Large Scale Multielectrode Recording and Stimulation of Neural Activity

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Abstract

Large circuits of neurons are employed by the brain to encode and process information. How this encoding and processing is done is one of the central questions in neuroscience. Since individual neurons communicate with each other through electrical signals (action potentials), the recording of neural activity with arrays of extracellular electrodes is uniquely suited for the investigation of this question. In addition to being able to detect simultaneous activity of multiple neurons, such recordings provide the combination of the best spatial (individual neurons) and temporal (individual action-potentials) resolution compared to other large scale imaging methods. Electrical stimulation of neural activity in turn has two very important applications; it enhances our understanding of neural circuits by allowing active interactions with them, and it is a basis for a large variety of neural prosthetic devices.

Until recently, the state-of-the-art in neural activity recording systems consisted of 64 electrodes spaced at hundreds of microns. Using silicon microstrip detector expertise acquired in the field of high energy physics, we created a unique neural activity readout and stimulation framework that consists of high density electrode arrays, multi-channel custom-designed integrated circuits, a data acquisition system, and data processing software.

Using this framework we developed a number of neural readout and stimulation systems; 1) a 512-electrode system for recording simultaneous activity of hundreds of neurons in retinas and brain tissue slices, 2) a 61-electrode system for electrical stimulation and readout of neural activity in retinas and brain-tissue slices, 3) a system with telemetry capabilities for recording of neural activity in the brain of awake-behaving animals.

We will report on these systems, their various applications to the field of neurobiology, and novel scientific results obtained with some of them. In the end we will outline future directions.

Introduction

Neuronal cells in the brain employ voltage-gated ion channels to produce action potentials (~1ms long electrical signals) in response to their synaptic inputs. These action potentials (commonly called spikes) propagate along the cell's axon and are "received" by the other neuronal cells through synaptic connections. Simultaneous detection of the spikes produced by a large population of neurons in the brain on a set of extracellular electrodes provides a unique insight into how the brain processes information.

While the exact specifications of a mulielectrode recording system have to be matched to the specific neural system under investigation (e.g. retina, visual cortex, hippocampus, etc), some general principles can be stated:

- The number of electrodes should be comparable to the size of the local population of neurons under investigation. This number varies for different neural systems, but usually is of the order of hundreds.
- Interlectrode spacing should be comparable to the distance between neurons: tens of microns.

- The signal detected on the electrodes has to be amplified, as it is usually of the order of hundreds of microvolts. The small input signal also requires the system to have a low internal noise.
- The full analog waveform detected on each electrode has to be digitized with sufficiently high sampling rate (several kHz) and recorded for further processing. This combined with a large number of electrodes calls for a data acquisition system capable of acquiring and storing data at a high rate (several MB per second).
- As it is necessary to identify recorded spikes that were produced by individual neurons, a robust data processing algorithm capable of processing large quantities of data has to be in place.

The field of experimental particle physics has developed sophisticated detector systems consisting of millions of channels. Using this expertise, the Large Scale Readout System (LSRS) collaboration of experimental particle physicists, engineers, and biologists developed a unique neural activity readout framework that adheres to the principles described above. The details of the concrete systems and the novel biology results obtained with them are described below.

Retina

Functional Organization

Motivation

As a system, the retina is very well suited for the study of neural activity. In the retina, initial detection of visual information is provided by the two-dimensional array of light sensitive rod and cone photoreceptors (input layer). The output layer consists of the retinal ganglion cells (RGCs) that receive information from the photoreceptors through intermediate retinal layers, encode it into a series of electrical spikes, and send it to the brain through the optic nerve. In general, each RGC receives inputs from many cones, and each cone supplies inputs to many RGCs. There are more than twenty distinct, morphologically identified, ganglion cell types. Populations of each of the RGC types sample the whole of the visual field and have their own unique spatial and temporal filtering properties providing for more than twenty representations of the outside world sent simultaneously to different targets in the brain. How is the visual information (spatial patterns, color, and movement) encoded by the diverse ganglion cell classes, or in the other words, what is the language the retina uses to relay visual information to the brain? The answer to this question is crucial for understanding the image processing that happens further in the brain and its behavioral implications.

Experimental approach

The experimental approach to addressing this question in-vitro has been developed in Meister *et al*., 1994 [1], and includes placing the separated retina, RGC layer down, on a planar array of microelectrodes. A computer-controlled image is then focused on the photoreceptor layer. The RGC responses to the visual stimulus are recorded as the retina is kept alive (up to 15 hours) by perfusing it with oxygenated physiological solution.

