The Journal of Experimental Biology 209, 2312-2319
Published by The Company of Biologists 2006
doi:10.1242/jeb.02163

Review

Neuronal networks and synaptic plasticity: understanding complex system dynamics by interfacing neurons with silicon technologies

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Accepted 8 February 2006

Summary

Information processing in the central nervous system is primarily mediated through synaptic connections between neurons. This connectivity in turn defines how large ensembles of neurons may coordinate network output to execute complex sensory and motor functions including learning and memory. The synaptic connectivity between any given pair of neurons is not hard-wired; rather it exhibits a high degree of plasticity, which in turn forms the basis for learning and memory. While there has been extensive research to define the cellular and molecular basis of synaptic plasticity, at the level of either pairs of neurons or smaller networks, analysis of larger neuronal ensembles has proved technically challenging. The ability to monitor the activities of larger neuronal networks simultaneously and non-invasively is a necessary prerequisite to understanding how neuronal networks function at the systems level. Here we describe recent breakthroughs in the area of various bionic hybrids whereby neuronal networks have been successfully interfaced with silicon devices to monitor the output of synaptically connected neurons. These technologies hold tremendous potential for future research not only in the area of synaptic plasticity but also for the development of strategies that will enable implantation of electronic devices in live animals during various memory tasks.

Key words: synapse, transistor, photoconductive stimulation, interface, neural circuits, biocomputational device, biomic hybrid.

Introduction

Synaptic and network plasticity

Synapses have innate propensity to alter the efficacy of synaptic transmission between neurons. These changes in synaptic strength can occur rapidly through mechanisms such as long-term potentiation and depression (LTP/LTD) (Malenka and Bear, 2004; Lisman and Spruston, 2005). In this form of plasticity, either the amount of neurotransmitter released from the presynaptic terminal or its receptor function in the postsynaptic cell is modulated. This provides a rapid and immediate mechanism for altering the strength of that particular synapse, and is usually initiated in response to a specific activity pattern, such as high frequency stimulation. In addition to such biophysical changes, the connections between neurons can also undergo direct structural modifications by altering the total number of synapses (Dillon and Goda, 2005; Chklovskii et al., 2004; Ruthazer, 2005). Such structural changes underlying long-term plasticity are more permanent, require longer time periods to develop and are contingent upon *de novo* protein synthesis. This form of synaptic plasticity has been the main focus of neuroscience research over the past decade and has provided greater insights into both cellular and molecular mechanisms by which synapses between subsets of neurons can be altered. These advances have not yet, however, resolved the attributes of network plasticity, nor have they elucidated the cellular basis of any given behavior – owing primarily to our inability to monitor direct cellular activity at the level of larger neuronal ensembles.

The network plasticity can be considered as the sum of individually summated synaptic changes that occur in groups of functionally related neurons. While this assumption holds true at the structural level, in terms of information processing and the network dynamics, the situation is actually much more complex. The output of a network is determined by the integrated activity of individual neurons, yet the activity-dependent changes at a single synapse are themselves driven by the complexity of the network itself. Numerous studies have demonstrated that changes in synaptic efficacy between a subset of neurons occur primarily in response to activity at that particular synapse. However, in the network environment the global activity that is distributed throughout many, if not virtually all, of the cells is equally important in determining

the functional output of the system. Neurons rarely receive input from a single source; the input signals are thus integrated from many different contacts and subsequently retransmitted in parallel to an equally larger number of targets. So the changes observed at a single synapse are actually the sum of a great number of inputs, and the change in efficacy of that synapse can likewise alter an equally larger number of target neurons. Therefore, the coordination of global activity throughout the network, which must ultimately be mediated by individual synaptic changes, determines which contact points should be modulated selectively.

An example of such a mechanism would be the balance between inhibitory and excitatory synapses within a cortical network, which is modulated both during development and also in the adult brain in an activity-dependent fashion (Graf et al., 2004; Levinson and El-Husseini, 2005; Liu, 2004; Prange et al., 2004). By shifting the inhibitory/excitatory balance, either temporarily or permanently, dramatic changes in network behavior can be observed. These changes not only generate seizure like activity (McCormick and Contreras, 2001; Romo-Parra et al., 2003), but can also produce synchronized *versus* uncoordinated firing patterns within the network (Aradi and Maccaferri, 2004; Kudela et al., 2003; van Pelt et al., 2005). Synchronized neural activity is an effective way to provide information to individual synapses regarding the network behavior. While somewhat cliché, the adage that 'neurons that fire together, wire together' holds true; and provides a viable mechanism for coordinating synaptic change in a large number of neurons dedicated to a specific information processing task.

