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Current Opinion in Neurobiology

Nanotechnology and neurophysiology

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Neuroscience would be revolutionized by a technique to measure intracellular electrical potentials that would not disrupt cellular physiology and could be massively parallelized. Though such a technology does not yet exist, the technical hurdles for fabricating minimally disruptive, solid-state electrical probes have arguably been overcome in the field of nanotechnology. Nanoscale devices can be patterned with features on the same length scale as biological components, and several groups have demonstrated that nanoscale electrical probes can measure the transmembrane potential of electrogenic cells. Developing these nascent technologies into robust intracellular recording tools will now require a better understanding of device–cell interactions, especially the membrane–inorganic interface. Here we review the state-of-the art in nanobioelectronics, emphasizing the characterization and design of stable interfaces between nanoscale devices and cells.

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Current Opinion in Neurobiology 2015, **32**:132–140

This review comes from a themed issue on **Large-scale recording technology**

Edited by **Francesco P Battaglia** and **Mark J Schnitzer**

For a complete overview see the Issue and the Editorial

Available online 15th April 2015

http://dx.doi.org/10.1016/j.conb.2015.03.014

A renaissance for neurophysiology?

The brain is considered by many to be the most complex biological system in existence, so it is not surprising that the technical demands of neuroscience research drive innovation across many fields. The last decade has seen an explosion of new optical methods for recording and stimulating brain circuits [1–5], particularly in the area of optogenetics [6–9]. Equally significant, new experimental and analytical tools for electron microscopy are now enabling high resolution, volumetric reconstruction of neural tissue followed by quantitative connectivity analysis [10–14]. Given the progress in these areas, it is surprising that direct electrical recording from neurons — long considered central to neuroscience research — has changed very little in the last three decades. Whole-cell patch recording [15], sharp microelectrode recording [16,17], and silicon-based extracellular electrodes [18,19] remain the state-of-the-art in neural recordings despite their well-known limitations: whole-cell patch recording causes dialysis over periods of minutes [20–22], sharp microelectrodes change input resistance and resting membrane potential [23–27], and extracellular recording cannot measure subthreshold changes in neuronal transmembrane potential [28].

The motivation for developing nanoscale tools for neurophysiology is to record intracellular electrical activity from thousands of neurons simultaneously without perturbing their physiological properties. Furthermore, to make these tools impactful to neuroscience, they should be robust, easy to use, and their performance reproducible across labs. Several groups have now demonstrated that solid-state nanoscale devices can record electrical activity in neurons and cardiomyocytes, which signals an important step toward parallelizable, non-dialyzing intracellular recording. The remaining challenge is to demonstrate that these devices can be used over timescales relevant to neurophysiological experiments while preserving native cellular function. In the following sections we will examine the technical capabilities to produce nanoscale devices, the intrinsic electrical properties of these devices, and their specific interaction with cells. We conclude that the cell–electrode interaction is most crucial aspect of designing next-generation intracellular electrodes.

The nanoscale toolbox

Many neuroscientists will already be aware of the exciting developments in field of *bionanoelectronics*, thanks in part to several existing reviews on the topic [29,30•,31]. Nanoscale devices incorporating nanowires [32•,33•,34•,35], nanotubes [36,37•,38•], and nanotransistors [37•,39••,40•] have been used to record electrical activity from neurons and cardiomyocytes. This field draws upon a broad range of state-of-the-art fabrication techniques, which means that building and manipulating devices on subcellular length scales (<500 nm) is now routine, with some devices being patterned at the length-scale of single proteins [41,42••]. Patterning using electron beam lithography at ~10 nm feature sizes is relatively straight-forward, if expensive, while patterns at 250 nm and above are readily accessible with optical lithography. Vertical nanowire growth is a field in and of itself, and numerous techniques are available for producing nanowires <100 nm in diameter and up to tens of micrometers in length, either by patterning and etching,