Until recently, the state-of-the-art retina recording systems consisted of arrays of 64 electrodes spaced at hundreds of microns. Such systems allow one to record activity of a total of only dozens of RGCs in a single preparation. While this technique significantly advanced our understanding of retinal processing, the small detected number of RGCs of each particular type did not allow for the truly comprehensive study of it.

We have developed a unique 512-electrode planar array readout system (Litke et al., 2004 [2]) capable of recording and analyzing the simultaneous activity of several hundred retinal ganglion cells in one preparation.

A photograph of the complete system is presented in Figure 1. The system has been described in detail in (Litke et al., 2004 [2]) and consists of the following components:

- A high density planar multielectrode array with 5μm diameter electrodes spaced at 60μm. The array has a glass substrate with the electrodes and leads microfabricated from the transparent

conductor, Indium Tin Oxide. To reduce the impedance at the electrode-solution interface, we electroplate the electrodes with platinum.

- Two custom-designed VLSI microchips. First, a 64-channel Platchip that provides AC coupling, DC current for electroplating, and current pulses for neuronal stimulation. Second, a 64-channel Neurochip that provides amplification, bandpass filtering, and analog multiplexing of the signals. Both gain and bandpass are controlled by reference currents. Equivalent input noise of the complete system (including the electrode array immersed in physiological solution) is ~7μV: significantly lower than the typical spike amplitude of hundreds of microvolts detected on the electrodes. The full system consists of eight pairs of Platchips and Neurochips.
- A Data Acquisition System consisting of a personal computer with two four-channel 12-bit ADC cards. Each of the 512 channels is digitized with 20kHz sampling frequency. The data is simultaneously saved to an external firewire hard drive at a rate of 15MB/sec and sent over gigabit Ethernet connection to the second personal computer that partially analyzes it and displays a number of online diagnostic plots. This DAQ system is implemented using LabView software.
- Data processing software. Since each electrode detects spikes from more than one neuron, and in general each neuron is detected on more than one electrode, we use spike waveform information to uniquely identify spikes that originated from each individual neuron. First we identify spikes on all electrodes as the events when the electrode signals cross a fixed threshold of ~80μV. We then use a combined waveform detected on a group of seven adjacent electrodes every time a spike occurs on the central electrode of a group as an input for Principal Component Analysis. We identify spikes associated with individual neurons by performing automatic clustering in the principal components space. CPU time needed to process a dataset is only a few times more than the length of the data recording time. Neuron-finding is implemented using Java.

Scientific results

The system is now being successfully used by collaboration between physicists from SCIPP and biologists from Prof. E.J. Chichilnisky laboratory at the Salk Institute to perform recordings of guinea pig and primate RGC activity. Figure 2 shows an example of such a primate retinal recording. It reveals the highly organized and complete mosaics of spatial sensitivity profiles (receptive fields) of several RGC types.

Primate retina recordings on the 512-electrode readout system have produced so far the following published novel scientific results:

- Motion encoding by an ensemble of hundreds of parasol RGCs (one of the most populous RGC types) has been shown to provide about an order of magnitude more precise information about the speed of movement compared to psychophysical results (ref). This implies that the obvious degradation of the speed estimate resolution happens downstream of the retina, and might have two possible sources. First, an additional noise introduced by the downstream neuronal circuits can degrade the resolution. Second, the retina readout in the motion sensitive areas of the brain can be sub-optimal for the speed estimate in the specific motion paradigm used in the experiment (vertical bars moving in two directions) while being more efficient when a more realistic visual scene is present.
- The correlated activity of hundreds of parasol RGCs has been studied using maximum entropy method (ref). The study revealed that in the presence of visual stimuli with low spatial and temporal correlations, the observed correlated RGC activity can be almost fully explained by only pair-wise and nearest neighbor connections. The study of the correlated activity of different types of RGCs in the presence of a more realistic visual stimulus is underway.

Currently we actively work on characterizing RGC types which have not been functionally studied before, and on color encoding in the retina.

Retinal Stimulation

Motivation

Retinitis Pigmentosa and Macular Degeneration are manifest in the loss of sight due to degeneration of retinal photoreceptors. At least a portion of retinal ganglion cells remains intact. Stimulation of these cells with spatio-temporal patterns of electrical pulses has the potential to restore vision to millions of people. While pilot human trials show promise that such stimulation might be successful, the exact parameters of stimulation pulses and electrode arrays necessary for the prosthesis remain to be investigated and optimized.