To understand network function and plasticity, two levels of investigation need to be performed. On a synaptic level, the mechanisms underlying the basic process of synapse formation and loss, as well as the biochemical mechanisms to decide between excitatory *versus* inhibitory synapses, must be determined. On a network level, we need to characterize the activity signals received by individual neurons, which in turn, instruct them *vis-à-vis* their role in the final output. In order to do this several approaches have been used. While extremely varied in both the experimental paradigm and technology employed, they have one main feature in common: the goal of simultaneously recording activity from a large number of neurons. Inherent in this pursuit, however, is the caveat that as we increase the number of neurons in our recording paradigm, we decrease the resolution and the fidelity of our recordings.

Strategies to investigate network plasticity

Brain imaging technologies – all neurons but low resolution

Working towards the goal of simultaneously imaging activity in the entire brain, several newer technologies have been employed. For example, functional magnetic resonance imaging (MRI), which is based on the detection of blood oxygen levels using molecular resonance imaging technology, can determine non-invasively which areas of the brain are most active, as the increased metabolism is reflected by the change in blood flow. A similar principle is employed for positron emission tomography (PET) scans, where a radiolabeled glucose analogue is tracked in three dimensions; increased metabolism is again a measure of activity.

Several studies have used this technique to map brain regions responsible for specific behavioral functions following training or during recovery from stroke, and have addressed global plasticity by monitoring metabolic activity in areas assigned to a specific function (Jasanoff, 2005; Mechelli et al., 2005; Smirnakis et al., 2005; Sowell et al., 2004). While spectacular results have been achieved, the level of resolution is still restricted to large areas of the brain containing millions of neurons. To further enhance the resolution and interpretation of such metabolism-based imaging studies, these techniques have been combined with high-resolution electroencephalography (EEG) and magnetoencephalograpnhy (MEG) in order to correlate the anatomical images with more detailed firing patterns of the neurons. In addition to three-dimensional imaging technology, new biochemical techniques are also being developed to use voltage-sensitive and calcium-sensitive dyes (Baker et al., 2005) as well as fluorescence resonance energy transfer (FRET)-based systems (Chanda et al., 2005) to watch (or monitor) neuronal activity. Brain imaging technologies have a strong advantage in their ability to examine large ensembles of neurons simultaneously, thus giving a tremendous overview of the activity in the entire brain.

In vivo network dynamics – hundreds of neurons, better resolution

Investigation of how changes in neuronal connectivity alter network function as a whole requires a much higher level of resolution. During the past decade, advances in microscopy techniques offer greatly improved new strategies towards this goal. Two-photon imaging of living neuronal tissue expressing the fluorescent protein GFP has allowed detailed imaging of the neuronal architecture both before and after a learning paradigm (Mainen et al., 1999). These technologies have uncovered many details about the physical properties of neuronal connectivity and plasticity. However, to achieve this level of resolution one must limit the amount of tissue that one can monitor simultaneously. This is accomplished *in vitro* by taking slices of brain tissue, often from the hippocampus – a brain structure intensely studied due to its involvement in learning and memory. However, recent studies have demonstrated that this approach is also feasible in the intact animal, by using calcium-sensitive fluorescent indicators of activity in the visual cortex (Ohki et al., 2005).

Perhaps the greatest wealth of information about neuronal function in a network setting has been obtained by electrophysiological analysis of tissue slices. By recording both directly from single neurons using whole-cell patch-clamp techniques or from small groups of neurons using extracellular recording electrodes, we can gather detailed information about the electrical dynamics of functioning mammalian neural networks. Brain tissue slices can be kept

alive and functional for hours at a time, and have the advantage of maintaining the same network connectivity as is observed in the intact animals. Studies of intact tissue slices have uncovered phenomena such as LTP and LTD as well as spike-timing dependent plasticity, in which acute timing of activity in several parallel and convergent pathways can initiate plasticity in the network. Although these experiments have pioneered our understanding of how neurons function at a systems level, they are however drastically limited in the number of neurons that can be examined simultaneously.

