BIOELECTRONIC DEVICES

Parallel probing of intracellular neuron potentials

An array of 4,096 nanoelectrodes can record and stimulate intracellular action potentials, as well as excitatory and inhibitory post-synaptic potentials, from thousands of connected mammalian neurons in culture.

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Multielectrode arrays (MEAs) are increasingly used for studying the biophysical principles underlying neuronal-circuit operations, short-term and long-term neuroplasticity (learning and memory), as well as neuropathological processes and the actions of toxins and drugs on neurons. Sophisticated MEAs with high-density arrangements of thousands of addressable small-diameter sensors with low impedance can monitor extracellular field potentials for weeks and months[1–3]. However, the properties of the interfaces formed between individual neurons and planar electrodes limit the recordings to the extracellular field potentials generated by propagating action potentials. Hence, contemporary MEAs are 'blind' to the rich and critical 'landscape' of the sub-threshold inhibitory, excitatory and electrotonic (that is, graded) post-synaptic potentials that allow neurons to communicate, modulate their firing patterns, regulate their biophysical membrane properties and undergo activity-dependent changes. As a result, neurons that do not fire action potentials are not 'visible' to planar electrodes, and thus their contribution to neuronal computations cannot be examined. Furthermore, the inability to simultaneously record the diverse types of synaptic potentials from individual neurons within a network curtails the ability of researchers to study the basic principles underlying brain functions and to elucidate the basic synaptic processes of healthy and pathological circuits. It also hampers the ability to understand how toxins, drugs, metabolic processes and genetic modifications exert their effects. Reporting in *Nature Biomedical Engineering*, Donhee Ham, Hongkun Park and colleagues now show that a nanoelectrode array can simultaneously record and stimulate attenuated intracellular action potentials, as well as excitatory and inhibitory

