Neurobiology: rethinking the electrode

Vivien Marx

Electrodes and electrode arrays to record from neurons come in an increasing number of shapes and sizes, and engineers are continuously adding capabilities.

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Electrodes are the workhorses in many labs for recording electrical signals from neurons and also for stimulating these cells. Researchers use electrodes to record voltage changes in single neurons. They use electrode arrays to record from groups of neurons in either a dish, a model organism or a human.

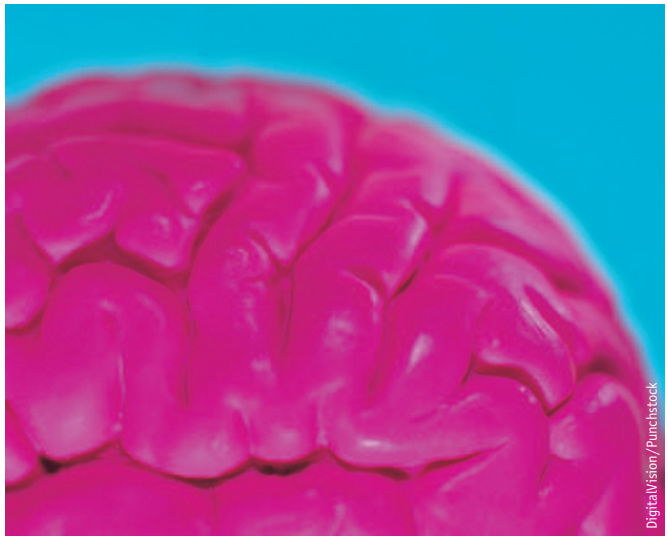
No current technology can record from many thousands of individual neurons with single-cell resolution. In addition, approaches are still emerging to link electrophysiological measurements with optogenetics: for example, to record voltage and calcium release from the same large group of neurons. These capabilities will also emerge from technologies developed in large-scale research projects such as the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative, which is funded by the US National Institutes of Health (NIH).

New types of electrodes are delivering ever more electrophysiological information from both intracellular and extracellular measurements. *Nature Methods* spoke with academic and commercial technology developers and users about emerging types of electrodes.

Listening inside cells

The patch-clamp technique developed by cell physiologists and Nobel laureates Bert Sakmann and Erwin Neher has given scientists the ability to dip a glass capillary into or onto a neuron and record electrical signals[1]. Although the technology is established, these are still tough measurements to make: the glass electrode must reach the cell and maintain contact, says neuroscientist Camilo Libedinsky, a postdoctoral fellow at Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), who studies how large networks of neurons located in multiple brain areas interact during mental processing tasks such as perception, attention and recall.

During patch-clamping, the cell and electrode must be still to keep in contact. Even top-of-the-line microelectrode technology is known to be extremely finicky, says Libedinsky. And even with well-placed instruments, experimental measurement can last only a few hours because of cell damage.



The brain's neurons are communication hubs, and new types of electrode arrays let researchers tune in.

Electrode technology has certainly advanced to record neural activity from single cells with nanoscale spatiotemporal precision, says neuroscientist Aviad Hai, who is a postdoctoral fellow in the lab of Alan Jasanoff at Massachusetts Institute of Technology.

As a graduate student in Micha Spira's lab at Hebrew University of Jerusalem, Hai co-developed an extracellular multielectrode array with capabilities that make it more like an intracellular electrode array[2]. The mushroom-shaped microelectrodes are around 1.5 micrometers (μm) high and embedded in a glass substrate. The neurons engulf the electrodes because a peptide on the electrode's surface induces phagocytosis. Spira and Hai describe their electrodes as ones that do not muscle their way into the neurons; rather, the approach is one of "swallow the bait microelectrodes," which brings the cells and microelectrodes to the needed level of physical intimacy for recording.

A separate approach to intracellular recording involves vertical nanowire electrode arrays, with each electrode made of silicon and equipped with titanium-gold tips[3]. Either these nanowires are engulfed by neurons, or the neuronal membrane is punctured with a small electrical pulse, says Hongkun Park, a bioengineer at Harvard University who developed the array. These electrodes record much like the patch-clamp technique, he says.

