A versatile all-channel stimulator for electrode arrays, with real-time control

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Abstract
Over the last few decades, technology to record through ever increasing numbers of electrodes has become available to electrophysiologists. For the study of distributed neural processing, however, the ability to stimulate through equal numbers of electrodes, and thus to attain bidirectional communication, is of paramount importance. Here, we present a stimulation system for multi-electrode arrays which interfaces with existing commercial recording hardware, and allows stimulation through any electrode in the array, with rapid switching between channels. The system is controlled through real-time Linux, making it extremely flexible: stimulation sequences can be constructed on-the-fly, and arbitrary stimulus waveforms can be used if desired. A key feature of this design is that it can be readily and inexpensively reproduced in other labs, since it interfaces to standard PC parallel ports and uses only off-the-shelf components. Moreover, adaptation for use with *in vivo* multi-electrode probes would be straightforward. In combination with our freely available data-acquisition software, MeaBench, this system can provide feedback stimulation in response to recorded action potentials within 15 ms.

1. Introduction

1.1. Background

Two-way communication between brains and computer systems has been an important goal in neural engineering for several decades, since it can significantly broaden the horizons in many research areas, such as cortical population coding (e.g., Salzman *et al* (1990)) or long-term plasticity *in vivo* (e.g., Staubli and Lynch (1987)). Over the last 30 years, technology to record from a large number of cells has been developed and applied to a wide range of model systems: multi-electrode arrays (MEAs) (Thomas *et al* 1972, Gross 1979, Pine 1980) have been used to record from many preparations from dissociated cortex (Potter 2001) to intact retina (Meister *et al* 1991), while their *in vivo* counterparts, silicon probes (Wise and Angell 1975) and multiwire probes (Nicolelis *et al* 1997) are also gaining popularity. Stimulation technology has not kept equal pace. Commercially available systems presently are limited to a relatively small number

of channels (typically 10 or less), or require programming ahead of time, making true two-way real-time communication impossible. Accordingly, many researchers have built their own custom devices: one of the first such devices used a manual switchboard to select 8 out of 61 channels for stimulation (Regehr *et al* 1989). Jimbo and Kawana (1992) describe a complete system with 18 stimulation channels. Pancrazio *et al* (1998) describe a 16-channel stimulation system for cardiac myocytes implemented in VLSI, while Zeck and Fromherz (2001) use FET technology to construct a similar system for invertebrate neurons. Another system that combines recording and stimulation capabilities for 64 electrodes was recently described (Jimbo *et al* 2003).

Our research focuses on re-embodied neural cultures (Potter 2001, DeMarse *et al* 2001) and the developmental impact of persistent stimulation on network formation. For both these projects, we needed a stimulator with the following properties:

- Ability to stimulate through any of the electrodes of our MEAs.

- Rapid switching between stimulation and recording through the same electrode.
- On-the-fly specification of stimulation sequences as a function of live neuronal activity.
- Compatibility with existing recording technology.

The 'real-time all-channel stimulator' (RACS) we present here has all these properties, and has the additional advantage that its assembly from off-the-shelf components is straightforward.

1.2. Design philosophy

Conceptually, stimulators are relatively simple devices: they consist of a voltage or current source, some logic to route the signal to the appropriate electrodes, and a set of isolator switches to allow recording from electrodes whenever they are not being used for stimulation. Most complexity comes in the form of the logic that controls the timing of the system. Commercial systems currently use micro-controllers for this task, but we have chosen an alternative design route: our stimulator is controlled externally by a computer running a real-time operating system, RTLinux (Barabanov 1997). This eliminates the need for a dedicated microprocessor on the stimulator board, and makes for much easier programming. Perhaps surprisingly, it is also extremely cost-effective, since even an old 100 MHz CPU is fast enough to provide the required real-time control. The total cost of our stimulator is about $250; $150 for components and $100 for printed circuit board fabrication. Assembling the system takes about one day and requires only basic electronics skills.