Newest paradigm

Dissociated cultures – fewer neurons, higher resolution

While tissue slices have the advantage of maintaining network connectivity, they suffer from a short lifespan during the experimental procedure. To overcome this problem, two main strategies have been developed. One is the use of organotypic cultures, where tissue slices are partially dissociated and allowed to grow on a nylon membrane, maintaining their basic connectivity and allowing nutrient access to all the neurons in the network. This technique has been used to demonstrate longer-term phenomena, which could not otherwise be observed in the isolated brain or tissue slices, due to their shorter lifespan (Dong and Buonomano, 2005; Hasenstaub et al., 2005).

Neurons dissociated in cell culture from their respective areas in the intact brain can recapitulate their connectivity patterns and remain viable for months, provided that appropriate substrate and trophic factors conditions are met (Potter and DeMarse, 2001). A caveat with this approach is that the structural and functional integrity of the original network is lost. However, neurons taken from embryonic or neonatal animals have a remarkable capacity to rewire themselves accordingly, and if provided with growth and synapse conducive conditions, they are likely to network in a manner similar to that *in vivo*. For example, the neuronal connectivity that endows the developing retina to generate waves of activity *in vivo*, is recapitulated in cell culture, when these neurons are grown in the presence of appropriate growth factors (Colicos et al., 2004). Such judicious use of culture techniques has enabled us to reconstruct networks of functionally connected neurons, which generate rhythmical patterned activity, in a manner similar to that seen *in vivo* (Syed et al., 1990). Similarly, many *in vivo*-like neuronal activity patterns, including LTP and LTD, as well as a large variety of spike-timing-dependent phenomena, have been observed in cultured neurons (Hasenstaub et al., 2005; Netoff et al., 2005; Aradi and Maccaferri, 2004). Additionally, cells in culture can be transfected with specific genes to express exogenous proteins or to lower the levels of endogenous molecules. Culture systems are therefore a practical way to directly determine the functional significance of various proteins in neuronal functions.

Various imaging and electrophysiological recording techniques employed *in vivo* can also be adopted for cultured neurons, albeit at a much higher resolution and with greater fidelity. Because dissociated cells can be placed in close contact with the substrate as compared with their *in vivo* counterparts, this provides a unique advantage with which to develop an entirely new way of interfacing recording devices with neurons.

The transistor–neurons interface

When transistor technologies were in their infancy, parallels were drawn between computers and neuronal systems everywhere from a laboratory setting to Hollywood. Specifically, researchers were struck by the similarity between the charge transfer in a biological membrane and the semiconductor elements of the transistor. The possibility of using the transistor in close contact with the biological membrane of neurons was first investigated in the late 1970s and early 1980s, when field effect transistors were used to record brain activity during normal animal behavior (Boyko and Bures, 1975; Fontani, 1981; Sasaki et al., 1983). The goal of these experiments was primarily to collect behavioral data from animals by transmitting information collected by standard electrodes in contact with the neurons in an intact brain. Using transistor devices, this information could be relayed to the recording unit. However, in 1988 the Rutledge lab developed a silicon-based microelectrode/neuronal connection, which marked the start of a more sophisticated method of interfacing the silicon materials with the transistor in direct contact with the biological membrane (Regehr et al., 1988). In 1991 Peter Fromherz of the Max Planck Institute succeeded in developing a neuron–silicon junction between an insulated gate field effect transistor and a Retzius cell of the leech (Fromherz et al., 1991). This landmark paper marked the beginning of the refinement of the neural–transistor technology, enabling interfaces between living neurons and silicon as a viable tool for information transfer between the two.

Interfaces between neurons and electronics have since evolved over the years and now take two basic forms, each with specific goals. Currently, commercially available multielectrode arrays with 64 points for stimulation and recording can function without direct physical coupling with neuronal membrane (Arnold et al., 2005; van Pelt et al., 2005). These devices are extremely useful for recording excitatory postsynaptic current (EPSC) field potentials, and can stimulate groups of neurons situated above the electrode. By using the same array principal, but incorporating a transistor and capacitor at each node rather than simple electrodes, one can directly contact the membrane of individual neurons, allowing intracellular recording and stimulation. Recently, in a collaborative effort between researchers at the Syed lab at the University of Calgary and Peter Fromherz (Max Planck Institute), a proof of principal experiment was performed using this technology (Kaul et al., 2004). Specifically, individually identifiable, pre- and postsynaptic neurons from the snail *Lymnaea* were directly