Together with the lab of Donhee Ham, an electrical engineer also at Harvard, Park and his team are now testing the next iteration of this microelectrode array. They have designed and fabricated their own semiconductor chips into which they are

Harvard Univ.

Hongkun Park and colleagues are exploring ways to record the events that drive a neuron to fire.

integrating nanowire electrodes. Park says this device can potentially handle massively parallel recordings from up to 10,000 sites.

Intracellular measurements track when neurons 'fire'—that is, transmit a voltage spike called an action potential—and release neurotransmitters, which are chemical messages that pass from one neuron to the next. And intracellular measurements can also detect inputs and fluctuations below the firing threshold. Measuring such "behind the scenes" events can help researchers understand what drives a cell to fire, says Libedinsky. It might, for example, show how short-term memories are stored, he says.

In the first generation of his microelectrode array, Park says that stray resistance and impedance hindered subthreshold recording, "but we are actively working on improving that." The new electrodes will have higher signal-to-noise ratio and be able to detect these subthreshold events.

Libedinsky's ideal electrode array would have many features. It would record from all neurons intracellularly, so there would be little need for extracellular recording, he says. This array of the future could be implanted into an animal's brain for an extended period and wirelessly transmit comprehensive neural information—both smaller fluctuations in voltage and action potential spikes—all while animals, or perhaps people, perform tasks. It might be an array of tens of thousands of electrodes at a price point not just for well-endowed labs.

It would also help to know much more about the geography of the recording. Except when using microelectrodes, scientists usually do not know exactly from which neuron they are recording. To understand circuits, it would be helpful to pinpoint the neuron one is recording from, to know which neuron feeds into it and which receive connections from it, he says. "This is one possible advantage of optical methods over electrode methods," he says. All of these approaches on Libedinsky's wish list are still distant.

Listening from the outside

The alternative to listening to neuronal signals from the inside is to record from the vicinity of the cells. Electrodes can measure action potentials, which typically lead to neurotransmitter release, from a distance of around 100 μm. When probing a neuronal network, Libedinsky says, he and other neuroscientists mainly use extracellular electrode arrays to measure action potentials.

Scientists can choose from several types of arrays, which differ in their geometries and materials. One early electrode array fabricated with silicon electrodes is the 'Utah array' developed by Richard Normann of the University of Utah[4]. Blackrock Microsystems, a company focused on neuroscience equipment, sells the array. The electrode tips are coated with either platinum or iridium oxide and insulated with a polymer called Parylene C. The array can both record and stimulate neurons and has up to 96 electrodes.

According to a company spokesperson, researchers can also ask for customized electrode lengths. Blackrock Microsystems is working on a number of new developments, among them varied-length microelectrodes and electrodes that can be used for optogenetic experiments.

NeuroNexus, originally a University of Michigan spin-out, sells several hundred electrode designs. This variety is needed for a growing scientific community with many applications for a diversity of anatomical structures and different types of experiments, says company founder Daryl Kipke, who previously ran a biomedical engineering lab at the University of Michigan. For example, he and colleagues designed a carbon fiber electrode able to collect signals from single neurons when implanted in a rat brain[5]. He was motivated to create microelectrodes about the size of a neuron's cell body, that would reduce injury and immune response in the brain.

His company also sells optoelectrodes for groups exploring how to combine optogenetic techniques with electrophysiology; the electrode can deliver light stimulation through an optical fiber and also record electrical signals.

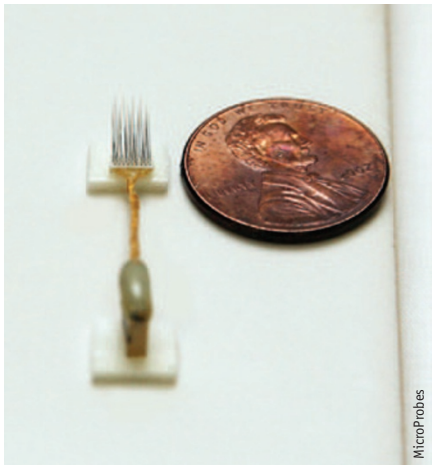
The BRAIN Initiative is enticing scientists to try new techniques and electrodes for recording and stimulating neurons, says Kipke. Traditionally, labs have preferred to use the electrodes that have worked for them in the past, but he sees increased requests for custom electrodes. "It's not 'one size fits all'," says Kipke. A lab might want electrodes to record from one brain area and to stimulate another: for example, to reach layer IV of the cortex and have longer electrodes that extend into deeper brain structures.

