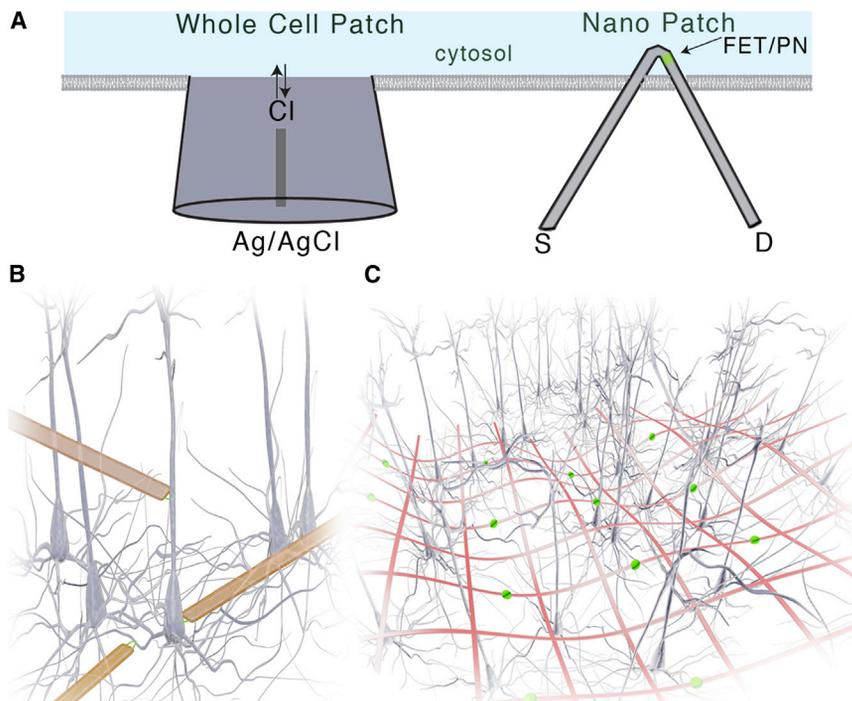
Figure 1. Nanowires as a New Approach to Electrophysiology
(A) New recording methods such as nanowires for localized intracellular recordings will have advantages compared to patch pipettes. Nanowire FETs require source (S) and drain (D) electrode connections for making high-sensitivity recordings.
(B) 3D polymer probes can direct nanowires to neuronal processes or targeted cell types.
(C) Nanowires may be integrated with 3D flexible electronics for experiments in cultures or, potentially, in vivo.

a semiconductor is termed "n-type," and conduction is by negative charge carriers; conversely, addition of dopants with one less valence electron/atom yields a "p-type" semiconductor with positive charge carriers. Spatial variations in the concentration of dopant atoms in a semiconductor allow for the implementation of different types of localized devices, including FETs and p/n junction diodes. The p/n diode has an added advantage that its function can change dependent on voltage polarity, which will be discussed further below.

A working FET device is configured by connecting source and drain electrodes, and when a voltage is applied between these electrodes, the measured conductance (which depends on device size and dopant concentration) varies as the local external potential changes due to events such as an action potential. This potential (voltage) dependent change in conductance—the device transconductance—represents the device sensitivity. Advantages of measuring local electrical

potential in this way compared to standard neurophysiological electrodes include: (1) they can be miniaturized to the 10-nm scale without loss of bandwidth, and (2) they do not exchange material with cells and do not depend on interface impedance (Duan et al., 2013).

In particular, smaller recording structures are an important goal because they offer the potential for increased stability and reduced invasiveness, as well as increasing the number of recording sites, as is important for probing functional relationships between neurons across multiple cell types. Single-ended electrodes such as microwires for tetrodes, metal pads for silicon probes, and similar devices suffer from impedance limits and related issues as the sizes of these probes shrink (Spira and Hai, 2013).

The unique scaling of FETs to ultra-small sizes relevant to new electrophysiology capabilities has been realized through the size-, dopant-, and morphology-controlled synthesis of semiconductor nanowires (Duan et al., 2013). For

example, single-crystal silicon nanowires have been grown with diameters as small as 5 nm and with dopant variations, which define the FET recording device, on the order of 10 nm. Using nanofabrication methods, including high-resolution lithography and deposition techniques, to configure these synthesized nanowire structures into nanowire FETs has led to 100-nm-scale, 3D devices small enough for isolated intracellular recordings (Tian et al., 2010), which are discussed in more detail below.

Nanowire FETs for Intracellular Recordings

One clear advantage of nanoscale devices such as nanowire FETs is their potential to be developed as novel intracellular recording tools, where several distinct approaches have been reported to date. Nanofabrication has been used to define passive metal nanopillar devices that can yield intracellular-like signals from cultured cells (Spira and Hai, 2013), while a combination of nanowire synthesis and nanofabrication have yielded several distinct types of 3D nanoFET devices that record true intracellular signals (Duan et al., 2013).