or chemical vapor deposition (CVD) [43,44]. Thin film thicknesses can be controlled to <1 nm on planar substrates, and with the advent of atomic layer deposition (ALD) oxide and metallic films can be conformally coated onto complex and high-aspect ratio structures with similar finesse. Nanostraws of wall thicknesses between 5 and 50 nm and diameters from tens to thousands of nm are also easily made with ALD [37•,41,45–47] and can potentially dissociate the membrane penetrant portion of a recording device from the electrically sensing portion [37•,41]. Another facile method for conductive nanostructures is templated electrochemical growth, which can produce nanotubes [38•,48] and nanorods [48], and microspines [49••,50,51], including banded structures of heterogeneous composition [52]. Nanoscale field effect transistors have also been fabricated for cellular recordings [37•,39••,40•,41], some in combination with nanostraw designs [37•,41]. Finally, thin-film techniques and self-assembled monolayers have been employed to create 3–5 nm hydrophobic bands on the sides of inorganic nanostructures, allowing them to integrate into the lipid bilayer like membrane proteins [42••,53,54]. In summary, the 'nano toolbox' is capable of producing almost any design down to the 10 nm level, making the central question not how to build, but what to build.

The nanoscale advantage

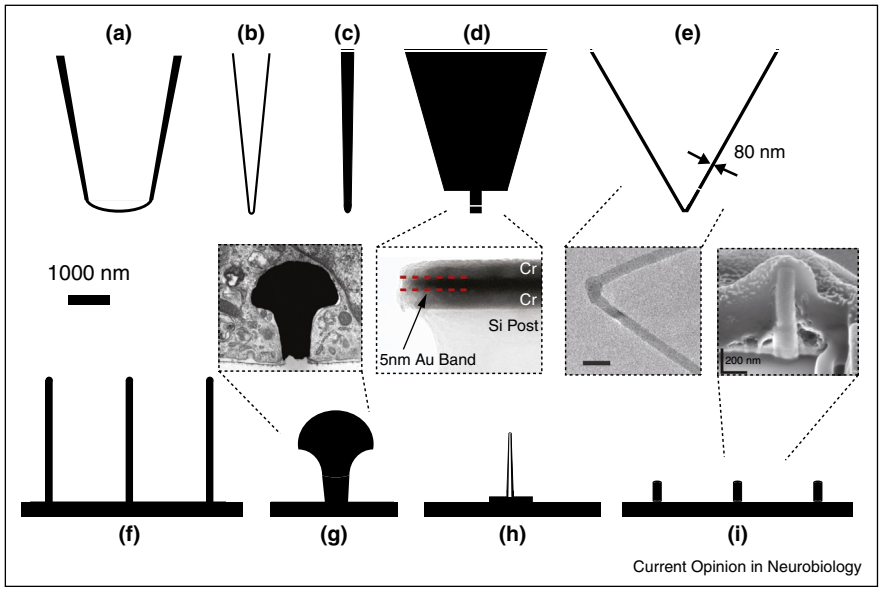
The most obvious argument for using nanofabricated recording devices is one of scale: smaller electrodes can record from smaller structures and be packed more densely to record from more cells with less volume displacement *in vivo*. Thus, all other things being equal, smaller devices are better for massively parallel activity mapping. Secondly, it is widely observed that cells spontaneously 'wrap', or engulf protruding nanostructures [33•,38•,49••,50,51,55]. This intimate contact between the cell and electrode simplifies signal source attribution (one electrode, one neurons) and improves signal-to-noise ratio as compared to planar recording systems. The nanoelectrode arrays described in recent literature are therefore not simply smaller microelectrode arrays, but rather interact with cells in qualitatively different ways. Another major advantage of using a solid-state nanoscale recording device is that that can be used intracellularly without disrupting the composition of the cytoplasm. Eliminating the liquid electrolyte conductor used in micropipette-based approaches immediately resolves the issue of interdiffusion between the cell and the pipette solution, which is especially problematic for whole-cell recording [21,22], but also for microelectrodes using high ionic strength internal solutions [56]. Because metallic conductors have greater than 10^7 times higher conductivity compared to typical electrolyte solutions, there is a specific scaling argument for reducing the cross-sectional area of the electrode leads by switching to solid-state devices. Finally, though impressive strides have been made toward the automation and parallelizing patch clamp technique *in vivo* [57], the mechanical requirements for pressure control (i.e. pipette rigidity) are not commensurate with massively parallel recordings. Nanofabrication on the other hand is *designed* for parallelization, routinely producing intricate structures at the same length scales as cellular components (3–30 nm), meaning that devices can be engineered to interact with cells at a subcellular level [37•,42••]. Integration into readout electronics is also greatly facilitated by working with solid-state electronic probes, and arrays of nanostructures can be bonded by flip-chip methods to high density CMOS electronics for recording [58,59] and stimulation [59,60].