The company's research and development team partners with academic labs working on new array components or materials, he says. The field is moving fast. "We solved that first-generation problem," says Kipke. The field has worked out how to make microelectrodes for a variety of anatomical structures and for experiments that have longer duration and larger scale, he says.

Efforts now focus on sophisticated manufacturing of highly specialized electrodes, which can mean fabricating silicon electrodes as microelectromechanical systems (MEMS)-based devices. These small devices allow finer resolution and higher channel counts, says Kipke. They are made by using lithography approaches that build up the device by etching and depositing material with methods borrowed from the semiconductor industry.

The largest array that NeuroNexus sells can record from 256 sites. Kipke and his team are now working to expand it to 1,000 electrodes. Its configuration will give electronic access to each electrode so that scientists can tailor the way current can be delivered or recorded. The difficulty of designing on that scale is connected to the manufacturing techniques that must be applied, he says.

A quest for longevity



MicroProbes

With extracellular electrodes, researchers try to get within 100 μm of a cell. Here, MicroProbes' Floating Microelectrode Array is shown.

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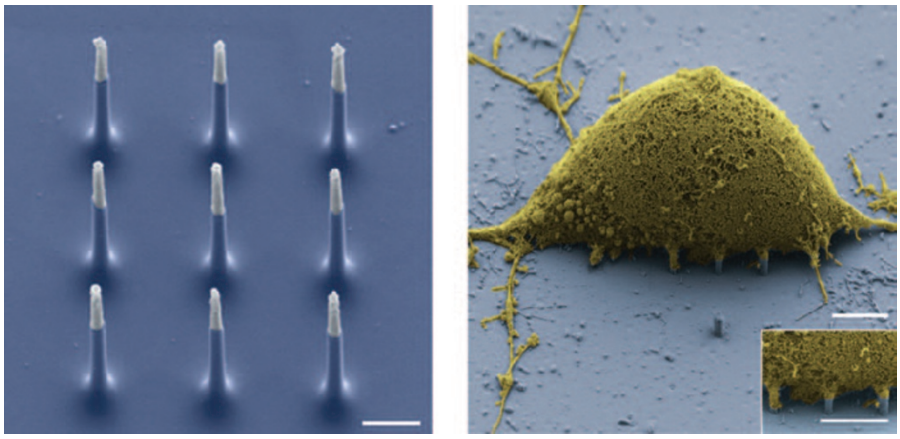


In vertical nanowire electrode arrays, the electrodes are made of silicon with titanium-gold tips (left). Neurons can engulf these electrodes (right), which allows recording. Reproduced from ref. 3, Nature Publishing Group.

The companies and scientists interviewed by *Nature Methods* all expressed the need for electrodes with greater longevity. One day, arrays might spend a lifetime in a person who uses a prosthesis controlled via an implanted array. Before that time, a number of biological and materials-based challenges must be addressed.

Electrodes elicit an inflammation response in the brain; glial cells surround the electrodes as a consequence of the inflammation, which can hinder signal recording and stimulation. There is also micromotion in the brain due to respiration and blood flow. "You want a recording electrode system that will move essentially as the brain pulsates up and down and still be able to hold single neuronal units in recording," says Martin Bak, CEO of MicroProbes for Life Science.

For extracellular electrodes, researchers try to get an electrode within 100 μm of a viable cell. The strength of the signal depends on which cell one is recording from; neurons vary greatly in size, from cortical striate neurons that are 8–10 μm in diameter to pyramidal cells in the motor cortex that can be 50 or 60 μm in diameter. "The larger the neuron, the more intense the field that will emanate from the cell," he says.