Experimental approach

Simultaneous stimulation with arbitrary spatio-temporal patterns of electrical pulses and the recording of the elicited activity is the crucial step in creating a retinal prosthesis. Using the framework developed for retinal recording we created a 61-electrode retinal stimulation system capable of simultaneously providing stimulation currents and recording neural responses. The system consists of a planar array of 61 5-10μm in diameter electrodes spaced at 60μm, and the same readout chips as in the 512-electrode system. Stimulation currents are provided by the Platchips under computer control.

Scientific results

The system was successfully used in the laboratory of Prof. Chichilnisky to show for the first time that small electrodes (<10μ) can be used to safely stimulate retinal ganglion cells (Sekirnjak et al., 2006). This finding is extremely important because it allows the possibility of prosthetic devices consisting of a large number of small densely packed electrodes, each capable of stimulating very few retinal ganglion cells at a time. Such a device will provide significantly better spatial and time resolution of the stimulated visual perception than the existing devices with large (hundreds of microns in diameter) and very few (dozens) of electrodes.

Further development

The current stimulation system has two important shortcomings rooted in the design of the Platchip. Firstly, a stimulation pulse causes a large electrical artifact that makes it difficult (and sometimes indeed impossible) to record stimulated spikes. Secondly, only one stimulation pattern can be applied to any number of electrodes. These two problems were addressed in the design of the new 64 channel stimulation chip (Stimchip). Stimchip uses DACs to allow for computer-controlled independent and arbitrary stimulation pulses on all 64 channels. Special circuitry disconnects the Neurochip's amplifier input from the electrode for the duration of the stimulation pulse, significantly reducing stimulation artifact. The Stimchip prototype is currently being tested. We plan to use the Stimchip as the basis for a new stimulation system capable of supplying arbitrary stimulation patterns while simultaneously recording neural activity.

Spontaneous and stimulated activity in cultured and acute brain slices

What are the functional properties of various brain areas? The study of acute and cultured brain slices provide us with the opportunity to investigate this question. The 512-electrode LSRS is ideally suited for recording local neural activity in acute and cultured slices. The parameters of the system are well matched to these recordings. The inter-electrode spacing of 60μm is of the same order as the average distance between directly connected cortical neurons. The large number of electrodes allows us to monitor local neural activity on a scale not previously possible. The new stimulation system described above will allow us to stimulate neural tissue using arbitrary patterns of currents and electrodes while simultaneously recording the spontaneous and elicited activity.

A series of pilot experiments that we conducted in collaboration with Prof. John Beggs (Indiana U.) showed that we can successfully use the 512-electrode LSRS to record activity of neurons in both cultured and acute brain slices.

Retinal development

How are the complicated connections between multitudes of neurons in the brain formed? This question is the subject of extensive and ongoing research in the field. We know that both molecular cues and experience are responsible for "wiring up" the brain.

An application of the multielectrode readout system allows for a comprehensive description of the retinal functional properties, which makes the retina a very promising system for studying neural connectivity development. Uncovering the molecular cues of the retinal neural circuitry formation will contribute to our understanding of the formation mechanisms of the entire nervous system.

In collaboration with Prof. David Feldheim (UCSC) we have started a study aimed at understanding the developmental mechanisms responsible for the formation of the retinal architecture. The study will be done in the mouse retina, so we can take advantage of mouse molecular genetic techniques. We will investigate the requirement for different axon and dendrite guidance molecules in the development of retinal circuitry.

Recording the brain neural activity of freely behaving animals

The large scale recording of neural activity in awake, freely behaving animals provides an absolutely unique opportunity to investigate the encoding and processing that happens in the intact brain.

Using the retina readout system framework, we developed a multielectrode system with telemetry capabilities for in-vivo recording of neural activity (in-vivo system). The work is carried out in collaboration with Prof. Markus Meister (Harvard U.) and Prof. Thanos Siapas (Caltech).

This system amplifies, filters, and transmits wirelessly the neural activity detected by a set of tetrode wire electrodes inserted in the brain of a freely behaving rat. A photograph of the system is shown in Figure 3. The system consists of the following components:

- A set of microdrives that guides up to 24 tetrode wire electrodes for chronic implantation into the rat's brain (not shown in Figure 3). The microdrive assembly will be mounted on the rat's scull and the electrodes will be inserted in the brain by Siapas's group.
- Amplification, bandpass filtering, and multiplexing of the signals from 12 tetrodes (48 channels in total) is accomplished in the new NeuroPlat chip that has built-in AC coupling capacitors, and which gain and bandpass are digitally controlled through internal DACs. The NeuroPlat chip is mounted directly on top of the electrode microdrive. The chip's gain and bandpass are set and the multiplexer commands (clock, hold, and reset) are produced by a programmable logic chip (Xilinx) mounted on the same printed circuit board.
- An electronic board that supplies the necessary power and batteries will be mounted on the rat's back.
- A multiplexed signal is sent and received by a commercially available FM transmitter and receiver. The transmitter/receiver combination was assembled, adjusted, and tested by Meister's group.
- The data acquisition system consists of the FM receiver connected to a National Instruments ADC card installed in a personal computer. The received multiplexer signal is digitized and software demultiplexed online. The resulting sampling rate is 20kHz for each channel. The demultiplexed data is written out to an external firewire drive.