Electrical recording from nanoscale devices

There are several distinct approaches to nanoscale electrical recording. To show the breadth of sizes and shapes, the silhouettes of some representative devices are compared in Figure 1 alongside a patch pipette and sharp microelectrode. The devices shown in the bottom row of Figure 1 are attached to a flat substrate and interact with cultured cells that are either grown on the substrates or are delivered to them. The devices shown in the top row of Figure 1 are inserted devices, delivered by micromanipulator to the cell, where recording moiety penetrates the plasma membrane to achieve cytosolic access. Figure 2 compares several electrical recordings using nanoscale devices where the transmembrane potential was independently measured by either a patch-pipette or sharp microelectrode. These early successes have stimulated a great deal of interest for expanding nanoelectrode designs into massively parallel device arrays for *in vivo* recordings.

From the standpoint of electrical recording, the most important distinction between these nanoscale recording devices is whether or not they penetrate the cell membrane. This determines whether the electrode will directly measure the transmembrane potential (Figure 3a) or whether it will measure the local extracellular potential (Figure 3b–d). If a nanoelectrode is merely adjacent to a cell, it behaves as a conventional MEA would (Figure 3b), measuring a extracellular action potential waveform and virtually no signal from subthreshold changes in potential [61,62]. When engulfment of a nanoelectrode results in a loose seal between the membrane and electrode [33•,38•], a canonical extracellular waveform is also observed (Figure 3c), but when the seal is very tight [49••,50], the invaginated pocket of solution may closely mimic the intracellular potential and low-frequency components of the action potential will be visible (Figure 3d). In fact, nanoelectrode–cell interactions may be complex with a device partially penetrating into the membrane and partially engulfed, but for clarity and to introduce the most important electrical properties of various configurations, we here separately describe two general recording configurations: cell-penetrating electrodes and cell-engulfed electrodes.

Figure 1



Size and shape of nanoscale devices. Different nanoscale devices are shown to scale for comparison with each other and with conventional intracellular recording methods. **(a)** Conventional patch pipette; **(b)** sharp microelectrode; **(c)** nanoelectrode (Ref. [34•]); **(d)** stealth probe (Ref. [42••]); (e) nanotransistor (Ref. [39••]); **(f)** nanoelectrode array (Ref. [32•]); **(g)** microspine electrode (Ref. [49••]); **(h)** nanotube–nanotransistor device (Ref. [37•]); and **(i)** nanotube electrode (Ref. [38•]).

