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A retrofitted neural recording system with a novel stimulation IC to monitor early neural responses from a stimulating electrode

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Abstract

Extracellular electrical stimulation is increasingly used for *in vitro* neural experimentation, including brain slices and cultured cells. Although it is desirable to record directly from the stimulating electrode, relatively high stimulation levels make it extremely difficult to record immediately after the stimulation. We have shown that this is feasible by a stimulation system (analog IC) that includes the feature of active electrode discharge. Here, we piggybacked the new IC onto an existing recording amplifier system, making it possible to record neural responses directly from the stimulating channel as early as 3 ms after the stimulation. We used the retrofitted recording system to stimulate and record from dissociated hippocampal neurons in culture. This new strategy of retrofitting an existing system is a simple but attractive approach for instrumentation designers interested in adding a new feature for extracellular recording without replacing already existing recording systems.

Index Terms

neural stimulation; neural recording; stimulus artifact; multielectrode array; analog VLSI

1. Introduction

Electrical stimulation is a popular method to induce neural responses in experimental and clinical neurophysiology. In many cases, there are separate channels dedicated to neural recordings during the stimulation. Stimulus artifacts interfering with the recording can be suppressed or removed by software based (Blogg and Reid, 1990; Parsa et al., 1998; Hashimoto et al., 2002; Wagenaar and Potter, 2002; Paul and Gnad, 2003; Montgomery et al., 2005; Whittington et al., 2005) or hardware based systems (Freeman, 1971; Roby, 1975; Babb et al., 1978; Walker and Kimura, 1978; Novak and Wheeler, 1988; Curtis et al., 1991; Hentall, 1991; Grieve et al., 2000; Wichmann, 2000; Liang and Lin, 2002; Jimbo et al., 2004).

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Post-stimulus recording from the stimulating electrodes is challenging due to stimulus-induced electrode polarization. This effect is worse when microelectrodes are used for localized stimulation and recording (Patterson and Kesner, 1981). The electrode polarization can overload the high gain recording amplifier and input information is lost during the overload period, which can last more than tens of milliseconds. There have been several attempts to solve this problem. Hentall minimized the stimulus artifact by blanking the recording amplifier during the stimulation and stimulating through a pair of matched glass pipettes under electrolyte injection (Hentall, 1991). He was able to record spikes that appeared 0.5 ms after the stimulation; however, the stimulus level (up to 6 μ A) and the recording amplifier gain (50) were relatively low. Jimbo et al. (2004) reported a multichannel recording and stimulation system that had a sample-and-hold circuitry in the recording amplifiers to blank stimulus pulses while electrodes were held at the pre-stimulation level for stimulation and post-stimulation discharge. By minimizing the electronic switching artifacts and discharging the electrodes for 1 ms after the stimulation, direct neural responses were recorded 3 ~ 4 ms after stimulation from the stimulating electrodes. Recently, we reported an analog VLSI integrated chip (IC) that has multiple stimulators and recording units (Brown et al., 2008). Each unit had a novel programmable electrode discharge circuitry to minimize the stimulus artifact due to electrode polarization.

Here we demonstrate the idea of retrofitting an existing multichannel recording amplifier system with this novel stimulation IC so as to record immediately after the stimulation through the same electrode. The idea underlying the present scheme is that a high gain amplifier output can be controlled to operate in a linear range so that neural information would not be lost by amplifier saturation (overload). As long as the output is not saturated, the input signal can be recovered in the analog or digital domain using various types of filtering techniques. To demonstrate the proof-of-operation, we used cultures of dissociated neurons grown on planar microelectrode arrays, recorded with a commercial multichannel amplifier system, and used the IC for stimulation. Results show that neural recordings are possible as early as 3 ms after the stimulation from a stimulating electrode. This simple idea is attractive to both instrumentation designers and electrophysiologists who are seeking for an easy way to add a new feature to already purchased conventional recording systems.