cultured on the silicon chip in a soma–soma configuration (Feng et al., 1997; Hasenstaub et al., 2005; Munno and Syed, 2003; Smit et al., 2001) (Fig. 1). The presynaptic neuron visceral dorsal 4 (VD4) makes excitatory, cholinergic synapses with its postsynaptic partner left pedal dorsal 1 (LPeD1) in cell culture and this synapse exhibits short-term synaptic plasticity (Fig. 1a). When their neuronal somata were juxtaposed on a silicon chip, in the presence of the brain-conditioned medium (Fig. 1), excitatory synapses reformed that were similar to those observed under normal conditions (Fig. 2). Most importantly, the silicon chip technology allowed us not only to stimulate the presynaptic cell *via* the capacitor located underneath the neuron, but also to monitor postsynaptic action potentials non-invasively. The chip was used to induce synaptic potentiation, which was successfully recorded through the chip (Fig. 2B). Thus, by stimulating the presynaptic neuron with a capacitor, and by recording the post-tatanic potentiation in the postsynaptic cell through the transistor, the biological synapse between two neurons was modulated by computer control (Kaul et al., 2004). Repetitive stimulation of the first neuron strengthened its connection with the second neuron and this change in the synaptic efficacy was detected by the output capacitor

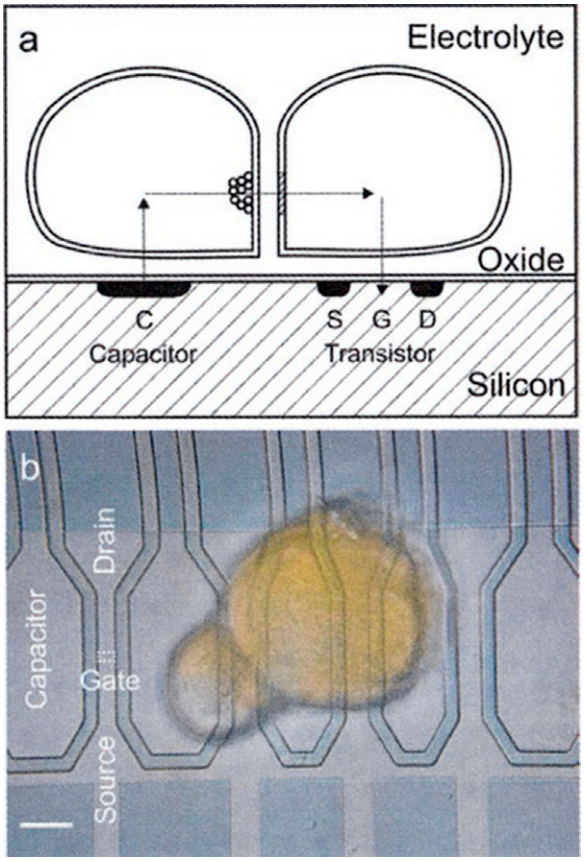

Fig. 1. *Lymnaea* neurons on a silicon chip paired in a soma–soma configuration. (a) A hybrid design depicting the relationship between neuronal connectivity and its interfacing with various chip components. G, gate; S, source; D, drain. (b) Photomicrograph of soma–soma paired presynaptic neurons visceral dorsal 4 (VD4-left) and its postsynaptic partner left pedal dorsal one (LPeD1 – larger cell) interfaced with silicon chip on a linear array of capacitors and transistors. Scale bar, 20 μm. Figure taken from (Kaul et al., 2004).