Preliminary measurements show that the equivalent input noise of the full system (with terode wire electrode immersed in physiological solution) is of the order 10-15μV with most of the noise coming from wireless signal transmission. This noise level is significantly smaller than the few hundred microvolt signals typically detected on the tetrode wire electrodes inserted into the rat's brain.

The total weight of the system is approximately 50g, with more than half of it coming from the

batteries that enable the system to run continuously for 10 hours. Preliminary tests show that the rat can be trained to comfortably carry this weight. The receiver-transmitter combination has been tested to reliably transfer signals at a range of up to 60 meters.

We plan soon to do a pilot recording of the brain neural activity in the freely behaving rat.

Future Directions

In the future, we plan to:

- Develop a stimulation system based on the new Stimchip. The system will be used both for retinal prosthesis studies, and for stimulation and recording of the neural activity in acute and cultured brain slices.
- Further develop the in-vivo system, increasing the number of readout channels and adding stimulation capabilities.
- Develop new electrode arrays with larger inter-electrode spacing for recording activity of sparse retinal ganglion cell types (the larger spacing will provide for the larger number of sparse cells at a price of subsampling denser ones).
- Develop bed of nails electrode arrays with electrodes located at the tips of nails (few hundreds of micrometers high) that can be inserted into acute brain slices or into the surface of the brain.

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Figure 1:
Magnified view of the 512-electrode large scale readout system. 512-electrode array is located in the center of the chamber that is filled with physiological solution during the experiments. Common reference electrode for all channels is a platinum wire encircling the circumference of the chamber. For scale, the inner diameter of the chamber is 14mm.