2. Methods

2.1. Cell culture

Details have been described before (Potter and DeMarse 2001). Briefly, cortices from E18 rat embryos were dissected and dissociated using papain and trituration. Cells—neurons and glia—were plated at a density of 5000 cells mm^{-2}, on MEAs coated with poly-ethylene-imine (PEI) and laminin. Cultures were maintained in a serum-containing DMEM-based medium, in Teflon-sealed dishes. Stimulation experiments were performed from *in vitro* day 8 onwards.

2.2. Recording system

Signals were recorded through 30 μm titanium nitride electrodes spaced at 200 μm. Each MEA (MultiChannel Systems, Reutlingen, Germany) has 59 such electrodes, and one large ground reference electrode that also served as return electrode for stimulation. Signals were pre-amplified 1200x, then sampled at 25 kHz using MultiChannel Systems hardware. Data acquisition and online analysis, including visualization, artifact suppression, spike detection and storage were controlled through MeaBench (Wagenaar 2002). Artifact suppression was performed by subtracting third-order polynomials locally fitted to the recorded voltage trace, using the SALPA ('suppression of artifacts by local polynomial approximation') algorithm (Wagenaar and Potter 2002). A C++ implementation of this algorithm is available

upon request from the authors. Further analysis was performed using Matlab (MathWorks).

2.3. Stimulation system

In order to keep the fabrication as simple as possible, we used only standard 0.1 inch DIP technology, avoiding surface-mount technology. Furthermore, we opted for a modular design, which made it easier to test a variety of isolation switches as well as several types of digital-to-analog convertors (DACs). This also facilitates adaptation to other recording hardware. Circuit layout was performed using software from ExpressPCB (www.expresspcb.com), who also machined the printed circuit boards.

The RACS consists of one main board containing a DAC and some interface logic, as well as four modules each of which contains isolation switches to gate signals to one of 16 electrodes (figures 1 and 2). It is controlled from a PC's parallel port, which provides four 'control' pins and eight 'data' pins. The control pins are used to route signals from the data pins to either of two DAC channels—one for the stimulus proper, one for an auxiliary analog output—or to either of two latches—one to control the stimulation switches, one for 8 auxiliary digital outputs. Potential uses for the auxiliary outputs include tagging stimulus identities and triggering external equipment.

The electrodes are grouped into eight banks of eight, each bank being serviced by one 1-to-8 electronic switch with very low leakage and charge injection (MAX338, Maxim). A 3-to-8 decoder (74HCT238, Philips Semiconductor) is used to select which bank (if any) is activated. A copy of the stimulation voltage is available at the 'V' monitor terminal. The stimulation current passes through a 5 kΩ resistor, and the voltage across this resistor, amplified 11x, is available at the 'I' monitor terminal. Multi-turn potentiometers are included to tune the range of stimulation voltages as well as to center the range to zero. The maximal selectable range is ± 10 V; we trim the range to ± 1.0 V to prevent electrolysis and damage to electrodes. At that setting, the 8-bit DAC (TLC7628, Texas Instruments) allows specification of stimulation voltages to 8 mV precision. On-board latches (74HCT374, Texas Instruments) and switches are all fast enough (<100 ns propagation delays) that operation speed is effectively limited only by the controlling computer. The circuitry around the DAC, by contrast, was purposefully designed to have a relatively slow slew rate of 110 mV μs^{-1}. This ensures that the signal generated by the DAC can be accurately reproduced by the output op-amps, without distortion due to large capacitive currents at sharp voltage transitions.