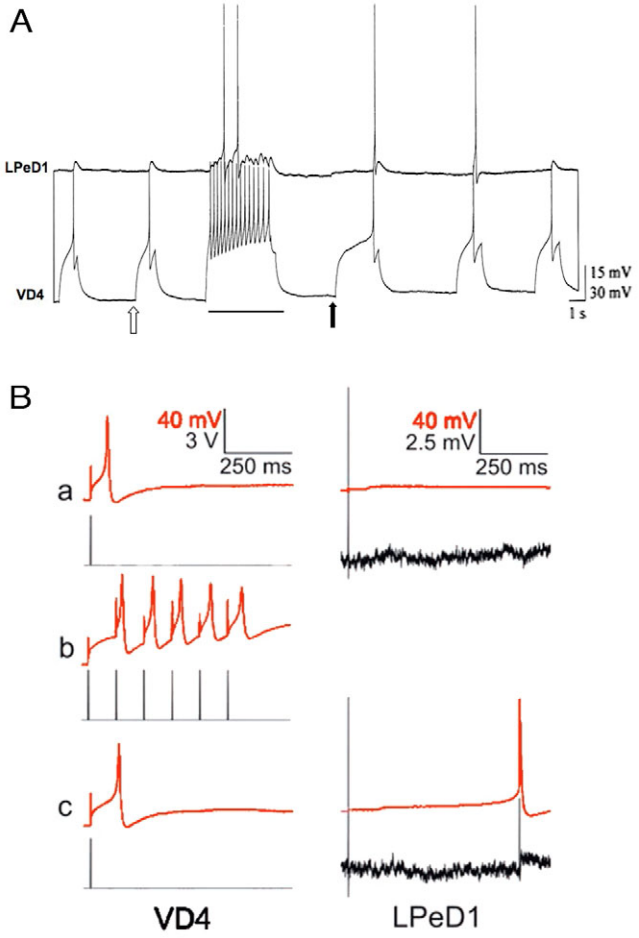

Fig. 2. (A) Short-term synaptic plasticity between soma–soma paired *Lymnaea* neurons. Presynaptic neuron visceral dorsal 4 (VD4) and its postsynaptic partner left pedal dorsal 1 (LPeD1) were paired in a soma–soma configuration and cells allowed to develop synapses overnight. Simultaneous intracellular recordings revealed excitatory synapses where induced action potentials in VD4 (first open arrow) generated 1:1 excitatory postsynaptic potentials in LPeD1. Following a burst of action potentials in VD4 (at bar) the subsequent action potentials in the presynaptic cell (at closed arrow) induced 1:1 spikes in the postsynaptic cell. This short-term change in the postsynaptic cell's response to the presynaptic action potentials illustrates the plasticity in the system. (B) Synaptic potentiation on a silicon chip. Cells were soma–soma paired overnight and synaptic physiology studied through the chip. The upper traces show intracellular voltages in red, the lower traces represent capacitor stimuli (left) and transistor records (right) in black. (a) Control recordings. Capacitor stimulation of the presynaptic neuron VD4 generated action potentials that did not elicit a detectable response in the postsynaptic cell LPeD1 (right). (b) Stronger capacitive stimulation through the chip induced bursts of spikes in VD4. (c) Post-tetanic action potential in VD4 now induced 1:1 action potentials in LPeD1 (right). Figure taken from (Kaul et al., 2004).

