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NeuroRighter: Closed-loop Multielectrode Stimulation and Recording for Freely Moving Animals and Cell Cultures

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Abstract—**Closed-loop systems, where neural signals are used to control electrical stimulation, show promise as powerful experimental platforms and nuanced clinical therapies. To increase the availability, affordability, and usability of these devices, we have created a flexible open source system capable of simultaneous stimulation and recording from multiple electrodes. The system is versatile, functioning with both freely moving animals and *in vitro* preparations. Current- and voltage-controlled stimulation waveforms with 1 µs resolution can be delivered to any electrode of an array. Stimulation sequences can be preprogrammed or triggered by ongoing neural activity, such as action potentials (APs) or local field potentials (LFPs). Recovery from artifact is rapid, allowing the detection of APs within 1 ms of stimulus offset. Since the stimulation subsystem provides simultaneous current/voltage monitoring, electrode impedance spectra can be calculated in real time. A sample closed-loop experiment is presented wherein interictal spikes from epileptic animals are used to trigger microstimulation.**

I. Introduction

Multielectrode recording offers rewarding insights into the function of the nervous system, insights otherwise unattainable with alternative techniques [1]. The same can be said of electrical stimulation, without which the discovery of long-term potentiation [2] or the motor and sensory maps of Penfield [3] would not exist. We believe that these two modalities, multielectrode stimulation and recording, will be even more powerful when coupled in closed-loop systems [4, 5]. In fact, research with closed-loop stimulation has already proven useful for suppressing clinical seizures in humans [6] and exploring new modes of network-level learning in neuronal cultures [7].

Unfortunately, no commercial system for closed-loop stimulation and multielectrode recording exists. Furthermore, commercially available systems for multielectrode recording are hampered by long, saturating artifacts, which prevent recording from electrodes following stimulus pulses.

Here we present an integrated system for simultaneous multielectrode recording and stimulation. The software and hardware designs are open-source[1] and freely available online (http://www.johnrolston.com/), reducing the *in vivo* system's cost to <$10,000 for 64-channels of closed-loop recording and stimulation, an order of magnitude less than comparable commercial systems that cannot stimulate. The system recovers from stimulation artifact rapidly and is flexible in use, with both *in vivo* and *in vitro* versions. The current work is an extension of our lab's previous efforts in creating powerful yet inexpensive tools for studying closed-loop systems [8, 9].

II. System Design

A. System Overview

The system has four components: 1) multichannel amplifiers, 2) stimulation channel selection circuitry, 3) interface boards for analog filtering, power filtering, and stimulation control, and 4) a standard desktop computer with multifunction data acquisition cards (DAQs). Components 1-2 differ for freely moving animals and *in vitro* preparations, but the software, computer, and DAQs are identical (Fig. 1). Further construction details are available online (http://www.johnrolston.com/).

B. In Vivo *System*

A custom-built stimulation headstage (designed with the free ExpressPCB software) connects to a chronically implanted microwire array (Tucker Davis Technologies). A 16-channel 100× gain head-mounting amplifier (Triangle Biosystems) attaches in turn to the stimulator headstage, buffering the signal and minimizing movement artifacts. Solid-state switches (Maxim, Inc.) direct stimuli to the appropriate channel. Current-control and diagnostic circuitry take place off-chip.

Extracellular signals, amplified by the headstage, are band-pass filtered in the interface boards, using a 2-pole active high-pass voltage-controlled voltage-source topology with a cut-off of 1 Hz, and a passive low-pass filter with a cut-off of 8800 Hz.

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[1] The software is licensed under the GNU Public License (GPL), version 3 (http://www.gnu.org/copyleft/gpl.html), and hardware under the Creative Commons Attribution-Share Alike 3.0 license (http://creativecommons.org/licenses/by-sa/3.0/us/).

C. In Vitro *System*

A 64-channel MultiChannel Systems preamplifier (1000× gain) amplifies extracellular signals from 60-channel substrate-integrated multielectrode arrays (MEAs; note that the inclusion of the MCS preamp significantly increases the *in vitro* system's cost over that of the *in vivo* system). Custom-designed stimulation modules (ExpressPCB) deliver stimulus waveforms to the appropriate channel.