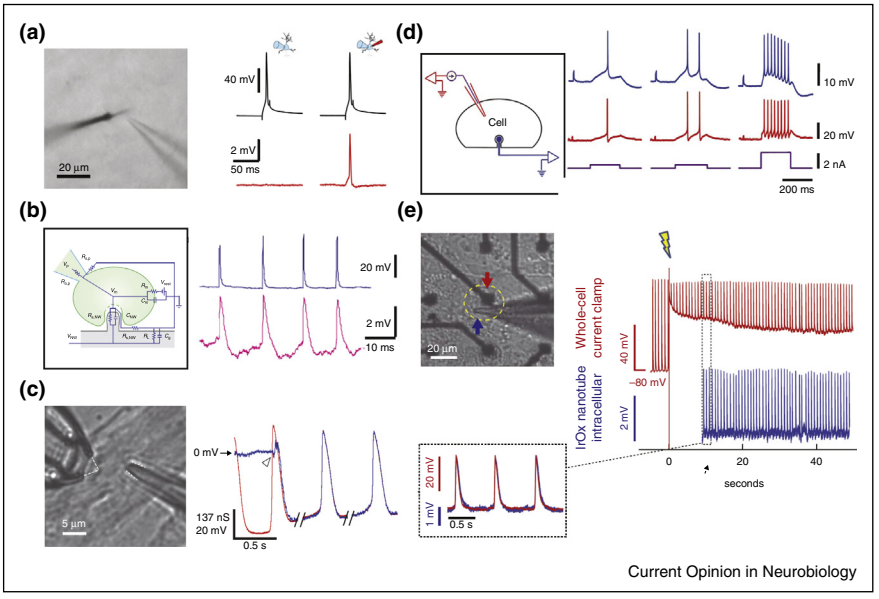
Method 1: direct intracellular access

Electrical recording with cell-penetrating nanoelectrodes follows the tradition of intracellular recording using metal microelectrodes that dates back to the 1940s [63–65]. Electrode insertion can be achieved either through mechanical force [29,34•,35,36] or by using fusogenic agents [37•,39••,40•]. Upon penetration, nanoelectrodes suffer similar disadvantages of their predecessor the metal microelectrode; insertion can cause a leak current at the site of penetration. For example, Angle and Schaefer [34•] report intracellular recordings from pyramidal neurons in hippocampal organotypic slice cultures, using inserted tungsten nanoelectrodes (Figure 1c). These electrodes are capable of measuring both action potentials and large (tens of mVs) DC offsets. In ~10% of cases they find that inserting these electrodes does not effect cellular physiology (as assessed by a whole-cell recording during nanoelectrode penetration (Figure 2a). The other 90% of recordings, however, showed measurable perturbation upon recording even with this small scale device. Given that sharp microelectrodes with tips < 100 nm in diameter cause measurable leakage [23–26], a concern is that recently reported nanoscale devices that are larger than 100 nm may also cause membrane leakage.

Reducing electrode leakiness favors going to even smaller electrodes, but this leads to higher electrode impedance, causing increased signal distortion [30•,66,67] and poorer signal quality [68,69]. One way of minimizing signal distortion [32•] is using nanoscale field-effect transistors (FET) [39••] instead of a metal nanoelectrode. The almost negligible input impedance of the devices allows for extraordinarily small probes (<100 nm) with excellent bandwidth. Using FETs for extracellular recordings was first proposed in 1968 [70], though it was not demonstrated until many years later [71,72]. These early FET devices were planar and recorded conventional extracellular action potential waveforms. The issue with creating non-planar FETs at the nanoscale is that they are three terminal devices, requiring two electrical contacts in addition to solution contact; a simple post or nanowire at the end of an electrode is insufficient. How to fabricate such devices remained elusive until Tian *et al.* demonstrated a 'kinked' nanowire growth in which a nanowire could be made into a loop, with a FET located near the tip (Figure 1e) [39••,40•]. When brought into contact with a cardiomyocyte, a transition from an extracellular to an intracellular signal was observed, and the magnitude quantified by appropriate calibration of the FET transconductance. These waveforms showed excellent agreement with simultaneous patch clamp recording (Figure 2c). This work was later extended to create nanoscale FETs on a substrate with a sharp hollow tube that could insert into the cell like a sharp microelectrode [37•]. These sharp tubes can be fabricated as small as a single membrane protein (Figure 1h) [41].

Another way to reduce electrode distortion effects is to impose a large voltage on the electrode, thus enabling a new set of electrochemical processes to mediate charge transfer processes at the electrode–electrolyte interface.

Figure 2



Electrical recording capabilities of nanoscale devices. These recordings were chosen in particular because they were performed alongside simultaneous control recordings by either patch pipettes or sharp microelectrodes. **(a)** Adapted from Ref. [34•]. Intracellular recording from a pyramidal neuron in hippocampal organotypic slice culture, using an inserted tungsten nanoelectrode. Here an action potential waveform is recorded by whole-cell patch clamp (black) before and after nanoelectrode penetration (occurring during the ~30 ms interval between the traces); **(b)** adapted from Ref. [32•]. Recording from culture neuron growing on a nanoelectrode array of gold-coated silicon nanowires. Current injection by a patch pipette (blue) elicits action potentials which can also be measured on the nanoelectrode (pink); **(c)** adapted from Ref. [40•]. Intracellular recording from a cardiomyocyte using a nanoscale field effect transistor (FET) (adapted from Ref. [40•]). Here the electrical potential at the probe tip directly changes the FET transconductance (blue). This transconductance waveform can be overlaid with the simultaneous whole-cell patch recording (red) and reveals how accurately the FET tracks the membrane potential. Also evident from the control recording, upon FET insertion into the membrane, a change in resting membrane potential is observed; **(d)** adapted from Ref. [49••]. 'In-cell' recordings, though extracellular (blue) track the transmembrane potential of an aplysia neuron with remarkable accuracy, as shown by a simultaneous sharp microelectrode recording (red voltage, purple injected current); and **(e)** adapted from Ref. [38•]. Electrical recordings lasting tens of minutes could be performed periodically on cultured cardiomyocytes over periods of days using iridium oxide nanotube arrays. To gain transient intracellular access, the nanotube electrode was driven at high voltage to electroporate the membrane ($t = 0$). This resulted in a change of resting membrane potential as indicated by the simultaneous patch recording (red) but it also lead to excellent recording properties from the nanotube (blue) as evidence by overlaying the two recordings (inset).