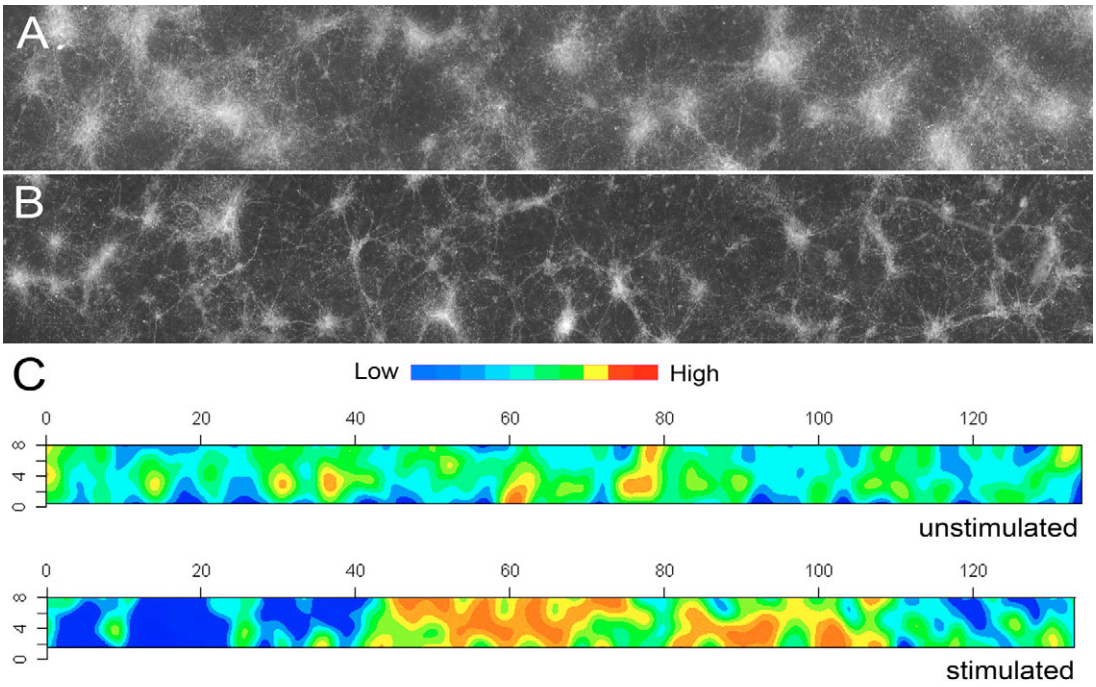

Fig. 3. Long-term stimulation of neuronal cultures. Rat hippocampal neurons were isolated from newborn pups and plated at high density on silicon wafers. After cultures were established (~7 DIV) the chips were placed into parallel stimulation devices, the control chip receiving no stimulation whereas the second chip received periodic high frequency stimulation in a circular region at the center of the wafer. Following stimulation for 1–3 days, chips were removed from the device and fixed, and then processed for immunostaining with anti-bassoon antibody. Bassoon is a presynaptic active zone protein, allowing the visualization of synapses. Images are acquired at low magnification and joined together to span the entire chip. (A) Unstimulated network, (B) central region of stimulated chip. Several mathematical methods were used to analyze the synaptic distribution; the first steps in analysis include deconvolution, thresholding and watershed analysis of the synaptic distribution images. (C) Sample synaptic density profiles represented by density distribution, illustrating altered patterns that result from long-term stimulation. Pseudo-color scale represents regions of low to high synaptic density.

(Fig. 2B). This essentially created a biological neuronal memory on a silicon chip, demonstrating a complete loop whereby both biological and electronic circuitries were successfully interfaced.

In parallel with the development of transistor and capacitor based neural interface technology was the emergence of a new form of neuronal/silicon interface, pioneered at University of California, San Diego, in the lab of Yukiko Goda. This new device harnesses the photoconductive effect of silicon; when light shines on a silicon wafer, the connectivity of the material increases. By targeting a single neuron grown on a silicon wafer with a narrow light beam, one can change the conductivity of the surface beneath it. If a brief electric pulse is then applied across the wafer, the majority of the current flows only through the illuminated region underneath the targeted neuron, thus causing that specific cell to fire. This noninvasive method can target single or multiple neurons in the network, irrespective of their location on the silicon. Using this technology, the Goda group documented the formation of new synaptic connections in response to specific activity patterns (Colicos et al., 2001), which had long been hypothesized to occur during memory formation but had never been visualized directly. The photoconductive stimulation technology is currently used to study neural network pathologies such as autism spectrum disorders, and can be adapted as an *in vitro* model to examine the network effects of long-term drug abuse.

Long-term stimulation of neuronal networks

One of the main advantages of photoconductive stimulation is the robustness of its interface and the non-invasive nature of the protocol. We have recently developed an incubator-mounted version of the interface that can provide target stimulation of neurons for long periods of time. As a proof of principal experiment, we have chosen a simple paradigm to test its functionality and to determine the basic effects of patterned stimulation on neuronal network development. Neuronal cultures are grown on 1 cm square silicon wafers, and when mounted in the device, a central region of the network is stimulated periodically at high frequency. A large body of evidence suggests that developing neural systems use activity to define their final patterns of connectivity. By determining the structure of a neural network in the regions of the stimulated *versus* unstimulated wafer, we can determine the effect of the activity on neuronal connectivity patterns. Fig. 3