A kernel module for RTLinux ('Open' version 3.1, FSMLabs, www.fsmlabs.com) was written to allow control over output voltages and switching with microsecond-level precision. Driver architecture was based on code examples in the RTLinux documentation, as well as in Rubini and Corbet (2001). To enhance maintainability, only minimal functionality was implemented in the driver, with most of the higher level control left to user-space programs: we used perl (www.perl.org) to write software for specifying pulse shapes in more convenient terms.
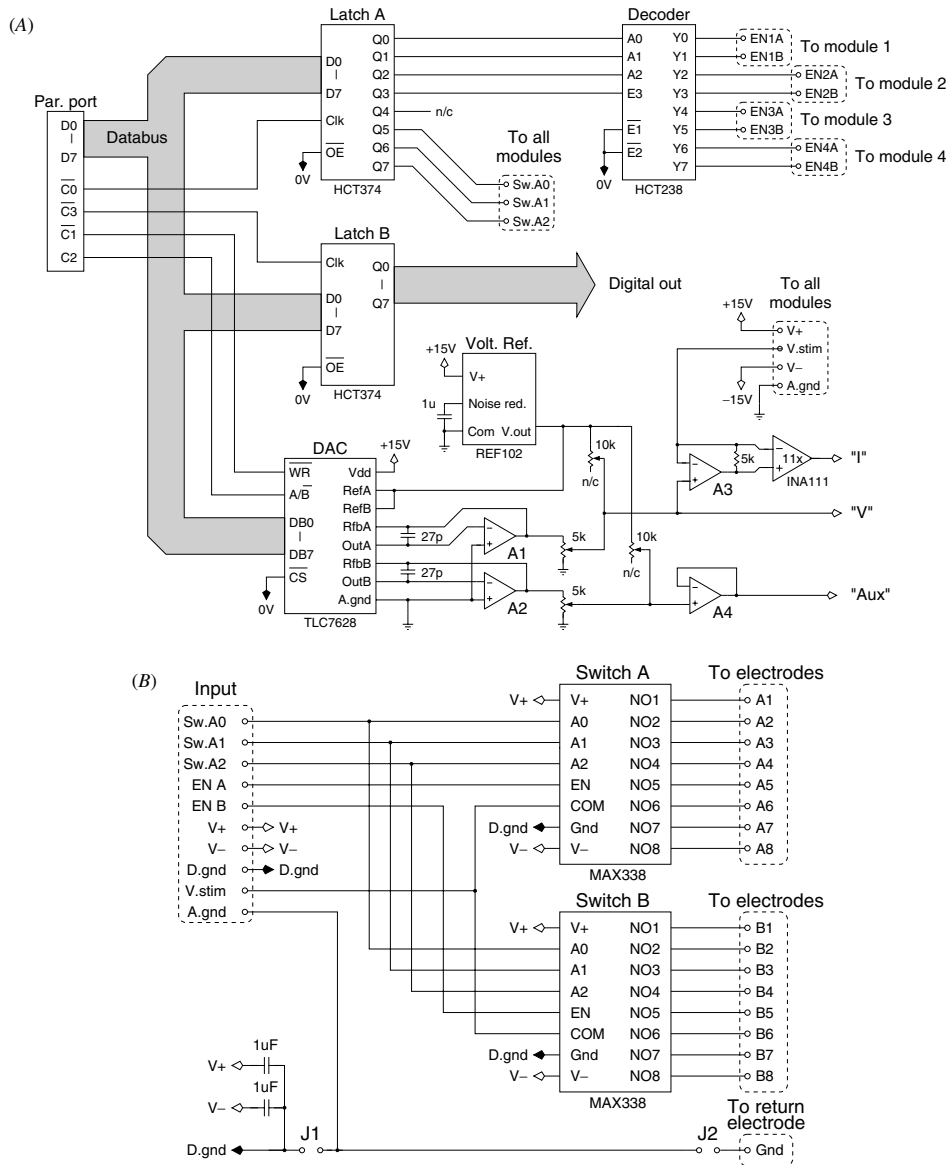
Figure 1. Circuit diagrams. (*A*) Main board. The system is controlled through a standard PC parallel port, and takes power from an external ±15 V supply. Terminals 'V' and 'I' monitor the voltage and current to the currently selected electrode. 'Aux' provides a buffered auxiliary analog output. ⏚ represents digital ground; ⏚ is analog ground. Op-amps A1–A4 are 1/4 LM348 (Fairchild Semiconductor); REF102 is a precision 10 V voltage reference (Texas Instruments); INA111 is a high-speed instrumentation amplifier (Burr-Brown). Other ICs are described in the text. For clarity, the +5 V digital supply and decoupling capacitors have been omitted from this diagram. (*B*) One of four identical modules which deliver stimuli to the MEA. In our set-up, they plug into a set of terminals on the pre-amplifier (MultiChannel Systems) which in turn directly connect to the MEA. Jumpers J$_1$ and J$_2$ can be used to choose different grounding schemes. We leave both open on all four modules. Power lines are connected as indicated by the arrows (⊸ and ⊸).

