

PORTABLE STAND-ALONE INSTRUMENTATION FOR INTRACELLULAR *IN VITRO* RECORDINGS

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Abstract - A portable stand-alone system for continuous intracellular recordings *in vitro* is presented here. An off-the-shelf operational amplifier is used to illustrate the feasibility and reliability of the proposed method. The amplifier is powered by two coin batteries and directly connected to a traditional intracellular glass electrode. The brain of the sea slug *Tritonia diomedea* is used as the experimental model. By simultaneously impaling one neuron using two electrodes, and recording with two independent systems, namely, a commercial intracellular amplifier and the portable stand-alone circuit, intracellular signals are acquired for more than 20 hours without affecting cell spontaneous spiking patterns. The implemented system presents signal-to-noise ratio higher than 30. Signals recorded with the stand-alone circuit are shown to reliably reproduce subthreshold activity after a second order low-pass filter stage. Possible applications for this system include multichannel intracellular MEMS-based arrays and portable implantable electronics for drug-delivery.

Keywords – intracellular recordings, stand-alone system, *Tritonia diomedea*, implantable electronics.

I. INTRODUCTION

Several multichannel extracellular electrodes have been developed over the last three decades [1],[2], aiming mainly at basic neural research for *in vitro* as well as *in vivo* experiments [3],[4]. Despite their promising multiple application areas such as drug delivery, alternative sensing techniques, drug discovery, and controllable implantable systems, issues such as manipulation of cells (*in vitro*) or of arrays (*in vivo*) and control of the biological environment are still not completely understood and thus impede the widespread application of multi-microelectrode arrays. Moreover, the complex origin of multiunit signals prevent a straightforward implementation of the necessary instrumentation to acquire, preprocess and store data [5]. The main reason for the difficulty in developing appropriate instrumentation relies not on the number of electrodes, but on the extracellular signals themselves, which require elaborate analysis for identification of spiking units [6]. One possible way of enhancing the ability to probe further into cellular behavior is the development of micromachined intracellular arrays of electrodes [7]. The advantages of such arrays are obvious by contraposition to the extracellular electrodes: signal strength, stimulation control, finer analysis options regarding subthreshold activity.

In order to make use of implantable intracellular electrodes a necessary step is to be able to record signals by means of a portable, stand-alone system which eventually is intended to be fully integrated on the chip with an intracellular electrode. As a proof of concept, in this paper a commercial miniature operational amplifier is used in experiments showing reliable intracellular recordings from *in vitro* preparations.

The sea slug *Tritonia diomedea* was chosen as the experimental model for reasons elaborated below. Invertebrates present bigger cell somata than vertebrates, in the case of *Tritonia*, neuronal diameters can easily reach 100 μ m. The ganglionic structure of the brain, similar to other mollusks such as *Aplysia*, provides a simple system in which one can not only identify and repeatedly target the same specific neurons, but also effortlessly extract the whole brain and keep it in a functional state over a period of several hours or days [8]. Furthermore, initial tests performed with chip implantation [9] show that *Tritonia* can survive a two-week implantation period without major effects on the neuronal network or other pathological reactions. This is thus an adequate experimental model for *in vitro* and *in vivo* testing of intracellular implantable systems.

II. METHODOLOGY

An off-the-shelf operational amplifier, AD8602 (Analog Devices), SOIC (small-outline integrated circuit) package, was connected as a voltage follower and mounted inside a glass tube. The glass tube was held by a micromanipulator (WP-I Instruments). This rail-to-rail amplifier presents a bias current of 0.2pA, what ultimately enables the direct coupling to the intracellular space without either charging the cell or instantly depolarizing the membrane. Pilot experiments had been performed with several other operational amplifiers presenting similar characteristics as to noise, power consumption, and CMRR (common-mode rejection ratio), but none of them could be successfully used for long-term neuronal recordings. Even with bias currents as low as hundreds of pA the cell is driven to an unstable state over time. For comparison, the AD623 (Analog Devices), a common instrumentation amplifier, with an input bias current of 17nA, immediately depolarized the cell after implementation.