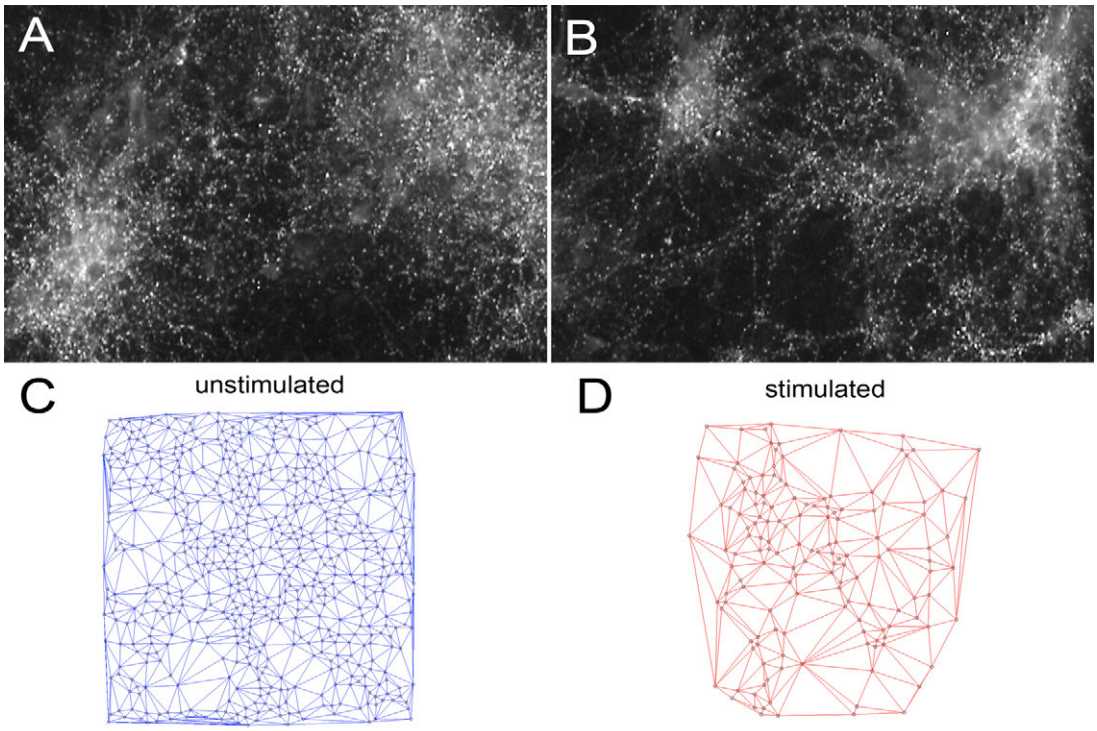

Fig. 4. Analysis of heterogeneity. (A) Unstimulated and (B) stimulated example close-ups of different network configurations resulted from activity driven plasticity. A variety of methods can be used to analyze synaptic distribution, including analysis of heterogeneity, variance and clustering, as well as more sophisticated modeling of the spatial distribution, which incorporate techniques such as Fourier transforms. (C,D) Examples of a Delauney triangulation of regions of clustered synapses, induced following overnight stimulation of the network, illustrating how pattern analysis can be performed to address network development using this technology.

shows an image of a region of the neural network cultured on the silicon wafer. To detect changes in the pattern of synaptic distribution following the stimulation paradigm, we labeled synapses with antibodies to the active zone protein bassoon. Images spanning a strip across the entire wafer are acquired and joined. Fig. 3C shows a representative density map of the distribution of synapses in a stimulated *versus* an unstimulated wafer. In the center of the wafer is a visible change in the pattern of connectivity as a result of the stimulation. Interestingly, while the pattern appears to have increased in complexity, there is a decrease in the overall number of synapses present. This complements observations indicating that decreased neuronal activity results in an increase in the number of synapses (Lauri et al., 2003; Nakayama et al., 2005). Intuitively this makes sense, in that if there is a specific pattern (information) within the stimulus, in order to represent this information only specific synapses are desirable in the final network. The ability to manipulate and visualize such large regions of neural network demands detailed statistical and mathematical processing of the data. Fig. 4 shows the Delaney triangulation pattern of synaptic cluster distribution for both stimulated and unstimulated neuronal cultures. Stimulation results in grouping subsets of neuronal blocks, and we are currently investigating the correlation between the geometric structures and the underlying stimulation frequency underlying network plasticity. By using techniques to analyze variance and heterogeneity, regions of nonrandom patterns can be extracted from these images. Fig. 5 shows an example of an analysis of directional variance (colored strings) overlaid on top of an image of the original network. These analyses will allow us to correlate structural changes that occur in response to specific frequency patterns. It is through such changes that the network structural and functional plasticity may be achieved.

Future developments

Perhaps the most exciting prospect currently under development is a fusion between the photoconductive stimulation and transistor/capacitor based technologies. By combining the highly adaptable and high-resolution targeting system of light with the intracellular recording capacity of the transistors, we are currently adopting a unique approach to provide large-scale integration of biological neural networks with digital technology. Photoconductive electronics will provide a means not only to understand the dynamics of neural networks, but will also form the basis for harnessing the massively parallel information processing capacity of neurons (Fig. 6). This will allow us to move back up the scale of resolution, to study large ensembles of neurons at a