2. Methods

2.1 Microelectrode arrays and cell culture

Planar microelectrode arrays (30 μ m TiN, spacing 200 μ m) were purchased from Multi Channel Systems (Reutlingen, Germany). They were cleaned with organic solvents (acetone, iso-propanol), and the mixture of poly-D-lysine (MW 70 000 – 150 000, Sigma-Aldrich, 0.1 mg/ml in borate buffer, pH 8.4) and FITC labeled poly-L-lysine (MW 30 000 – 70 000, Sigma-Aldrich, 0.1 mg/ml in 1xPBS, pH 7.4) at the ratio of 4:1 was printed on MEA surface using the micro-contact printing process (see detail in (Nam, 2006)).

Dissected hippocampal tissues (18-day gestation Sprague/Dawley rat hippocampus) were purchased from Brain Bits™ (<http://www.brainbitsllc.com/>). The tissues arrived in a 2 ml tube containing embryonic day 18 hippocampus in B27/Hibernate. This was immediately stored at 4–8 °C until cell plating (typically within 7 days). Tissues were mechanically dissociated and plated in serum-free B27/Neurobasal medium (Invitrogen, Gaithersburg, MD) with 0.5mM glutamine and 25 μ M glutamate at the density of 75–200 cells/mm². Cultures were stored in an incubator at 37 °C, 5% CO₂, and 9% O₂. After 4 days *in vitro* (DIV), the medium was changed to serum free B27/Neurobasal medium with 0.5 mM glutamine. Thereafter, half of the medium was changed weekly. All animal procedures were done in accordance with approved animal use protocols at the University of Illinois. An MEA MEM cover (ALA Scientific Instruments

Inc., Westbury, NY) was installed to decrease water evaporation and minimize contamination during the experiment outside the incubator. Data shown here were collected at 70 DIV.

2.2 Neural recording and stimulation

The MEA was connected to an MEA1060 amplifier (Gain 1200, 10 Hz – 3 kHz, Multi Channel Systems, Reutlingen, Germany) and the stimulation IC was directly connected to the amplifier input node. Therefore, the MEA1060 amplifier's input was connected to both the electrode and the stimulation IC. When there was no stimulation, the IC was effectively open-circuit so that it did not affect the recording quality of the MEA1060 amplifier.

The stimulation IC operates in three successive modes: electrode tracking mode, stimulation mode, and discharge mode. Under normal recording conditions, the stimulation IC tracks the electrode (input node voltage of the amplifier, node A in Fig. 1a) storing the average electrode voltage in C_{in} (Fig. 1b). The top amplifier of the IC, together with C_{in} , C_f and R_f , form a high-pass gain stage, storing the average electrode voltage in C_{in} . The input impedance is mainly determined by C_{in} (8 pF) and it is comparable to the input capacitance of the existing recording amplifier. Its loading effect on the recording amplifier would not affect the signal attenuation since the electrode impedance is much lower than the input impedances (~ 30 k Ω at 1 kHz). R_f is adjustable to tune the low-cut frequency of the amplifier and its calculated value is about 20 G Ω for 200 Hz ($C_f = 40$ fF).

During the stimulation mode the tracking circuitry is turned off and a voltage-controlled, positive-first biphasic pulse is delivered through a stimulation buffer. After the stimulation, the stimulation buffer is disabled and the feedback discharge circuitry is enabled. The bottom transconductance amplifier in Fig. 1b, connected directly to the electrode, is activated only during the discharge phase. The discharge amplifier forces the electrode voltage back to its pre-stimulation level. In this mode, the discharge circuitry behaves as a low value resistor (R_{disch} in Fig. 1c) connected to the pre-stimulation average electrode voltage. R_{disch} , which can be as low as 10 Ω in this implementation, is inversely proportional to the maximum discharge current ($G_{m_{disch}}$) and the gain of the top branch (see detail in Brown et al., 2008). For neural stimulation, we routinely used magnitudes in the range of 0.1 ~ 0.8 V with pulse widths of 100 ~ 200 μ s. The IC was controlled by a dedicated microcontroller communicating to a custom graphic user interface program built in Matlab 7.0.

The discharge phase was controlled by specifying the discharge time and maximum current for two phases (phase 1 and phase 2, see Fig. 1d for an example). Discharge times correspond to the on-time of the discharge circuitry, and the maximum discharge current is set through the discharge amplifier tail current ($G_{m_{disch}}$ in Fig. 1c).