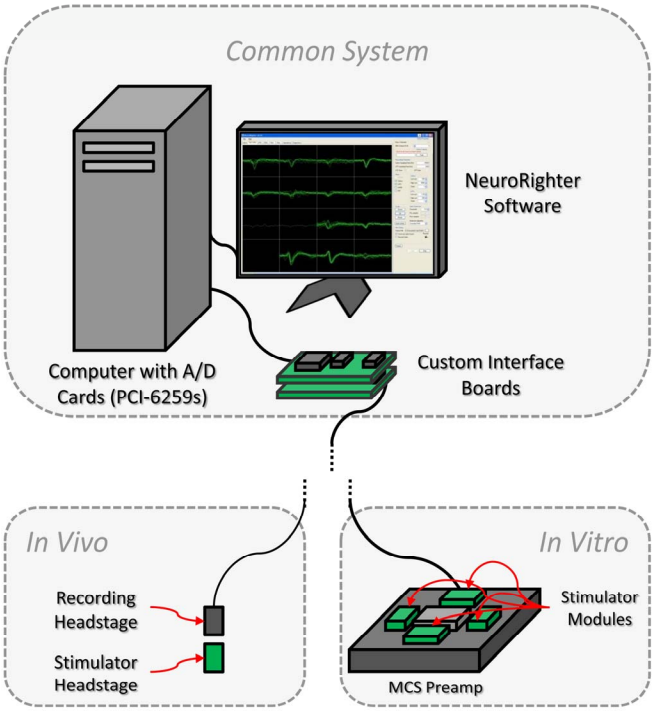

Fig. 1. System schematic. A common system (top), shared by *in vivo* and *in vitro* applications, consists of custom software, data acquisition cards, and a desktop computer. Interface boards handle stimulation control and filtering. In freely moving animals (bottom left) a recording headstage amplifies neural signals and a stimulation headstage routes stimuli to the appropriate channel. *In vitro* (bottom right), an MCS preamp amplifies signals and stimulation modules handle stimulus routing.

D. *Common System*

Analog signals are digitized at 25 kHz with 16-bit resolution by PCI-6259 cards (National Instruments). Our NeuroRighter software, a multi-threaded Windows-based application (written in C#), handles online signal processing and stimulation control. Online processing separates the signal into spike and local field potential (LFP) bands and performs real-time spike detection.

Custom-designed printed circuit boards (PCBs; designed with the free PCB123 software) interface the data acquisition cards with the recording and stimulating headstages (*in vivo*) or preamplifiers and modules (*in vitro*).

Voltage or current-controlled stimulation waveforms are specified in software, generated by the PCI-6259 card's D/A converters, and propagate to the interface board. For voltage-controlled stimulation, the D/A signal is buffered and the delivered current is monitored with an instrumentation amplifier. For current-controlled stimulation, the voltage-controlled D/A signal is converted to current through precision resistors and a bank of operational amplifiers. The selection of voltage or current waveforms is made in software, which controls solid-state switches on the interface board.

Power is supplied by rechargable lead-acid batteries; however, the system's ground is tied to that of the acquisition computer (as in many commercial systems, e.g., Plexon). Future versions will incorporate additional isolation.

III. Experimental Methods

A. *Surgery*

All work with animals was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Emory University Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (350-450g) were anesthetized with isoflurane, several anchoring skull screws were implanted, and a craniotomy was drilled over the right dorsal hippocampus. After removing the dura, a 16-channel microwire array (33 µm diameter tungsten wires with polyimide insulation) with two rows of 8 electrodes (row 1, 4 mm long; row 2, 2.8 mm long) was carefully lowered into craniotomy, with the longer row of the array targeted to the CA3 region, and the shorter row to CA1. Proper depth (usually 3-4 mm ventral to pia) was determined by monitoring electrophysiological recordings during implantation, using the NeuroRighter system. The craniotomy was then sealed with dental acrylic and the rat was allowed to recover for 5-8 days before recordings began.

Tetanus toxin (25 ng; Sigma) suspended in 0.5 µl of phosphate-buffered saline with 0.2% bovine serum albumin, was used to induce epilepsy in the closed-loop experimental animals. The injection was targeted to the CA3 region of the dorsal hippocampus (3.3 mm posterior to bregma, 3.2 mm lateral to midline, and 3.1 mm ventral to pia). Spontaneous seizures began in 5-9 days following injection. Unlike status epilepticus models, the tetanus toxin model has no mortality and a shorter latency to seizure onset.

B. *Stimulation Experiment*

To screen the effects of different stimulus intensities, rats were moved to a custom-built wooden and Plexiglas enclosure for recording and stimulation. They were tethered but otherwise freely mobile. Biphasic current-controlled stimulus pulses were then delivered to a subset of the electrode array (those electrodes with single unit activity and some additional electrodes). All cathodic-phase first pulses had a duration of 800 µs (400 µs per phase). Several stimulus amplitudes were used: 2, 4, 6, 8, 10, 15, 25, and 50 µA. 10 trials with each pulse amplitude were delivered to each electrode in random order. Randomization helped to control for neural adaptation.