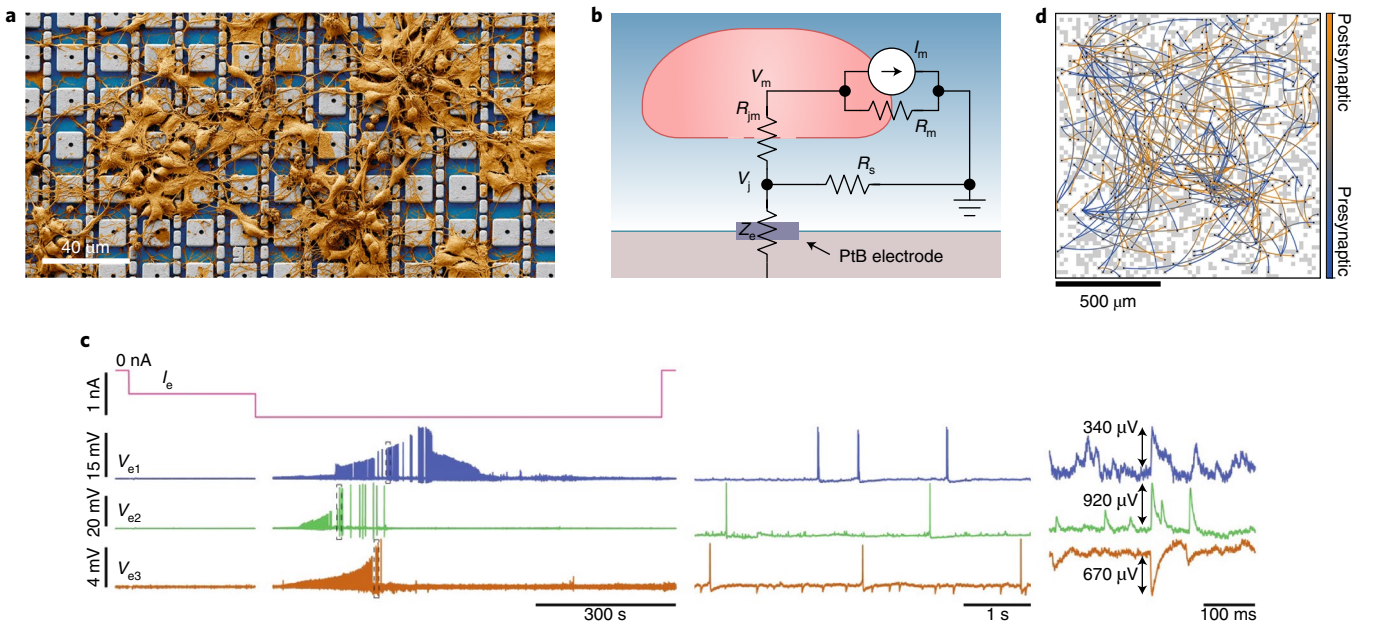

Fig. 1 | Parallel recording of intracellular neuron potentials. a, False-coloured micrograph of neurons cultured on top of the nanoelectrode array (the experiments were performed at much higher neuron densities). **b**, A passive analogue electrical-circuit model of the electrode–neuron interface. Z_e, resistance of the electrode; R_m, resistance of the neuron's membrane; R_{jm}, resistance of the membrane patch facing the electrode (the junctional membrane); R_s, seal resistance formed by the cleft between the electrode and the cell membrane; V_m, transmembrane potential; V_j, voltage at the cleft. **c**, Left, Intracellular access is achieved via the low-intensity and long-lasting injection of negative current (I_e). As depicted by the gradual increase in the recorded action-potential amplitudes (V_{e1}, V_{e2} and V_{e3}), effective voltage-recording configurations by the individual nanoelectrodes gradually develop, reflecting a gradual decrease in R_{jm} and an increase in R_s. Middle, Stretches of the intracellularly recorded attenuated action potentials and low-amplitude synaptic potentials. Right, Intracellularly recorded excitatory potentials (top two traces) and inhibitory potentials (bottom trace). **d**, Network-wide mapping of synaptic connectivity between pre-synaptic and post-synaptic elements via spike-triggered averaging. Pixels recording intracellular-membrane potentials during the experiment are indicated in grey. The arched lines connect pre-synaptic (blue) and post-synaptic (orange) neurons (a total of 304 synaptic connections between 396 neurons were mapped). Figure reproduced from ref. [4], Springer Nature Ltd.

post-synaptic potentials, from thousands of connected active neurons in culture[4].

Recent advances in material science, microelectronics and nanoelectronics, along with the realization that the planar MEA cannot reveal essential components of the brain's signalling repertoire, have prompted the integration of sharp intracellular microelectrodes with the non-damaging, durable and multisite-recording features of substrate-integrated planar MEAs[1,5,6]. To this end, arrays of 3D vertical nanoelectrodes with diameters of 50–500 nm and heights of 1–3 μm have been designed to perforate or penetrate the plasma membrane of cells cultured on the array, thus gaining direct ohmic contact with the cell's cytosol. Together with the formation of high seal-resistance (Fig. 1b) between the cell membrane and the vertical nanoelectrodes, the arrays generate configurations similar to those formed by a patch-clamp electrode or a sharp electrode. This technological concept has successfully progressed to enable recordings of attenuated intracellular action potentials from cultured cardiomyocytes and striated myotubes repeatedly over a number of days for durations of approximately an hour at a time[7–9]. In contrast, attempts to apply the same technological approach to cultured mammalian neurons have been surprisingly disappointing. In fact, the intracellular recording of attenuated action potentials and of subthreshold synaptic potentials from cultured mammalian neurons[10–13] were limited to the recording of single neurons at a time for short durations, and did not support the study of synaptic connectivity.