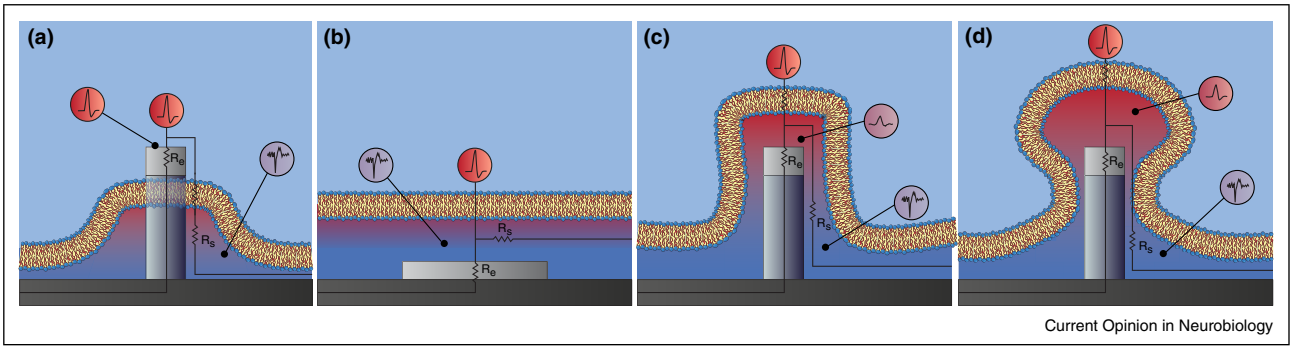
Robinson *et al.* [32•] achieve lower impedance on their gold-coated silicon nanowire probes (Figure 1f) by imposing a voltage of −1.5 V. This allows them to measure larger amplitude signals (Figure 2b) but unfortunately requires injecting constant current into the cell, and they report that the electrochemical processes that occur while recording in this way are deleterious to cell health.

Method 2: engulfment

When a device is engulfed by a cell without penetrating it, that device is less probably to effect the cell's physical properties, but it measures only the local potential of the invaginated pocket of extracellular solution. This 'pocket potential' may be closer to bath potential (ground) or cytosolic potential depending on the ratio of transmembrane conductance and seal conductance (Figure 3b,c). One can consider the cytosol and bath as two sources of competing electrical potential, the first influencing the pocket potential through the transmembrane conductance and the latter through the conductance of the membrane–device seal. In electrical engineering this is referred to as a voltage-divider, and because the membrane conductance (properly admittance) is both frequency and voltage dependent, the voltage divider is dependent on the signal being measured, acting as a filter [30•]. When seal resistance is low, the divider acts as a high-pass filter. When seal resistance is high [49••,50,73] the recording arrangement closely mimics tight-seal cell-attached recordings [74,75], and low-frequency coupling to the cell can be achieved. In both configurations, the local transmembrane potential can be quite different from the rest of the cell; the isolated membrane patch experiences the potential difference between cytosol and pocket.

To maximize signal in an engulfed recording, membrane conductance should be maximized and seal conductance minimized (i.e. a tight seal). There are two complementary strategies to improving these extracellular recordings. The first is to improve seal resistance by promoting cell–electrode adhesion [49••,50,73,76,77]. Early experiments

Figure 3



Electrode–membrane configuration. **(a)** Direct intracellular access; **(b)** typical planar extracellular electrode. The interaction between electrode and membrane is generally minimal, seal resistance is low, and consequently the electrode records a conventional extracellular waveform; **(c)** loose-seal engulfed recording. Essentially an extracellular recording, but the spike amplitude may be as large as milivolts due to isolation of the engulfed pocket from bath solution; **(d)** tight-seal engulfed recording. As the membrane–electrode seal resistance approaches the transmembrane resistance of the engulfing patch, the pocket potential can be very similar to the intracellular potential, thus the measured waveform more closely resembles an intracellular recording.

exploring the importance of adhesion and seal resistance on planar extracellular electrodes were performed by Fromherz and colleagues [76–79], who found that sealing is depending on cell type and substrate-coating [77]. More recently, Spira and colleagues have developed gold microspine electrodes that promote engulfment and subsequently high seal resistance [49••,50] (Figure 1g). These 'In-cell' microspine electrodes, despite being extracellular, are able to measure subthreshold membrane dynamics with high signal to noise and minimal filtering, as assessed by simultaneous microelectrode recordings (Figure 2d). In-cell recordings have been applied to several different cell types [49••,80,81], with the best results so far occurring in large aplysia neurons [49••]. Since they do not require membrane penetration, this route is very promising if slightly higher signal can be achieved, for example by increasing seal resistance.