The positive input of the AD8602 was directly hooked up to the Ag/AgCl wire from the glass electrode, as it is schematically represented in Figure 1, by means of conductive silver paint (Circuit Works[®], CW2400). No external resistors have been used. Wires have been directly soldered to the integrated circuit lids with conductive nickel print (CG[®] Electronics). The recording setup thus comprises the traditional glass electrode (impedance in the order of 10M Ω) filled with a KCl solution (3M), the silver-chloride wire, one amplifier and the power supply. For the second set of experiments two coin batteries (Energizer CR1225, 3V, 12.5mm diameter) were used instead of the power supply, turning the recording setup into a lighter and stand-alone version of the first setup. Each battery weighs 0.9g and has a capacity of 50mAh. They are both mounted on the glass tube containing the amplifier. This arrangement enables the experiment to be run for at least 71 hours, considering that the supply current for the SOIC amplifier used is of 700 μ A.

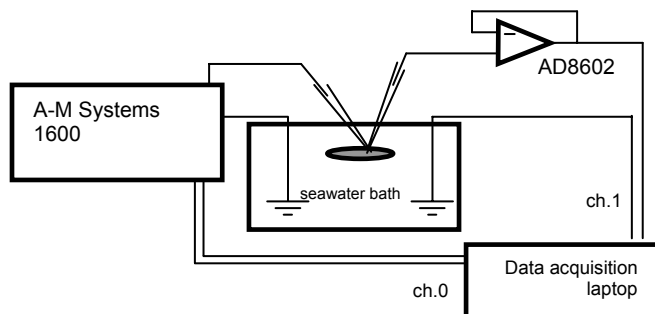


Figure 1. Schematic representation of the experimental setup. Two glass electrodes impale the same cell in the isolated brain of the sea slug *Tritonia diomedea*. The intracellular signal is simultaneously acquired by the commercial amplifier (Neuroprobe Amplifier) and the AD8602 connected as a voltage follower. Grounding is provided by a Ag/AgCl wire in the bath. The data acquisition system comprises a PCMCIA DAQ board and a laptop computer.

As a basis for signal comparison the Neuroprobe Amplifier (Model 1600, A-M Systems[™]) was used simultaneously during the experiments, connected to one intracellular electrode independently of the stand-alone system. Recording was performed in parallel by both systems.

Tritonia diomedea was prepared as previously described [8]. Briefly, the animal was cut open, the brain isolated by cutting the nerves, pinned down onto a sylgard-base dish, and finally the cells were exposed by dissecting away the overlying connective tissue. The brain was maintained in natural seawater for up to three days. This same arrangement can be used with the animal being held still in seawater, the skin open, and the cell impaled without the nerves being cut [10].

The right pleural cell (RPI1) was identified based on location relative to other cells, size and color [11]. This neuron was simultaneously impaled by two glass electrodes. The bath was grounded using an Ag/AgCl wire connected to both systems.

Signals were acquired by means of a PCMCIA DAQ board (AD16E4, National Instruments[®]) connected to a laptop computer. A virtual instrument was developed in LabVIEW[®] 6.1 (National Instruments[®]) in order to record signals from the two channels simultaneously at a sampling rate of 1kHz/channel, and to count spikes during acquisition. An example of a recording is illustrated in Figure 2, where the identified action potentials are also presented. This figure shows the decreasing spike rate along time. This behavior was also verified in control experiments (data not shown).

Double recordings were compared to control experiments in which only the commercial system was used. Neurons were stimulated by current application with the Neuroprobe Amplifier. Offline analysis such as filtering and interspike interval quantification were performed with Matlab[®] (Mathworks).



Figure 2. Example of intracellular spontaneous action potentials continuously recorded over 17 hours. Virtual instrument extracts timestamps for identified spikes, filters and subsamples signals. Scale bars stand for 20mV (vertical) and 30min (horizontal). Bottom trace indicates identified spikes. This signal is further used for interspike interval evaluation.

III. RESULTS

Recordings obtained with the miniaturized circuit are presented here, along with the control signals which were used not only to validate the proposed system but also to point out which optimization parameters have to be taken into account for the development of intracellular implantable MEMS and associated electronics.

Figure 3 shows an example of signals recorded from both electrodes. Although the noise level from the operational amplifier is high (20mVpp), as it is powered by a regulated power supply, action potentials are clearly identifiable, as it is illustrated here, with a signal-to-noise ratio of 5. In this case, measured amplitudes from both sources agree to within 10%. The discrepancy is due to the fact that the baseline variability of the operational amplifier set-up is higher than the commercial system. The action potential form and time course is the same for both systems (data not shown).