Together with Philip Troyk of the Illinois Institute of Technology and Richard Andersen at Caltech, Bak developed the Floating Microelectrode Array (FMA) for cortical recordings. The name of the array originated in a NASA (National Aeronautics and Space Administration) project in which electrode arrays would be subjected to various types of gravitational forces.

The FMA, now sold through MicroProbes, which Bak founded, uses electrodes made of platinum and iridium and coated with Parylene C. Bak says that the material has a good track record and is used in many implantable medical devices. He and Troyk keep reengineering the array to address researchers' needs.

A*STAR, Singapore

An array of the future might have tens of thousands of electrodes at a price point not just for well-endowed labs, says Camilo Libedinsky.

Bak has seen the electrode array field grow dramatically. While he was a researcher at NIH, his projects included stimulating the visual cortex in people undergoing neurosurgery for epilepsy. "They saw spots of light called phosphenes in their visual field," says Bak of the volunteers. This experience fuels his commitment to develop arrays potentially for neuroprosthetic implants, which could, for example, help people who are visually impaired.

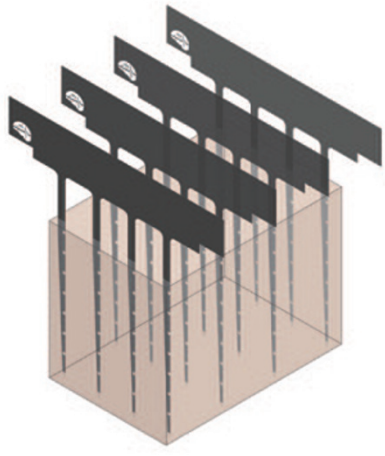
Over time, electrode arrays have got smaller, and stimulating arrays have been designed to need less power. But they still have cables for power supply and signal transmission. "Eventually, you want a totally wireless system," says Bak. Early next year he hopes to have a prototype of a wireless array ready for customers to test. In its first iteration, it will be for stimulating neurons, but he says a recording wireless FMA is also in the works.

Libedinsky uses FMAs for his experiments and says he can obtain good signals for extended periods of time. He also likes being able to flexibly define the length of each electrode. "When it comes to comparing to other technologies, I am not in a position to do so, as I have only used these electrodes," he says.

Hansjörg Scherberger, a researcher at the German Primate Center in Göttingen, also uses implanted FMAs to record from monkeys' brains. In one animal, the experiment has lasted over four years.

Scherberger started his work with Utah arrays but needed to also target areas beneath the cortical surface and use a combination of shorter and longer electrodes. At the time, the Utah array could not be configured. During his postdoctoral fellowship in Andersen's Caltech lab, he worked with early FMA models and has stayed with this technology since leaving Caltech ten years ago.

The FMAs are 32 electrodes arranged on a 2-mm × 4-mm plate. The electrodes can have variable length from 1 mm to 10 mm. The geometry Scherberger has chosen has some electrodes that are 1.5 mm high, and the longest is 7 mm long. "We put in six of those in three different brain areas so we can cover premotor, motor and parietal cortex simultaneously," says Scherberger. He uses the arrays to record the interaction of neuronal networks when, for example, a monkey moves his or her hand. He hopes his research will one day help people who need a hand prosthesis.



B. Surguine, NeuroNexus

NeuroNexus is increasing the resolution of its largest array (shown here, the Matrix Array). The new array will not be larger in size but will have 1,000 or more electrode recording sites (dots).

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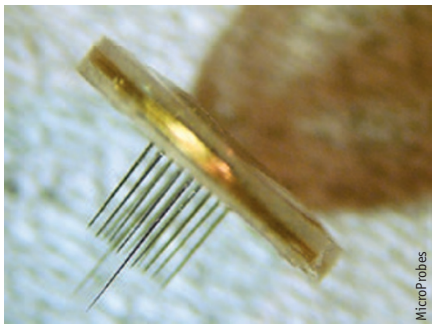

Early next year, MicroProbes plans to let scientists test a wireless array. Here, a prototype, currently under development.