PCB designs, part lists, and driver and application software are available on request by e-mail to the authors (wagenaar@caltech.edu).

3. Results

3.1. The real-time all-channel stimulator

The RACS provides the following:

- Stimulus outputs for direct connection to 64 electrodes, all driven from a single DAC, with high-quality isolation switches to select stimulation channels with microsecond timing.
- One independently controlled auxiliary analog output channel that may be used, e.g., to provide stimulus markers.
- Eight digital output lines that may be used, e.g., to trigger external equipment.
- Op-amp buffered outputs that allow monitoring the actual voltage and current being delivered to the currently selected electrode.
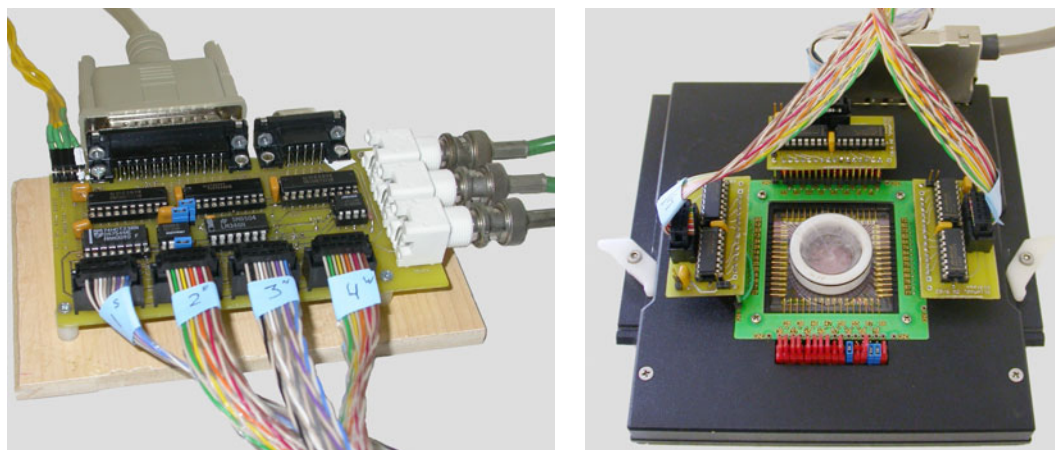
Figure 2. Photographs showing the main board (left) and three modules connected to a MultiChannel Systems pre-amplifier with MEA in the center (right). (The fourth module has been unplugged to allow a better view.) To facilitate stimulation and recording experiments lasting several months, the MEA is sealed with a Teflon membrane which prevents evaporation and infection (Potter and DeMarse 2001).
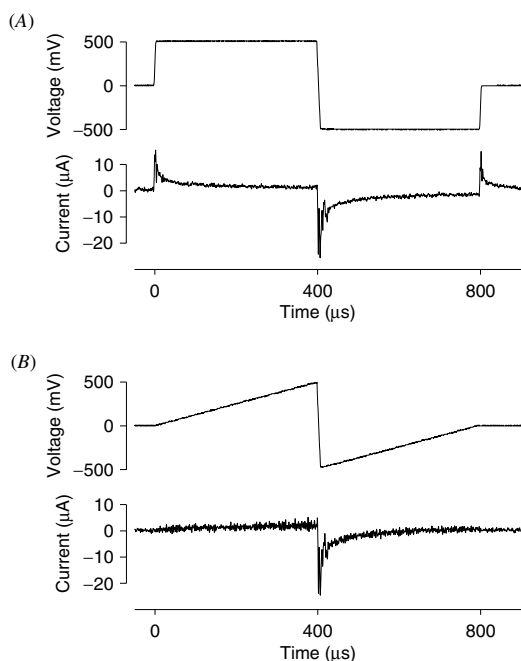