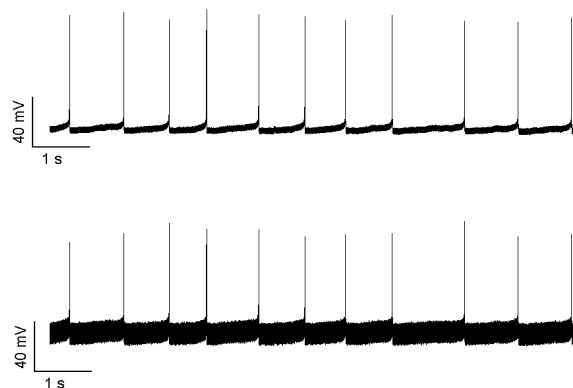


Figure 3. Spontaneous activity recorded from the right pleural cell (raw data). Upper signal refers to the commercial amplifying unit (A-M Systems™ 1600); measured system noise: 1mVpp. Lower trace shows recording from the operational amplifier powered by a regulated power supply; measured noise: 20mVpp.

Cells were stimulated under double recording conditions. Although the amplifier could have been used as a stimulation output stage, stimuli presented here were performed with current application by means of the A-M Systems setup. As Figure 4 shows, during the depolarization as recorded by the commercial system (channel a), the operational amplifier trace (channel b) does not reproduce the artifact. However, action potentials are present in both signals, as well as postsynaptic potentials. This phenomenon has two possible interpretations: either the initial forced depolarization of the membrane as triggered by the applied current is a local occurrence from the perspective of the soma, and as such would not be sensed by the second electrode, or the

capacitive coupling between both electrodes acts as a low-pass, thus precluding higher frequency signals from being recorded. In order to test for the second hypothesis without the occurrence of a spike, a hyperpolarizing current was applied. As a result the signal on the second electrode still shows a small depression (Figure 4), pointing towards the capacitive coupling hypothesis.

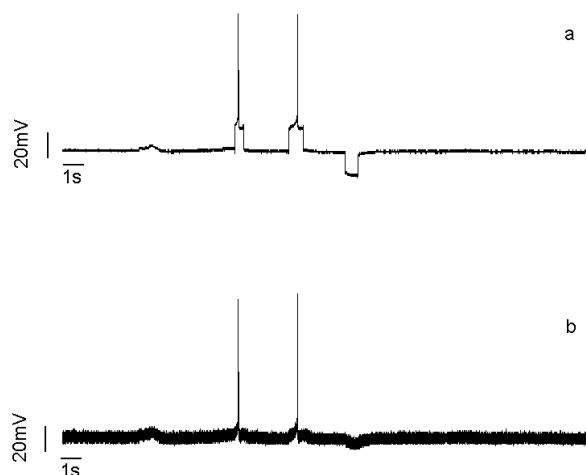


Figure 4. Stimulated action potentials by current application. (a) Impaled cell is stimulated by 1nA through the A-M Systems Neuroprobe Amplifier. (b) Lower trace shows recording from stand-alone system. Post-synaptic potentials are identifiable in both traces, although stimulation artifact is not visible in the stand-alone system.

In order to be able to detect postsynaptic potentials and to turn the system into a stand-alone unit, coin batteries were used to power the AD8602. This setup was continuously used for 20 hours, and the cell was spontaneously active during the whole experiment, allowing for recordings without external stimuli. Figure 5 presents an example of a raw signal acquired with this setup. Noise level decreases to 3mVpp. With the proposed system, small amplitude signals are identifiable between spikes (see inset in Figure 5), strictly following the signal recorded with the commercial amplifier. In this case, spike amplitudes agree to within 2%.

Once the recorded signal is stored, usual signal processing techniques may be applied and validated as for isolation and identification of postsynaptic potentials, along with the determination of spiking frequency over long recording periods. The ability to reliably record subthreshold oscillations is particularly interesting and specific to intracellular recordings, as this is virtually impossible in the case of extracellular microelectrode arrays.

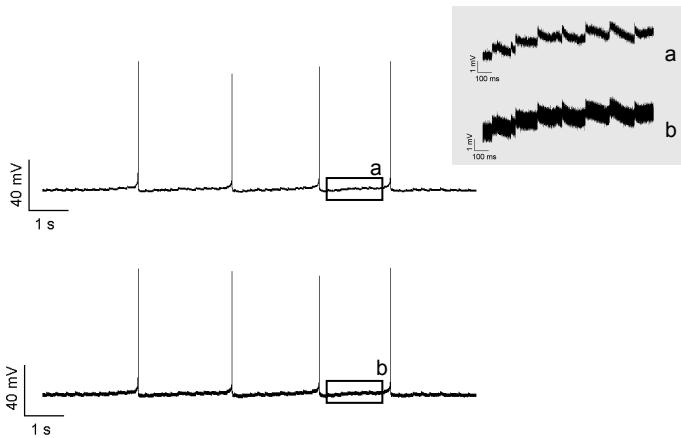


Figure 5. Raw intracellular recordings show comparable performance for action potentials and subthreshold signals in both systems. Upper graph shows signal from the Neuroprobe Amplifier (system noise: 1mVpp). Lower graph shows signal from the operational amplifier powered by two coin batteries; measured noise: 3mVpp. Inset shows postsynaptic potentials as recorded from (a) Neuroprobe Amplifier and (b) from operational amplifier.