Another approach to achieving intracellular-like recording using engulfed electrodes is to increase local transmembrane conductance in the area surrounding the electrode. An increase in transmembrane conductance could be achieved chemically by incorporating pore-forming antibiotics into the plasma membrane, as in perforated patch recording [82,83]. More recently, carbon nanotubes [84] and DNA-based nanopores [85] have also been used to introduce synthetic channels into lipid membranes, suggesting another possible route toward intracellular access. Alternatively, physical breakdown of the lipid membrane during and after electroporation provides a mechanism for transient and reversible poration of the membrane [33•,38•,46,51].

Electroporation refers to the formation of tiny membrane pores (10–20 nm) that drastically increase the transmembrane conductance. By increasing membrane conductance, electroporation can increase the recorded signal amplitude by more than 100 fold. Electroporation has been combined with gold microspine recording [51] as well as with nanowires [33•] and nanotubes [38•]. Cui and colleagues transiently electroporated cells with nanowire arrays and recorded from them periodically over several days [38•]. These electroporated holes remained open for several minutes, with the detected signal decreasing over time (Figure 2e). Modification of the electrode shape to a hollow cylinder (Figure 1i) dramatically extended this period to over an hour, and recordings on the same cardiomyocyte could be performed periodically for seven days. While leakage during this process may be detrimental to cell health, it was still possible to phenotype induced pluripotent stem cell-derived cardiomyocytes [86].

Finally, when membrane conductance is increased dramatically without concurrent increase in seal resistance, the result can be a membrane shunt similar to that which can occur during cell penetration. Accordingly, transient electroporation in the absence of a tight seal is associated with simultaneous loss of input resistance and change in resting membrane potential [38•,51] (Figure 2e), as has been shown previously when neurons are electroporated for dye loading [87]. Though some basic cellular properties can be distinguished during this porated phase [86], it follows that detailed investigation of cellular physiology will require both high membrane conductance and a tight seal.

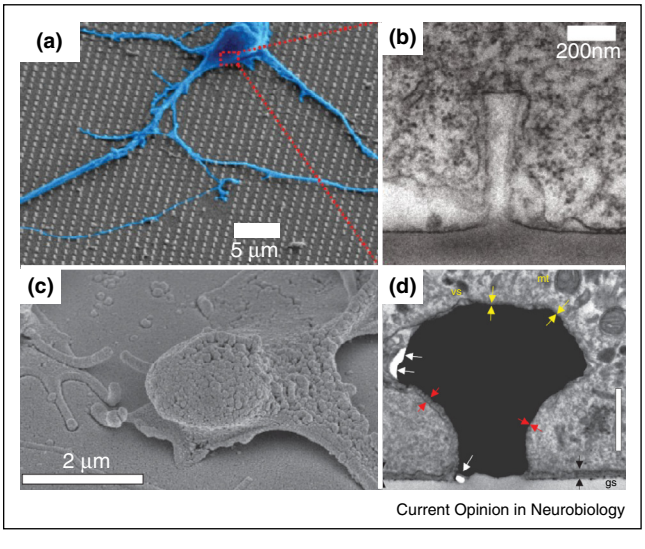
Seal resistance at the device–membrane interface

For both cell-penetrating and cell-engulfed electrical recording, it is crucial to control the interaction of the recording device with the cell, specifically the cell membrane. This kind of engineering will only be possible

however with better characterization of the interfaces that existing devices form with cell membranes. Even assessing whether or not a device is intracellular can be nontrivial. Because engulfment and penetration look nearly identical at the resolution of light microscopy, the distinction often requires special characterization methods such as electron microscopic reconstruction [38•,55,88•,89], force microscopy [90,91], or a second, simultaneous intracellular recording [32•,34•,38•,40•,51].