C. *Impedance Measurements*

Impedances were measured by delivering current-controlled sine waves across a spectrum of frequencies to

each electrode in turn. The delivered voltage was measured, a matched filter was applied, and the ratio of RMS voltage over RMS current was used to calculate the impedance at a given frequency.

D. Closed-loop Experiment

To illustrate the closed-loop experiments possible with the NeuroRighter system, we created an algorithm that delivered stimulation to a single electrode when an interictal spike was detected in the LFP. Interictal spikes were defined as deviations exceeding 7.5× the signal's RMS. This high threshold ensured a very high specificity (100% based on 45 minutes of test data immediately preceding the experiment) but at the expense of sensitivity (10%), as determined by an expert reviewer. Biphasic 10 µA, 400 µs per phase pulses were delivered after each detection.

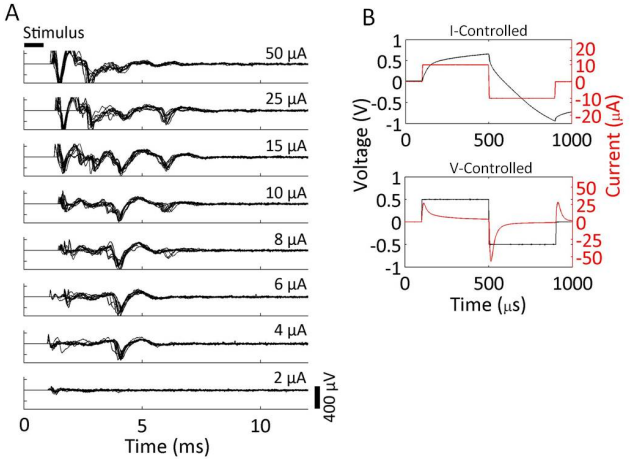

Fig. 2. Stimulation evokes action potentials (APs). (A) Biphasic current-controlled pulses of varying amplitudes are delivered to an electrode. Ten trials of evoked activity are overlaid for each intensity. The first APs are evoked at 4 µA, with additional APs emerging at higher intensities (15 µA). Spikes are detected less than 1 ms after stimulus offset. (B) The stimulator can operate in current- or voltage-controlled modes. The delivered current and voltage are simultaneously monitored during each pulse. This information can be used to measure impedance spectra when sinusoidal waves are used rather than biphasic pulses.

IV. Results

We created a combined recording and stimulation system for multielectrode arrays. The system is capable of recording LFPs and APs from freely moving animals. Both current- and voltage-controlled stimulation waveforms can be delivered to any recording electrode, and impedance spectra can be acquired in real-time. Stimulus pulses readily evoke APs which are recorded at short latency (Fig. 2A).

A. Noise and Cross-talk

Broadband root-mean-square (RMS) noise values for the *in vivo* system are 6.1 ± 0.2 µV (mean ± standard error across channels) when using a grounded reference, and 8.4 ± 0.2 µV when using an active reference. Restricted to bands containing action potential data (>300 Hz), the RMS noise is 3.9 ± 0.1 µV for grounded reference, 5.5 ± 0.1 µV for true reference. The increased noise when using a true reference arises from the superposition of the reference channel's noise and that of the signal channel (the combined noise is a factor of √2 larger). The headstage manufacturer, Triangle Biosystems, specifies the broadband RMS noise as 6.2 µV, so our system is not introducing additional noise through the interface boards, cables, or A/D conversion process.

For the *in vitro* setup, where an MCS preamplifier is used, the broadband RMS noise is 3.2 µV.

The observed cross-talk was −66 dB for adjacent channels, −69 dB for non-adjacent. This is in agreement with the headstage manufacturer's reported cross-talk (−63 dB for adjacent channels; personal communication with TBSI).

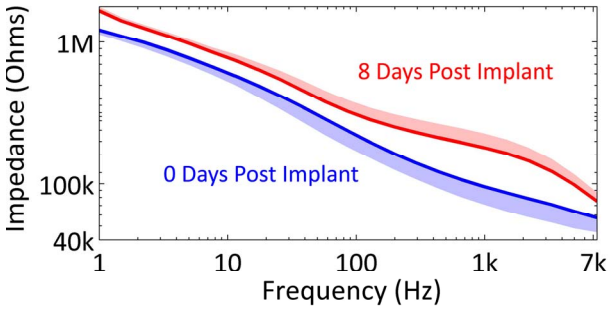

Fig. 3. Impedance spectra. The ability to simultaneously monitor delivered current and voltage allows the NeuroRighter system to calculate impedance spectra in real-time from freely moving animals. The above impedance spectra were taken from a microwire array implanted in rat hippocampus immediately after surgery (blue) and 8 days post-op (red). Dark lines indicate population averages (across the array's 16 electrodes); shading indicates one standard deviation (shown on ones side only, for clarity).