One of the most promising designs comes from kinked nanowires (Tian et al., 2010), where the "kinked structure" and nanoFET recording element (Figure 1A) are encoded directly during synthesis. In this way, source and drain electrical connections can be made remote from the cell membrane, which contrasts with nanofabricated linear FETs and allows the highly localized FET isolated at the "kink" tip to be inserted into a cell without damage. Interestingly, it has been found that kinked nanowire devices coated with phospholipids can directly penetrate the membrane of cultured cells without application of mechanical forces and record intracellular action potentials (Tian et al., 2010 and Jiang et al., 2012). Due to the small and highly localized nature of the FET on the kinked nanowire probe, the intracellular potential can be well isolated.

The above nanodevices have been fabricated primarily on planar substrates, thus facilitating in vitro cell culture. Substrate-based nanodevices are, however, difficult to target independently to specific cells or processes and cannot exploit

much of the "nano" advantage. This limitation can be overcome by developing free-standing probes that are manipulated in 3D similar to patch micropipettes. While more difficult than simply pulling a pipette, because one must bridge from the nanoscale element all the way to a convenient input/output connection, reliable kinked nanowire electrophysiology probes (Figure 1B) have been reported (Qing et al., 2014). A unique characteristic of these new probes involves embedding the metal interconnections to the kinked nanowire device element at the probe tip within a relatively soft polymer support that is defined by standard lithographic processes. The polymer support structures can be fabricated with submicron thicknesses and bending stiffnesses much closer to that of brain tissue than current commercial neural probes, which can be especially attractive in chronic and/or awake animal studies. Measurements made with these new free-standing kinked nanowire probes have demonstrated (1) the same intracellular signal amplitude-time response as patch-clamp micropipette in simultaneous measurements, (2) capability for subcellular resolution targeting, and (3) the ability to use the same probe to acquire intracellular signals from multiple cells (Qing et al., 2014).

Potential advantages of free-standing kinked nanowire FET probes compared to patch pipettes include the absence of solution exchange during measurements and the highly localized size (tens to hundreds of nanometers) of the nanowire FETs. Standard whole-cell patch clamp involves solution exchange between the intracellular space and the pipette reservoir, which can affect the functioning of the cell. While the internal solution ideally mimics the intracellular environment, the solution components may alter recordings. For example, electrophysiological properties such as after-hyperpolarization potential and input resistance may change over time using potassium-ion source compounds potassium gluconate or potassium methylsulfate (Kaczorowski et al., 2007). Perforated patch methods can ameliorate such problems, but can introduce other issues such as inherent access resistance.

The absence of solution exchange issues and the highly localized nature of

nanowire FETs in combination with independently controllable 3D probes should open up unique opportunities for elucidating submicron "intracellular" heterogeneity. Studies probing the differences in membrane fluctuations have revealed potential computational operations by dendritic processes (London and Häusser, 2005). It is also well known that different cell types target different localized areas of the post synaptic neurites (Silberberg et al., 2005). Interestingly, there are suggestions that inhibitory axo-axonic cells may be excitatory in local microdomains of the neuron around the axon initial segment (Szabadics et al., 2006).

Moreover, there are many questions around subcellular electrical domains that nanowire FETs could help to address. For example, dendrites can involve active non-linear channel activations with the capacity for processing of information (London and Häusser, 2005). Additionally, imaging studies have shown that there may be a relationship between microcircuitry and synaptic dendritic targeting (Takahashi et al., 2012). Local dendritic electrical potentials continue to be of interest, in particular with respect to their role in plasticity (Clopath et al., 2010), as underlying naturalistic subthreshold fluctuations and their dendritic modification likely contextualize synaptic weight changes (Kruskal et al., 2013, Clopath et al., 2010). Overall, the unique characteristics of nanowire FETs could allow direct electrical recording to yield new insights into a range of questions surrounding subcellular electrical heterogeneity that impacts neuronal signal processing, functional microcircuits, and computations.

Nanowires for Stimulation

Semiconducting nanowires may be used to generate highly localized electric fields for stimulation. For example, modulation of the dopant during nanowire growth can yield a p/n junction diode at the tip of a kinked nanowire probe (Jiang et al., 2012). In forward bias, the conductance of the p/n junction is modulated by the local potential like an FET, and p/n kinked nanowire probes can record intracellular action potentials. In reverse bias, current flow is blocked and a voltage-dependent electric field is generated across the p/n junction. Significantly, calculations have

shown that localized fields generated by these p/n junctions should exceed the threshold for opening voltage-gated sodium channels.