Figure 3. Pulse shapes. (A) Rectangular voltage pulse (top) with current response (bottom). (B) Sawtooth voltage pulse (top) with current response (bottom). Stray currents due to cabling capacitance have been removed from these graphs.

The major anticipated use of the device is to output (multiphasic) rectangular stimulation pulses, but it is possible to construct waveforms of arbitrary shape (figure 3). While the single-DAC design does not allow for truly simultaneous stimulation through more than one electrode, different electrodes can be stimulated with less than 10 μs between stimuli. We wrote software that provides several levels of abstraction of the stimulation hardware. At the lowest level, one controls the switches and DACs directly, stating, e.g., 'at time $t = 500$ ms, switch to channel 37; 50 μs later,

set the DAC to 700 mV; 400 μs later, set the DAC to -700 mV; 400 μs later, set the DAC to 0 mV; 50 μs later, release all switches'. At a higher level, one could specify the same command as 'at time $t = 500$ ms, send a biphasic pulse with amplitude 700 mV and width 400 μs to the electrode at position (6, 3)'. Since we found it convenient to use a special recording channel to mark the time and identity of stimuli, the highest level software automatically provides such markers through the auxiliary analog output.

Stimuli like those shown in figure 3 could be used to evoke neuronal responses through almost any of the electrodes on a densely plated MEA. Stimuli delivered to different electrodes each elicited distinct array-wide spatiotemporal response patterns (figure 4). More details on the efficacy of pulses of various shapes for eliciting action potentials may be found in a separate report (Wagenaar *et al* 2004).

3.2. Benchmarks

We tested the performance of the RACS in several key areas, including noise injection, stimulation artifacts, timing precision and integration with recording hardware. This section describes the results of those tests.

3.2.1. Switching time, real-time control. The timing of individual switching events could be controlled with very high precision: timing accuracy was 0.5 μs RMS, with a worst case deviation of 2.0 μs ($N = 5000$). Smooth stimulation waveforms could be approximated by controlling the DAC output voltage at a maximum rate of 130 kHz.