B. Impedance Spectra

Impedance spectra were calculated at several time points across several animals. Example traces are shown in Fig. 3, highlighting the gradual increase of electrode impedance after the initial array implant.

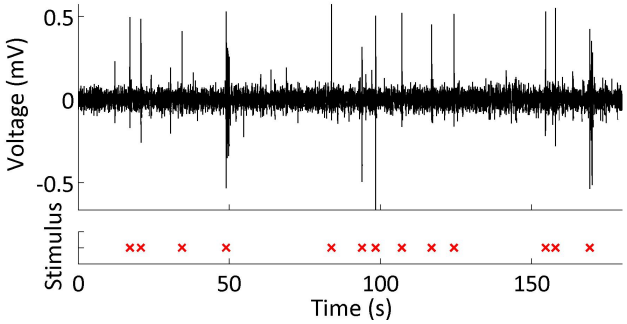

Fig. 4. Closed-loop stimulation. Stimuli (red x's) are triggered by the detection of interictal spikes in the LFP (top trace). The animal was freely mobile during this experiment.

C. Closed-loop Experiment

To test our system's closed-loop capabilities, we programmed a sample experiment wherein a single 10 µA stimulation pulse was delivered to an electrode when interictal spikes were detected (Fig. 4). The mean time between detection of interictal spikes and stimulus delivery was 4.4 ± 1.2 ms (± standard deviation). Though stimulation pulses readily evoked neural responses (Fig. 2), stimulation nevertheless had no discernible effects on interictal spikes in CA1 or CA3 of epileptic animals (the stimulating channel was located in CA3).

V. Discussion

The NeuroRighter system is an integrated software and hardware suite for conducting closed-loop multielectrode experiments. It has several notable features. 1) *Open source software and hardware.* Because the software and circuit layouts are free and permissively licensed, users can readily modify the code or circuitry to add features (e.g., new device drivers, new spike detection methods), improve functionality, or customize their experiments. The free licensing also leads to a total *in vivo* system cost of less than $10,000 for 64-channels, 4-10× cheaper than comparable commercial systems that cannot conduct closed-loop stimulation. 2) *Flexibility*. NeuroRighter has circuitry for both *in vitro* and *in vivo* experiments, providing a consistent platform for both fields of research. Stimulation is also flexible, with software toggling between voltage and current control, and the capability to produce waveforms of arbitrary complexity (e.g., "replaying" a previously recorded LFP). 3) *Stimulation.* Others have previously reported multielectrode stimulation systems with short stimulation artifacts [10-12], though these use specialized (in one case patented) artifact-reduction circuitry. Our system relies only on a low-gain front-end (100×) with no subsequent second-stage amplification, possible due to our 16-bit A/D resolution. This simplicity improves cost while still recovering from artifacts within 1 ms (the stimulating channel takes longer (~60 ms) to recover, however, due to capacitive coupling to the medium). 4) *Real-time impedance spectroscopy*. Impedance spectroscopy has the potential to reveal additional information about electrode viability and tissue composition proximal to the electrode [13, 14]. The NeuroRighter system provides push-button acquisition of these spectra. Preliminary results (Fig. 3) corroborate previous reports of increased impedance following electrode implantation [14], illustrating the system's utility. 5) *Closed-loop experimentation*. Few systems exist for conducting closed-loop multielectrode electrophysiology, and there are fewer still for use in freely moving animals. To illustrate the system's capabilities, we conducted a simple experiment wherein stimulation was delivered to CA3 upon detection of interictal spikes [15] in a freely moving rat. Some authors propose that stimulation following seizure detection can prevent full-blown seizures or shorten their duration [16, 17]. Though our stimulation evokes APs (Fig. 2), interictal spikes, which share pathophysiological features of seizures, were unaffected, even in the downstream CA1 region. This implies that the amount of tissue we affect is insufficient to suppress these spikes, or that the generating mechanism is resistant to perturbation by stimulation.

VI. Conclusion

The NeuroRighter system offers a streamlined platform for closed-loop experimentation using microwire arrays both in culture and in awake, behaving animals. It is our hope that the features and usability of the system will encourage additional researchers to capitalize on the exciting possibilities inherent in closed-loop devices.

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