Amplified raw data was digitized at 25 kHz and stored through MC Rack software (Multi Channel Systems) for post analysis. Post analysis was done by either a custom Matlab program or MC Rack.

3. Results

3.1 Suppressed artifact at the amplifier output

Figure 2 shows the recording amplifier (gain 1200, bandwidth 10 – 3000 Hz) output with and without the discharge phase. Without the discharge phase, the amplifier output suffered from long lasting amplifier saturation (about 45 ms in Fig. 2a). Even a relatively small stimulus (0.5 V) easily saturated the amplifier output. While the amplifier output is saturated, the amplifier output remained at the maximum analog-to-digital converter input range (± 4 V). The corresponding input-referred range is ± 3.3 mV. With the discharge phases, the amplifier output remained in its linear range, and neural signals could be monitored immediately after the

stimulation (Fig. 2b). Figure 1d shows another example of saturation free recordings with a relatively strong stimulus pulse (0.7 V) followed by two discharge phases (phase 1: 0.5 ms @ 81 μ A, phase 2: 3.0 ms @ 3.2 μ A).

Although the amplifier output was prevented from reaching the power rail by controlling the electrode voltage (node A in Fig. 1a), it still suffered from a transient filter response. As can be seen in Fig 2b, small neural spikes (filled arrow heads, a few tens to hundreds of microvolts) are superposed on a larger transient signal. This transient baseline shift, which resembles the impulse response of the amplifier filter, can be removed or minimized using any of several methods. First, a different hardware setting could minimize the filter response. A narrower bandwidth filter setting (300 – 3000 Hz) would stabilize the baseline 15 times faster than the current setting (10 – 3000 Hz). Second, digitized data can be postprocessed by a proper digital filtering technique. For example, a 2nd order Butterworth high-pass filter (cut-off frequency 300 Hz) was used to remove the long lasting slow signal in Fig. 3. A baseline subtraction method based on a local curve fitting technique could also be used to remove the transient signal (Wagenaar, Potter, 2002).

3.2 Early recording after stimulation – TiN 30 μ m electrode

Figure 3 shows electrical stimulation induced neural responses from a stimulation channel. The experiment was done with a dissociated hippocampal culture at 70 days *in vitro*. Stimulation pulse parameters and discharge times were tuned to record as early as 2 ms. Digitized traces were filtered with a 2nd order Butterworth high-pass filter (cut-off frequency: 300 Hz). There is a trial-wise consistent sharp downward negative peak corresponding to the filter response. Immediately after the filter response, stimulation induced biological spikes could be monitored beginning at approximately 4 ms (grey shaded region).

4. Discussion and Conclusions

4.1 System design

This work successfully demonstrated the idea that an early post-stimulus recording from the stimulating electrode is possible through a conventional high gain recording amplifier as long as the electrode polarization effect is properly controlled by external circuitry. The results imply that it is not necessary to redesign the whole system to accomplish post-stimulus recording from the stimulation electrode as reported by others (Jimbo et al., 2004). The stimulation and discharge circuitry were piggybacked on an existing recording system, and neural responses were monitored from the stimulating electrode immediately after the stimulation. This would be a convenient and cost-effective approach for those researchers who have already invested in multichannel recording systems with multiple amplifiers but are still seeking for a way to record directly after stimulation.

The high sensitivity of the electrode artifact to circuit parasitic elements and imperfections, combined with the stability constraints, makes the implementation of this circuitry as discrete components impractical, although those willing to attempt it can refer to Brown et al. for some of the considerations that have to be followed.

4.2 Discharge circuitry

Electrical stimulation generally induces electrode polarization, and tens or hundreds of milliseconds are necessary to fully discharge the electrode (Merrill et al., 2005). Relatively large polarizations can be generated (a few tenths of a volt) for microelectrodes that are commonly used for neural recording *in vivo* or *in vitro*. Post-stimulus recording from the stimulating microelectrodes can be problematic unless the polarized electrode is sufficiently discharged not to overload the high gain amplifier. In the present work, the added discharge

circuitry accelerated the recovery process from the electrode polarization so that the electrode-electrolyte interface voltage would not overload the amplifier. As long as the amplifier is not overloaded, neural signals (input information) are available at the amplifier output and they can be recovered by other filtering techniques (Wagenaar and Potter, 2002).