Ham and colleagues' arrays consist of 4,096 platinum-black, low impedance (~300 KΩ at 5 Hz) vertical nanoelectrodes fabricated on a silicon chip that integrates 4,096 amplifiers[1,6] (Fig. 1a). The electrodes are configured either for current injection and voltage recordings (current-clamp mode; CC), or for voltage application and current recording (voltage-clamp mode; VC). Remarkably, the quality of the nanoelectrode array makes it possible to record from hundreds of neurons in parallel and to measure intracellularly evoked activity in either the CC or VC modes. The quality of the obtained recordings is excellent and sufficient to resolve quantal fluctuations of the evoked post-synaptic potentials, a characteristic feature of chemical synapses. The CC mode for parallel recordings from hundreds of neurons can be harnessed to extract connectivity maps from hundreds of neurons (Fig. 1d), whereas the VC mode can be used to document alterations in ionic currents, for example in response to applied drugs.

To obtain simultaneous intracellular recordings from thousands of individual neurons by a matching number of nanoelectrodes, Ham and co-authors used unorthodox neuron-plating densities to form roughly three cell layers across the MEA so as to increase the probability of direct contact between the nanoelectrodes and the plated neurons. In addition, the authors developed a protocol for the electroporation of the neurons' plasma membrane so as to gain intracellular access for recording and stimulation. Earlier studies had shown that cultured cells (including neurons) seeded on MEAs adhere to the substrate and engulf vertical nanoelectrodes to form a narrow cleft between the cell membrane and the engulfed electrode. The high resistance of this cleft (R_s in Fig. 1b) is in fact the equivalent of the GΩ seal formed between a patch electrode and a neuronal membrane. This configuration made it possible to perforate or pierce the plasma membrane that faced the nanoelectrode (the junctional membrane, R_{jm} in Fig. 1b) to gain intracellular access. Since conventional electroporation protocols that involve applying short current pulses lead, in most cases, to irreversible neuron damage, the authors opted to 'electroporate' hundreds of neurons in parallel via the continuous injection of low-intensity negative DC currents (from −0.5 nA to −3.0 nA). To maintain low junctional-membrane resistance, the current injection was continued even after attaining intracellular access (Fig. 1c). This protocol sustains a negative transmembrane potential, which prevents inactivation of voltage-dependent sodium channels, and provides the electrochemical driving force needed to drive the generation of post-synaptic potentials and action potentials for a median duration of ~8 min (and a maximum of 19 min). Alterations of the injected current intensity can be used to modulate the neurons' excitability and to fire action potentials.

Further improvements in Ham and colleagues' technology are needed before it can be implemented effectively. In particular, a significant increase in the stability and durability of the intracellular recording configuration from minutes to months would be needed to enable the analysis of the electrophysiological signals underlying different forms of network plasticity (learning and memory), neuropathological processes, and the long-term effects of drugs and toxins.

The unstable transmembrane potential of individual neurons and the relatively short-term intracellular access, ending in irreversible damage to the neurons, are the outcomes of a number of processes that are potentially tractable. For example, whereas the relatively low R_s and low R_{jm} configurations obtained by electroporation are sufficient to support attenuated intracellular recordings, these elements unavoidably form a pathway for the influx of calcium ions from the culture medium into the neurons' cytosol[14]. Continuous injection of negative current is routinely used to 'help' neurons recover after a sharp microelectrode insertion or patching. Nevertheless, it also increases the driving force for calcium influxes. The elevated free intracellular calcium concentration may trigger conserved repair mechanisms of cell membranes, but at somewhat higher concentrations it may activate acute neurodegenerative processes (calcium-activated neutral protease calpains)[14]. In cultured cardiomyocytes, membrane electroporation by vertical nanoelectrodes is followed by hour-long intracellular recordings of action potentials, which ends when the porated membrane is repaired and the resistance of the junctional membrane returns to normal. This process is associated with the parallel recovery of the free intracellular calcium concentration. As a consequence, intracellular recordings from cardiomyocytes can be repeated a number of times, for an hour at a time, for days[7,8] (however, this is not the case for cultured neurons). Further challenges include the development of durable high-resistance seals between neurons and nanoelectrodes, and the regulation of innate membrane-repair mechanisms to maintain a low junctional-membrane resistance. ❐

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