Due to the noise inherent to the stand-alone circuit and to the connections to the data acquisition board, the SNR for these potentials drops significantly. However, the targeted signals contain a low frequency component easily filtered out of the noisy raw signal. An example of this process is presented in Figure 6, where the illustrated traces show two raw signals and one low-pass filtered region where there is no spike. The activity represented in this figure contains thus solely subthreshold potentials, which are readily identified. The filter used in this case was a second order, low-pass Butterworth filter [12], with a cutoff frequency of 100Hz. The tradeoff of any filtering procedure comes out in the form of loss of spike definition. The action potential itself presents a higher frequency content than the interspike activity. By filtering the signal, action potentials are clipped and the original form is lost (data not shown). This leads to an alternative way of treating recorded intracellular data, e.g., a hybrid solution in which timestamps of spike occurrence are recorded, along with a typical spike form, for the sake of identifying cellular activity, and a filtered signal, in order to account for the synaptic inputs.

IV. DISCUSSION

While the voltage follower configuration allows for low-noise recordings, and easy connections to be made inside a glass tube, within the same chip it is possible to mount a second amplification stage using the other available amplifier. The drawback of implementing a second stage is the power

consumption, which would decrease the life time of the stand-alone configuration to less than 50% of the initial value. Moreover, most analog-to-digital converters would reliably detect the intracellular action potentials. Although the subthreshold activity detection would not be easily implemented in a miniaturized circuit, as it has been shown above, with the addition of passive filters the signal can be recovered and postsynaptic potentials identified.

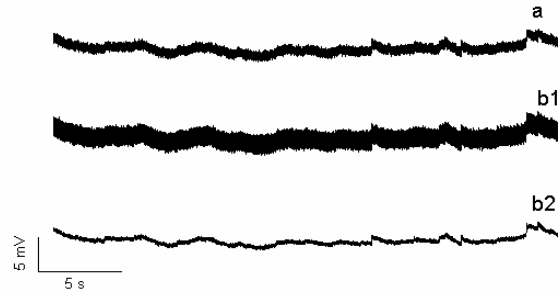


Figure 6. Representative subthreshold activity demonstrated by raw and filtered signals. The trace in (a) was obtained with the commercial system, whereas the trace in (b1) was recorded with the AD8602 setup. Filtering has been applied to the signal in (b1), resulting in trace (b2). The three signals shown are to scale (refer to bottom scale bars). A second-order Butterworth filter was used, with a cutoff frequency of 100Hz. Sampling rate was 1kHz. Note that the features from curve (a) are clearly visible in (b2), although only slightly recognizable in (b1).

Although the experiments reported here deal with the *in vitro* brain, because of the particular experimental model chosen, the *in vivo* testing is straightforward in what concerns the animal preparation. These slugs can be cut open and heal in a short period of time [9], the whole nervous system, located right underneath the skin [11], is easily accessible, and an adult slug can weigh 250g. In addition to that, they can be sustained in tanks containing seawater for periods of weeks or months, providing an ideal experimental model for studying the relationship between the nervous system and behavioral tasks while simultaneously monitoring the performance of engineered systems.

V. CONCLUSIONS

The presented method of acquiring intracellular signals with mini-circuits and on-board power is modular and scalable, and is promising for the development of implantable intracellular electrode arrays and computers. This solution also overcomes the lack of spatial and temporal resolution typical of extracellular microelectrodes. Future experiments will focus on the development of a stand-alone recording system based on a PSoC (programmable system-on-chip) design and on implant procedures for invertebrates.

Previous studies on *Tritonia diomedea* have pointed to interesting issues related to the nervous system of this particular animal model, such as magnetic field induced responses [13], relationship between behavioral tasks and spiking of specific identified cells [10]. By developing adequate implantable and stand-alone instrumentation we intend to increase our ability to investigate neural connections and cellular-to-behavioral relationships and to enlarge the spectrum of available tools with which one can tackle questions posed by *in vitro* as well as *in vivo* models.

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