4.3 Performance limitation

Discharge time had to be tuned to obtain the earliest possible recordings. The optimal discharge time limited the earliest recording time after the stimulation. As the discharge time increased, the amplifier output became more stable. In some electrodes, it was possible to monitor neural signals as early as 2 ms after the stimulation. The typical recordable time after the stimulation was 5 ~ 8 ms. In some cases, discharge time had to be set over 5 ms to obtain saturation free recordings, which implied that neural responses within 5 ms were not recordable; however, even suboptimal discharge conditions resulted in much smaller, shorter artifacts than the no-discharge condition (Fig. 2a). This tuning process can be automated to minimize manual operation.

The question arises as to whether this concept applies to other manufacturers' amplifiers and other designs for stimulus artifact suppression. The amplifier used in this work had an input range of ± 3 mV and output swing of ± 5 V. Results with other systems should be similar provided the input range and the frequency response are similar. If amplifier gain is too high, it will limit the input linear range, requiring the electrode discharge time to be increased to avoid amplifier saturation. The earliest recordable time is fixed by the required discharge time. For advanced recording systems integrated with electronic switches or sample-and-holds for the purpose of artifact suppression (Novak and Wheeler, 1988) or automated multichannel stimulation (Wagenaar and Potter, 2004), the piggybacking idea presented here can also be used without any modification in the systems. These systems operate in such a way that electronic switches are triggered to disconnect the electrodes from the amplifier during the stimulation and to reconnect them after the stimulation. As long as the trigger signals are properly synchronized with the stimulation IC operations, similar results are expected from these systems; therefore, the piggybacking idea can be generalized to other type of systems.

4.4 Future application

Direct recordings from the stimulating channels would be beneficial to understand neural activity near stimulating electrodes, especially when no recording electrodes are available close to the electrodes. This provides valuable information about the neural interface between an electrode and neural tissue. The neural response would be different depending on which part (dendrite, axon, soma) of the neuron is stimulated. Accessing the excitability of neural tissues would be useful in clinical and experimental neurophysiology.

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(a)

(b)

(c)

Figure 1.

Schematic of stimulation and recording setup. (a) Dotted parts are a piggybacked stimulation and discharge system implemented by the analog IC. The IC includes a controlled voltage pulse generator (stimulation buffer) and a feedback discharge circuitry. Amp: Existing recording system (MEA 1060 system). Z_e : electrode-electrolyte interface impedance. V_{cell} : Extracellular field potential generated by nearby neurons. (b) Schematic of the electrode discharge circuitry. See texts for details. (c) Equivalent circuit during the linear portion of the discharge phase. (d) An amplifier output during the stimulation and discharge. Two discharge phases (Dsch, 0.5 ms and 3.0 ms) following a biphasic stimulus (± 0.7 V, 100 μ s pulse width). The arrow shows the end of first discharge phase (0.5 ms). Scale bar: 1 mV, 1 ms.

Figure 2.

An amplifier output with and without an electrode discharge phase after the stimulation phase. (a) Without the discharge phase, the amplifier output suffers from long lasting output saturation (recording possible only after 45 ms in this case, arrow). (b) With two brief discharge phases (0.5 ms and 1.5 ms respectively), recording is possible after 3 ~ 4 ms (arrow). Stimulation parameters: positive first biphasic voltage pulse, ± 0.5 V, pulse width 200 μ s / phase). The blank arrow head shows the stimulation. Filled arrow heads show neural spikes. Scale bar: 500 μ V, 10 ms.

Figure 3.

Stimulation and recording from a stimulating electrode. Fifteen traces aligned to the stimulus are shown here. The arrow indicates the electrical stimulus and the gray shaded region shows early time-locked spikes around 4 ~ 5 ms. The stimulus was a positive first imbalanced biphasic voltage pulse (+ 0.4 V / -1.0 V, pulse width 100 μ s / phase). There are sharp non-biological transient peaks at the end of the discharge phase at 1.7 ms. The discharge time was 1.5 ms. The original recording filter setting was 10 – 3000 Hz and post-filtering was done by a 2nd order Butterworth high-pass filter (f_c = 300 Hz). Scale bar: 5 ms, 500 μ V.