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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.

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 Gnadt, 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).

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 μA) 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

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(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 μm TiN, spacing 200 μm) were purchased from Multi Channel Systems (Reutlingen, Germany). They were cleaned with organic solvents (acetone, isopropanol), 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 1× PBS, pH 7.4) at the ratio of 4:1 was printed on MEA surface using the micro-contact printing process (see detail in Nam et al., 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 0.5 mM glutamine and 25 μM glutamate at the density of 75–200 cells/mm^2. Cultures were stored in an incubator at 37 °C, 5% CO_2, and 9%O_2. 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 to 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. 1(a)) storing the average electrode voltage in C_{in} (Fig. 1(b)). 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 kΩ at 1 kHz). R_f is adjustable to tune the low-cut frequency of the amplifier and its calculated value is about 20 GΩ for 200 Hz (C_f = 40 fF).

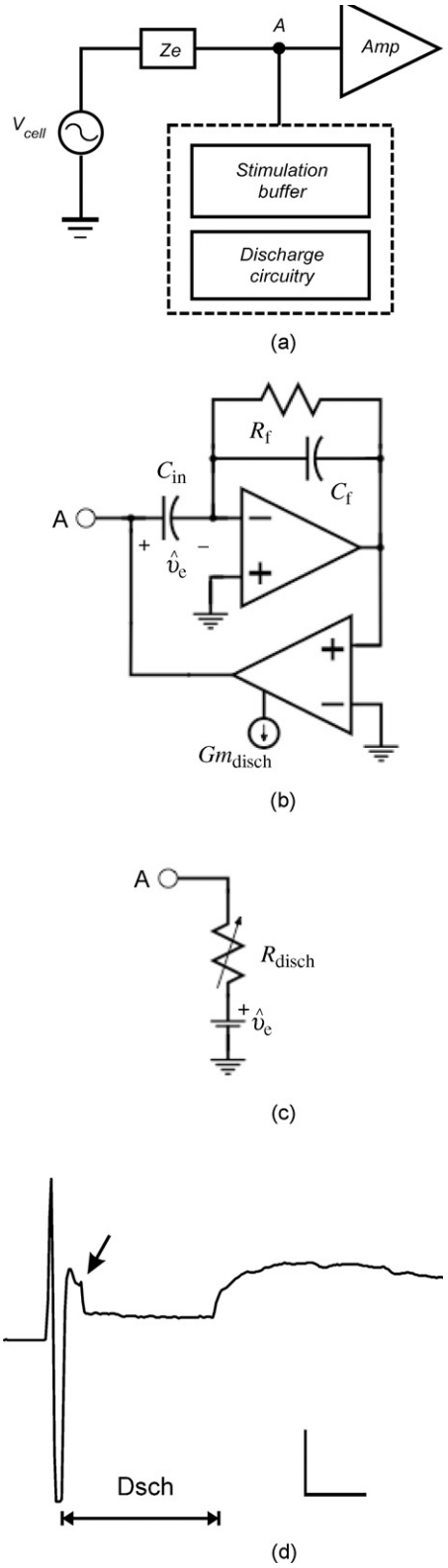

Fig. 1. Schematic of stimulation and recording setup. (a) Dotted parts are a piggybacked stimulation and discharge system implemented by the analog IC. The IC includes a controlled voltage pulse generator (stimulation buffer) and a feedback discharge circuitry. Amp: Existing recording system (MEA 1060 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 μs pulse width). The arrow shows the end of first discharge phase (0.5 ms). Scale bar: 1 mV and 1 ms.