The capability of applying such localized electric fields opens up several exciting opportunities. For example, an applied electric field may yield a sustained depolarization within localized areas or neural processes, where the precision of the local field is not expected to influence multiple neurons. The ability to affect subtle details of neural firing while maintaining neural recording could be exploited for testing microcircuitry, e.g., the examination of PSP strengths. Further, it has been shown that over time microstimulation of only one or a few cells has the potential to modify behavioral output (Houweling and Brecht, 2008). For brain-machine interfaces, using nanodevices for selective stimulation could allow the brain to remap different percepts.

Nanowires for Multiple In Vivo Recordings

There has been increasing interest in in vivo intracellular recordings, since these can provide greater insight into behavioral microcircuit dynamics than is possible with extracellular measurements. Early in vivo patch studies focused on anesthetized and then quiet resting or sleep states in order to maintain stable recordings (Steriade et al., 2001). By modifying how patch pipettes are secured to the skull, stable patch recordings have been performed during active movement, although only for short time periods (Epsztein et al., 2011). Such awake-state patch experiments have allowed a deeper understanding of the relationship between subthreshold dynamics, behavior, and brain states (Epsztein et al., 2011). Nevertheless, there are intrinsic limits to recording stability due to stiffness mismatch between glass pipettes and brain tissue, which yields maximal shear strains in the tissue at the patch site.

Nanowire FET probes, which are fabricated with soft polymer supports (see above), can overcome this mechanical limitation of patch pipettes. In addition, the small sizes possible for nanowire FET polymer probes are less likely to be rejected by the brain and to cause glial scarring and chronic damage. Hence, we expect that soft nanowire FET probe

structures may allow intracellular recordings to be maintained over the course of days though multiple brain states and behavioral paradigms. By improving the stability of intracellular recordings, these methodologies could advance our understanding of neuronal processing in extended behavioral contexts between many brain states.

Key advantages of such multiple-probe measurements are as follows. First, common analysis of multi-neuronal intracellular recordings can yield the functional or sub-threshold correlative relationships between neurons, in contrast to extracellular methods (Lampl et al., 1999). A second advantage comes with the potential for neuronal stimulation with p/n junction kinked nanowires described above. The ability to evoke local neuronal activity by isolating a single cell can piece out microcircuitry and PSP dependencies on behavior state and the natural presence of various neuromodulators and help us to better understand the neural correlates of learning and memory.

Soft Polymer Meshes for Delivery of Nanowire Arrays: A New Frontier

The potential of flexible polymer electronic interfaces can be extended from individual nanoprobes to 3D macroporous flexible meshes, where addressable nanowire FETs and other nanodevices are integrated within the mesh scaffolds. The width/thickness dimensions of the mesh with embedded electrical interconnects can be on the order of a micron or less, and thus similar to neural processes. The large mesh area allows for a multitude of simultaneous electrical lines and recording sites on a single, free-standing, flexible support through which neurons/processes can interpenetrate (Figure 1C) and could confer a great advantage for scaled-up recordings. Our lab has already demonstrated the use of these meshes as 3D scaffolds for integrating FET electronics in combination with 3D cell culture (Duan et al., 2013). Recent efforts in the stem cell field have shown that it is possible to grow and differentiate 3D structures similar to that of the developing nervous system (Zhong et al., 2014). It will be interesting to combine this with 3D macroporous nanowire FET polymer meshes to study activity under different growth conditions.

More generally, we suggest that it will be possible to integrate 3D macroporous electronic meshes within the living brain. Structural plasticity in the form of spine growth over long time periods has been observed (Holtmaat and Svoboda, 2009), and such structural plasticity may be more extensive in terms of brain rewiring (Holtmaat and Svoboda, 2009). The brain is well known to be functionally dynamic and can adapt or reorganize after damage via structural changes such as new axonal sprouting or dendritic growth (Dancause et al., 2005, Holtmaat and Svoboda, 2009). As we gain increased control over development of cultures and greater understanding of structural plasticity, the potential of 3D polymer meshes with integrated devices such as nanowire FETs and p/n junctions could push the medical boundaries of brain machine interfaces and general neural prostheses.

Conclusion

The developments of recording techniques such as nanowire FET and p/n junction devices open up new avenues for novel discoveries and new perspectives on the complex circuity and computations in the brain. Improvements in intracellular recording methods by these emerging nanoelectronic tools are of great interest, particularly for in vivo studies. It is essential to also consider the ability of such methods to cause minimal interference to the functioning of the brain and their facile translation to potential medical devices without the need for genetic manipulations. As we move into a world of new neural medical devices, including cochlear implants, retinal implants, deep-brain stimulation, and general brain-machine interfaces, technologies with biomimetic properties are increasingly important. These sophisticated devices will be essential for gaining insight into the multitude of neural interactions as microcircuits throughout the brain, a puzzle that underlies the fundamental challenges in neuroscience today.

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