3.2.2. Noise injection. The RACS did not add a significant amount of noise to the recordings, in contrast to stimulation systems without high-quality isolation switches, which often increase the noise to well above useful levels. With the RACS connected to the MEA, we measured 2.32 $\pm$ 0.28 μV RMS noise (mean $\pm$ sample standard deviation (SSD), $N = 46$
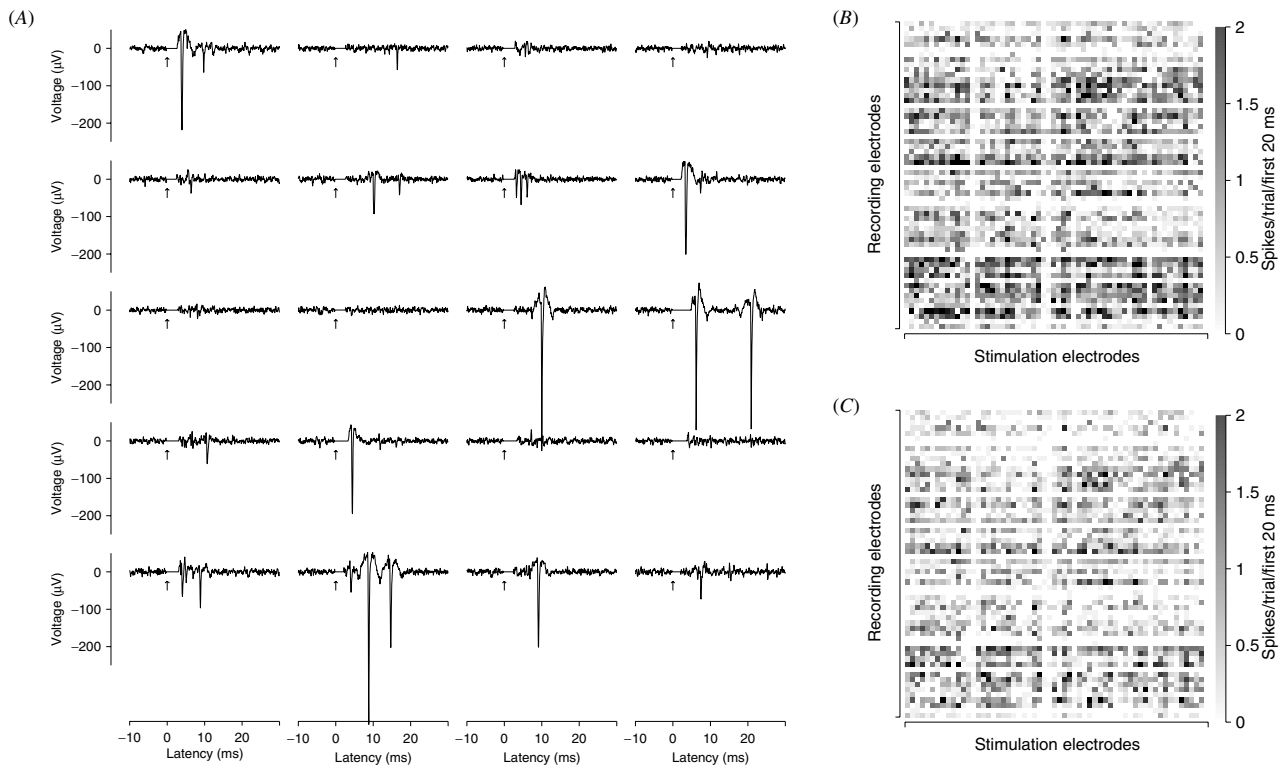
Figure 4. (*A*) Neuronal activity recorded on five selected electrodes (top to bottom) in response to stimulation on four different electrodes (left to right). Arrows indicate time of stimulation. SALPA (Wagenaar and Potter 2002) was used to suppress stimulation artifacts. Examples of recordings with and without artifact suppression may be found in that paper. (*B*) Number of spikes recorded in the first 20 ms after stimulation with biphasic rectangular pulses (as shown in figure 3(*A*)) through 59 different electrodes ($N = 10$ trials/electrode). (The diagonal is white, reflecting the fact that an electrode cannot be used for recording so quickly after stimulation.) (*C*) Same, for sawtooth pulses (as shown in figure 3(*B*)). The pattern is similar, but rectangular pulses elicit 77% more spikes on average.

electrodes) in the frequency range 150–2500 Hz used for spike detection. Without the RACS, the baseline noise was 2.26 ± 0.27 μV RMS on the same electrodes, not significantly different (*t*-test).

3.2.3. Switching and stimulation artifacts. Because stimulation involves voltages between 100 mV and 1 V while recorded neuronal signals are typically around 50 μV, even the most careful design cannot completely prevent stimulation artifacts. Such artifacts can be attributed to two sources: the stimulation hardware itself, and the electrode, which undergoes surface charging and electrochemistry. Both kinds of artifacts may affect the signal recorded from the stimulated electrode, as well as signals from other electrodes in the array.