TEM examination of the cell membrane–substrate interface by different groups show that engulfment results in reduced membrane–nanoelectrode separation [49••,88•, 89,92]. In particular, Hanson *et al.* [88•] (Figure 4a,b) quantified the membrane–nanoelectrode cleft to be ~18 nm, which is significantly smaller than the membrane-flat surface cleft at ~50 nm. From the measured distances, the leakage resistance is estimated to increase by more than 10 times for nanoelectrodes interfacing with cell bodies. They also found that neurites can engulf nanoelectrodes and form closer junctions as compared with flat surfaces. Fendyur *et al.* [80] (Figure 4c,d) find similarly that small neurites can even engulf much larger gold microspines with diameters >1 μm. Another recent study further suggests that the performance could be further improved by optimizing the 3D geometry [55].

Figure 4



Electrode–membrane characterization by electron microscopy. Adapted from Ref. [86]. **(a)** Scanning electron micrograph of a cultured cortical neuron on a nanopillar array; **(b)** transmission electron micrograph of a cross-section of a fixed, resin-embedded cortical neuron on a nanopillar from an array similar to that in (a). Adapted from Ref. [90]; **(c)** scanning electron micrograph of a neuron engulfing a gold microspine; and **(d)** transmission electron micrograph of a cross-section of a fixed, resin-embedded aplysia neuron on a microspine from an array similar to that shown in (c).

A different paradigm for membrane–inorganic interactions can be found in the work by Almquist and Melosh [42••,53], who modify the surface of their probes to interact directly with the hydrophobic bilayer. Using gold-thiol chemistry and patterning the probes with a 3–5 nm thick hydrophobic band, they create structures that can integrate into the bilayer like membrane proteins (Figure 1d). Similar applications where surface-assembled monolayers have been used to integrate with lipid bilayers have been described elsewhere [93,94] and have even been used to form electrically tight pore-spanning membranes [95]. This approach is promising because it potentially avoids complications in cell–surface heterogeneity that make engineering surface–adherent interfaces difficult, and it eliminates the aqueous layer that is responsible for seal region conductance.

Designing over orders of magnitude

Engineering the nanoscale interaction between cells and electrodes may be the fundamental challenge to massively parallel intracellular recordings, but a complete *in vivo* recording system presents additional technical obstacles. For instance, each recording site must transmit information out of the brain (>millimeters) to downstream electronics, which can become logistically challenging as the number and density of the electrodes increases. While up to 512 electrodes have been accommodated by flexible connectors [19], dramatically larger numbers of electrodes are probably to require multiplexing and/or wireless transmission. Electrode arrays of up to 26,400 electrodes have been achieved *in vitro* with multiplexing [96], but such a demonstration has yet to be achieved *in vivo*. Finally, electrode micromotions within the brain can disrupt the recording if the electrode moves relative to the cell [97]. Ideally the recording site could move independently from the rest of the electronics, such as a wireless device on the brain surface [98] or a soft physical tether to the outside world [19]. Active mechanical stabilization has been used to correct the micromotion of rigid glass microelectrodes *in vivo* [97], but high density recordings will more probably rely on the compliance of flexible micro-wires or nanowires arrays [99,100] to passively decouple recordings sites from recording electronics. Such a design will be particularly important for awake recordings, where animal movement requires special attention to electrode stabilization [101]. Indeed, motivated by the difficulty of interfacing rigid structures with brain, there is much ongoing work adapting flexible electronic materials for use in neuroscience [102,103]. Flexibly tethered recordings present a different problem, however, as they cannot be delivered deep within the brain without the aid of a rigid shuttle [102,103] or other means of providing reversible rigidity during insertion [104,105].

Conclusion

Nanoscale devices show great promise for intracellular electrical recording and have indeed already been used

for specific applications such as electrophysiological phenotyping of IPSC-derived cardiac cells [86]. Stable, non-invasive intracellular electrical recording will require much more careful attention interactions between nanoscale devices and the cell membrane. Some of this work is already underway, and several groups are now paying closer attention to characterizing, modeling, and designing cell–electrode interfaces. Given the capabilities of modern nanotechnology and increased interest in device membrane interactions, it seems highly probable that better intracellular recording technology is on the horizon.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors thank Jason Castro for helpful comments on the manuscript, and support from NSF IDBR-1063397 and HFSP-RGP0048 and the Stanford Neuroscience Institute NeuroFab (N Melosh, M Angle), as well as NSF DBI-1055112 and MPS-1344302 (B Cui).

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- •• of outstanding interest

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