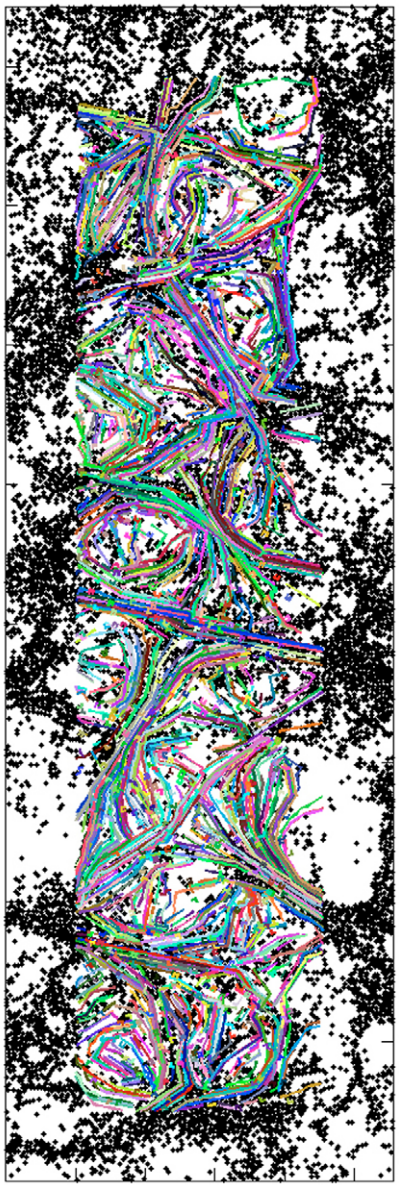

Fig. 5. Directional heterogeneity analysis. In addition to analysis such as synaptic cluster distribution, direction mapping along paths of maximum entropy can be used to trace connectivity on a large-scale. The figure shows experimental software designed to follow connectivity patterns in a stimulated network.

resolution of single neuron recordings. While neuronal networks formed from dissociated cells can provide valuable information, by incorporating this technology with brain slices, a more naive network structure can be analyzed. In this way, we can obtain a complete picture of how large neural networks not only process information, but also how they endogenously adapt to and change the network behavior. Finally, future technologies will also focus on designing implantable recording and stimulating devices for freely behaving animals.

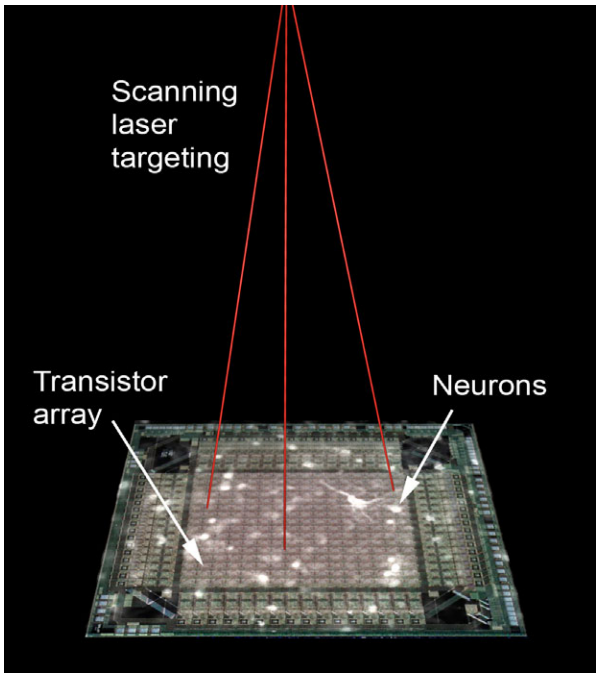

Fig. 6. Photoconductive electronics technology. By combining a massive array of transistor interface points, and the light-addressable specificity of photoconductive stimulation, a seamless interface with large neuronal ensembles can be achieved.

List of abbreviations

DIV	days *in vitro*
EEG	electroencephalography
EPSC	excitatory postsynaptic currents
FRET	fluorescence resonance energy transfer
GFP	green fluorescent protein
LPeD1	left pedal dorsal 1 neuron
LTP/LTD	long-term potentiation and depression
MEG	magnetoencephalograpnhy
MRI	magnetic resonance imaging
PET	positron emission tomography
VD4	visceral dorsal 4 neuron

We thank Wali Zaidi and Andrea Sullivan for technical assistance, and Johanna Hung and Audrey Kertesz for the network analysis studies. This work was supported by Alberta Ingenuity Fund grants to M.A.C., and Natural Sciences and Engineering Research Council of Canada (NSERC) grants to N.I.S.

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