We measured the switching artifact directly generated by the RACS by closing and opening a stimulation switch while outputting a 0 V signal through the DAC. This caused minor artifacts on the other channels: signals remained within the amplifier's dynamic range throughout the stimulus in >99% of trials, and the absolute value of the artifact 1 ms after the end of the stimulus was 10.6 ± 15.6 μV (mean $\pm$ SSD). These artifacts could be entirely suppressed in software using SALPA (Wagenaar and Potter 2002). The stimulated channel itself did record significant artifacts: in 55% of trials the signal was driven outside of the amplifier's dynamic range (± 683 μV) for 10 ms or more. This artifact is the combined

effect of charge injection by the isolation switch and the fact that the DC electrochemical equilibrium potential of the electrode is not necessarily precisely 0 V, so that a 0 V stimulus may still involve non-zero currents.

The combined artifact of stimulator and electrode caused by actual stimuli is of more direct relevance to research. To measure it, we presented biphasic pulses of 500 mV and 400 μs per phase, as commonly used during experiments. We found that the signal on the stimulated electrode transiently exceeded the amplifier's dynamic range in all cases, for 61 ms on average (figure 5(*A*)). On other channels, the artifact was outside the dynamic range only during the stimulus itself, and had absolute values of 16.8 ± 17.3 μV at 1 ms after the stimulus (figure 5(*B*)). Spikes could be detected within 1–2 ms after the stimulus by using the SALPA artifact suppressor.

3.2.4. Feedback loop time. Together with MEA, pre-amplifier, data-acquisition board and MeaBench software, the RACS forms a feedback loop allowing us to generate stimuli as a function of the observed activity in the culture, as required for our experiments with neurally controlled animats (Potter 2001). Thanks to the open, modular and extensible structure of MeaBench, it took only about ten lines of new code to generate stimuli in response to action potentials recorded through a particular electrode of the MEA, thus closing the feedback
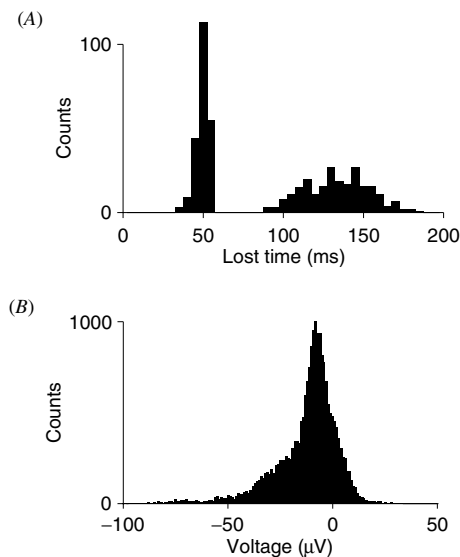
(A)

(B)

Figure 5. Stimulation artifacts for positive-then-negative biphasic stimuli of 0.5 V amplitude, 400 μs duration per phase. (A) Amount of time the stimulated electrode cannot be used for recording because the signal is driven outside the dynamic range of the amplifier. The histogram shows a bimodal distribution, because the recorded signal sometimes swings to the other rail after recovering from the first phase of the artifact. (B) Histogram of artifact sizes on other electrodes, measured 1 ms after the end of stimulation. These artifacts were well inside the dynamic range of the pre-amplifier, and could be suppressed in software.
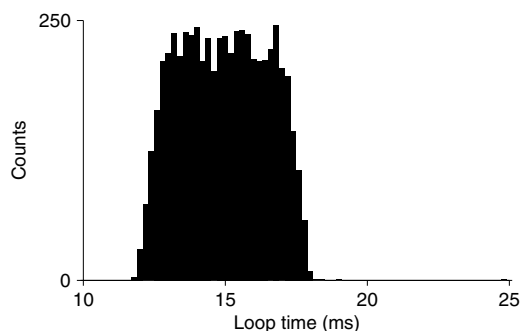