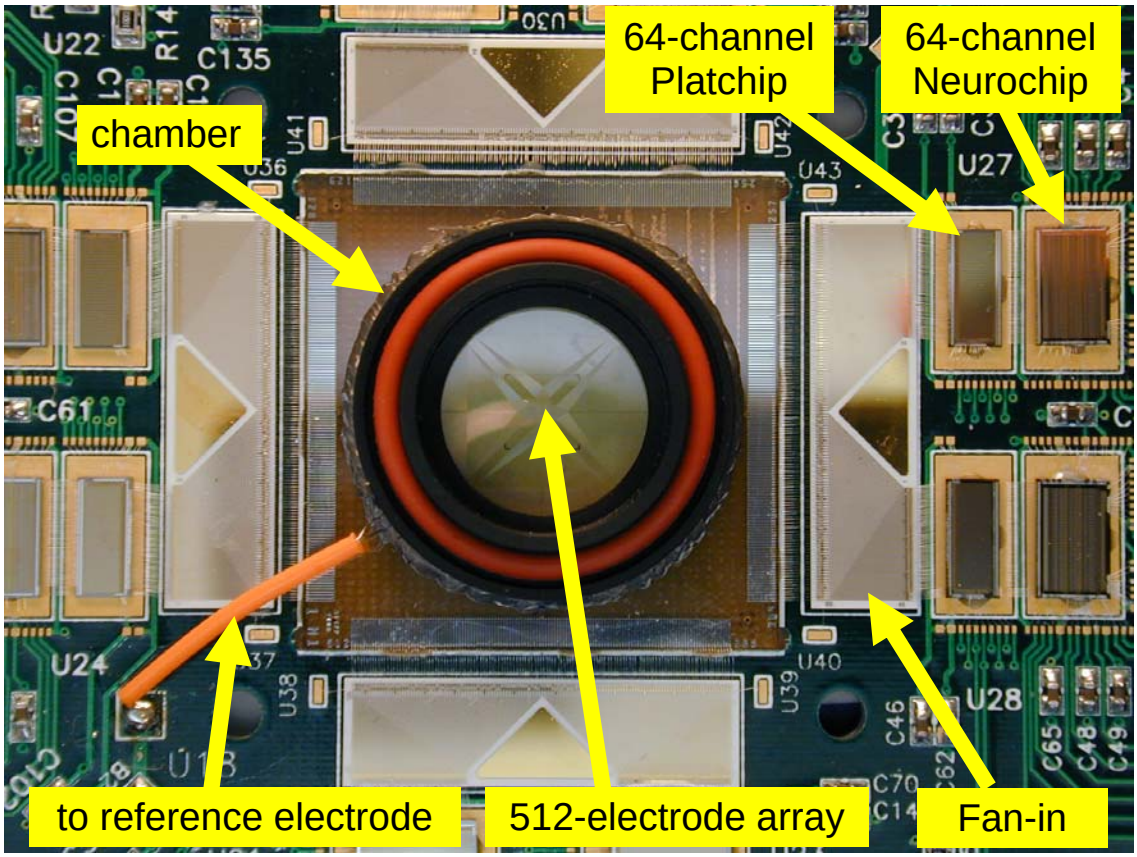

Figure 2:
Spatial sensitivity profiles (receptive fields) of the primate retinal ganglion cells (RGCs) recorded by the 512-electrode readout system in one retinal preparation. Each ellipse represents one sigma contour that was fitted to the receptive field of a single RGC. The middle panel shows all of the identified neurons. The four panels in the corners show the four RGC types classified based on their space and time filtering properties. These four types are most likely correspond to anatomically identified parasol and midget RGC types. The ON and OFF notation distinguishes cells that respond to increase and decrease in the light intensity, respectively. The boxes outline the 512 electrode array and have the size of 1860x900μm^2.

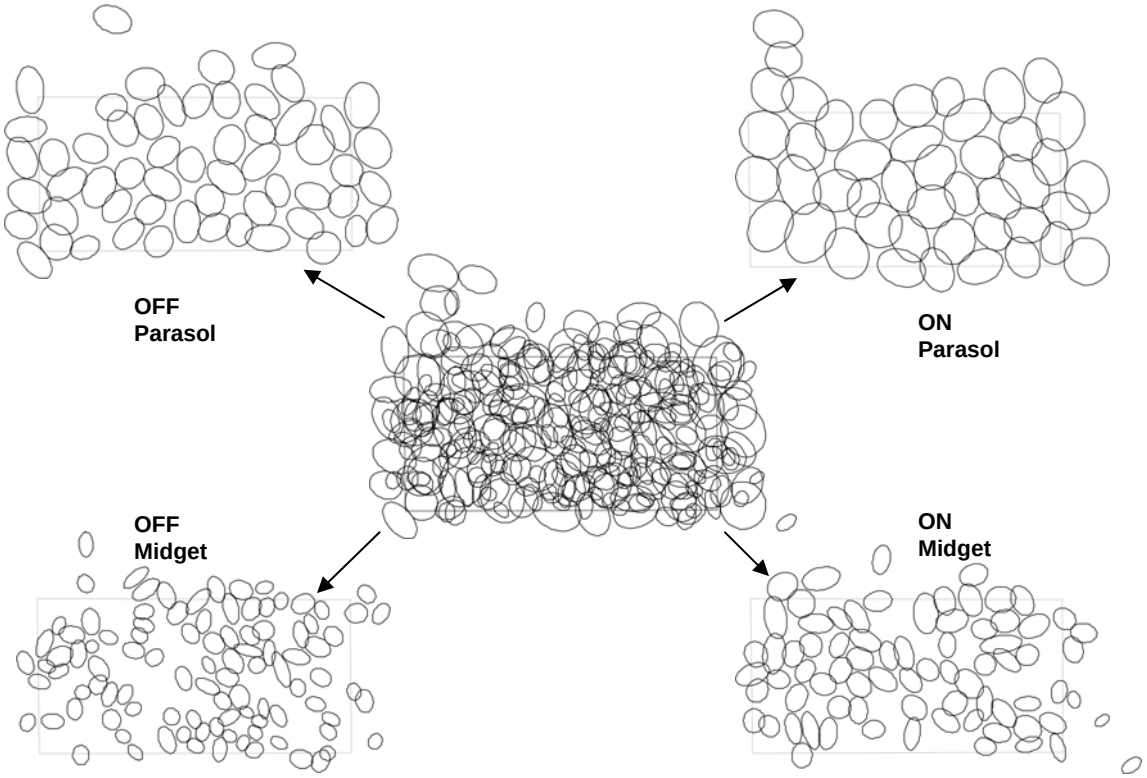

Figure 3: A photograph of the in-vivo system. The circular printed circuit board (headboard) contains readout NeuroPlat chip and programmable logic chip with supporting electronics. This board will be mounted directly on top of the microdrive assembly with tetrode wire electrodes inserted in the rat's brain. The rectangular board (backboard) provides the necessary voltages for the headboard and is powered by the two 3V batteries. The batteries, backboard, and the FM transmitter (shown in the lower left) will be mounted on the rat's back.