Any foreign material in the body—such as an electrode or a pacemaker—elicits an encapsulation reaction. "In the brain we call it a gliosis, and it's very thin, maybe 100 microns or so," says Scherberger, adding that it is enough to create too much distance between the electrode tip and the next neuron. "That can then decrease your recording capabilities up to a point where you can't listen to single neurons anymore," he says.

He knows of colleagues using both FMAs and Utah arrays who have lost signals after 1–3 months and is unsure why his luck his better. Wireless electrode arrays are promising, he says, for applications in people. And in the lab, these could let his team record from neurons in monkeys in many kinds of social situations as they run and tumble about.

Scherberger also has a technology wish list. It includes intracellular recording arrays that would tell him about the information arriving at a neuron, such as membrane depolarization and hyperpolarization, which helps in understanding the "computation" that neurons perform. These data could also benefit from ways to identify and label the exact neurons from which the array is recording.

View to the future

Bioengineer Ian Halpern, CEO of Modular Bionics, believes that a new type of electrode design is in order. With small-business funding from NIH, he and his team have developed a three-dimensional (3D) electrode array, called N-Form, intended for long-term implantation.

Traditional electrode arrays have rows of shanks arranged in a grid. The electrodes in the N-Form array are made of a proprietary material that is mainly a biocompatible polymer as opposed to arrays that use coatings, he says. The electrodes in the N-Form array are rounded, whereas in many popular arrays they are more angular.

The array's electrodes also have lower impedance than more classic designs because of the choice of materials—a blend of iridium and platinum and the coat of iridium oxide for the electrode sites—thereby allowing users to record from single firing neurons and also to capture local field potentials, says Halpern. These field potentials are the continuous change in voltage levels of entire groups of neurons near the recording electrode.

Last fall, the company launched a pilot *in vivo* testing program for researchers to order an N-Form array configured to their needs. Traditional electrode arrays have either a single electrode or multiple electrodes along the shanks; in a single N-Form, there can be 1–16 shanks and up to a total of 64 electrode sites. The shanks can range in length from 0.5 mm to 3 mm and can be placed at small intervals from the tip to near the base along the side of each shank.

Ian Halpern believes it is time for a new type of electrode design.

The design addresses scientists' need, for example, to record from different neurons at different depths of the cortex. Users need to try different configurations as they implant electrodes in areas suspected to be part of a circuit. They then configure the array as their knowledge grows about the circuit, says Halpern. Arrays have to be adapted to the 3D curvatures of the brain and its layers. After all, the brain "isn't a perfectly planar layer cake," he says. "Better 3D electrodes are needed to study 3D neural circuits."

What is crucial for electrodes' longevity is high-quality microscale manufacturing, says Halpern. "Microelectrodes have very small features, and it is difficult to manufacture these devices with a high level of quality—especially in a quickly delivered, customized manner," he says. At the same time, he and his team are working on approaches to accelerate manufacturing of microelectrode arrays and at a lower cost.

He and his team also explore ways to minimize the brain's immune response. In

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certain electrode designs, the insulation delaminates or cracks around electrode sites, which adds to the recording surface area[6]. The result is that nearby neurons are recorded, making the signal noisier. It can be hard or even impossible to record from the original single neuron.

Resolving these issues is important for basic neuroscience and neuroprosthesis development. And other applications are on the horizon. MicroProbes' FMA has been developed for cortical recordings, but Bak says there is increased interest in peripheral nerve applications, for less invasive neuroprostheses. Electrodes also play a role in the emerging area of 'electroceuticals'. GlaxoSmithKline, for example, is exploring how to use electrical signals to treat disease. MicroProbes is part of a GlaxoSmithKline effort to test the FMA to stimulate and record from the vagus nerve, which plays a role in blood pressure, obesity and diabetes.

Overall, researchers are still exploring which types of information—such as from intracellular or extracellular recordings, or completely different types of data—will be most useful, for example, when it comes to understanding cognition, says Libedinsky. Technology development is crucial. "And by this I don't mean improve existing technologies," he says. "I mean create completely new ones."

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