Figure 6. Histogram of loop times for delivering stimuli in response to recorded action potentials. The loop consists of data acquisition, spike detection and identification, and stimulus generation. This quick feedback time makes it possible to construct stimulus sequences as a function of observed neuronal activity with loop delay times equivalent to only a few typical cortical synaptic delays.

loop. We tested the speed of this loop and observed loop times of 12.2–17.7 ms (98% confidence interval), and a worst case of 24.8 ms ($N = 5873$ trials) (figure 6). Even the worst case is easily fast enough to provide feedback on biologically relevant timescales, since it corresponds to only a few typical neuron-to-neuron propagation delays.

4. Discussion

We have described a stimulation system for multi-electrode arrays which interfaces to existing recording systems, and
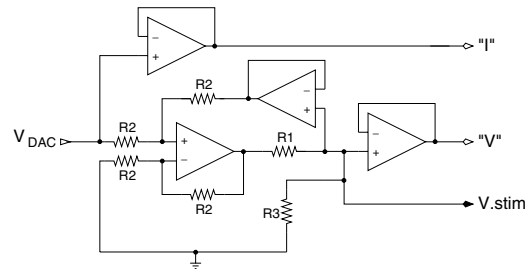
Figure 7. A voltage-to-current convertor to adapt the RACS for current-controlled stimulation. R_1 (10–100 kΩ) converts the voltage to current. R_3 (6.8 MΩ) acts as a shunt to prevent runaway voltages when all switches are off. R_2 are 150 kΩ. Op-amps are 1/4 LM348 as before. Based on a design by Horowitz and Hill (1996).

can be reproduced readily in other labs. While we designed this stimulator for use with MultiChannel Systems pre-amplifiers, it can be adapted to any other recording system that allows direct access to the MEA electrodes. The system could trivially be expanded to handle more electrodes by adding *decoder* chips (top right in figure 1(A)). Furthermore, adaptation for use with *in vivo* multi-electrode probes would involve nothing more major than miniaturizing the modules, probably by replacing the MAX338 switches by their surface-mount versions. For applications where current-controlled stimulation is preferable, the voltage output stage formed by op-amp A3 and instrumentation amplifier INA111 (bottom right in figure 1(A)) can be replaced by a voltage-to-current convertor (figure 7).

The RACS allows stimulation of all electrodes in the array, with arbitrary patterns and rapid switching between stimulation and recording through individual electrodes. Combined with artifact suppression algorithm, SALPA, we could detect spikes as early as 1–2 ms post-stimulation, using any electrode except the stimulated electrode itself. On stimulated electrodes, spikes could be detected after 40–160 ms: as soon as artifacts no longer saturated the pre-amplifier. To further reduce that time, sample-and-hold technology can be employed (Novak and Wheeler 1988). A simpler approach would be to isolate the amplifier from the electrode during stimulation using an additional switch. Unfortunately, we found that this was insufficient to prevent artifacts, most likely because artifacts are primarily due to the electrode slowly returning to its electrochemical equilibrium potential after stimulation. We chose not to use sample-and-hold circuitry here, because that would require an integrated design of stimulation and recording systems (Jimbo *et al* 2003), and one of our goals was to maintain independence between stimulator and recording system designs.

By allowing arbitrary stimulation patterns which can be modified during the course of an experiment, the RACS opens the way for a new experimental paradigm, in which cultures are probed continuously with naturalistic patterns of distributed stimulation. We formed a feedback loop that allowed stimulation in response to recorded action potentials within 15 ms on average, by combining this stimulator with MeaBench data-acquisition software. When cultures are used

as the brain of artificial animals (or 'animats') as in DeMarse *et al* (2001), this fast feedback will allow for much more direct interaction between the animat and its environment, which should result in greatly enhanced performance.

Acknowledgments

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