Simultaneous Stimulation and Recording of Cardiac Depolarization Enabled by High-Frequency Stimulation

Laurent Giovangrandi, *Member, IEEE*

***Abstract*— High-frequency stimulation techniques have been recently proposed for the pacing and control of excitability of cardiac tissues. This paper introduces a system designed specifically for such stimulation, and demonstrates the unique ability to record depolarization events on the same electrode used for stimulation, *during* the stimulus. Experimental results with HL-1 cardiomyocytes are presented, highlighting key concepts enabled by this system, such as direct strength-duration relationship measurement and beat-to-beat stimulation threshold monitoring following pacing onset or pharmacological modulation.**

I. Introduction

Electrical stimulation is the earliest historic form of electrophysiology experimentation, and is now routinely used clinically in cardiac pacemakers, neuronal stimulators for pain or Parkinson's control, transcranial magnetic stimulation (TMS) and many other tissue stimulators. Although often used in conjunction with electrical recording of cellular activity, the typically large difference in amplitude between the cellular signals (microvolts to millivolts) and the electrical stimuli (volts) precludes simultaneous stimulation and observation of evoked potentials at the same location. Instead, recording of evoked potentials is typically performed distal to the stimulation electrode or starting after a blanking period, in both cases missing the response of the tissue directly stimulated. The spatial or temporal separation is typically required because of saturation of the amplifier facing the large stimuli, and slow electronic (amplifier) and electrochemical (electrode) recovery post-stimulus.

Many artifact mitigation strategies have been developed over time, including blanking circuits (decoupling of amplifier during stimulation), charge balancing circuits, signal processing (filtering, template subtraction), pulse shaping and electrode positioning (see [1-3] for some examples). However, none of these approaches have made recording during stimulation possible. Using two different modalities for stimulation and recording (such as optical stimulation offered by optogenetics [4] or light stimulation [5]) remains the most effective way to achieve this, although at the price of a much more complex system.

A novel stimulation technique based on high-frequency (HF) bursts of alternating current was recently proposed by the authors [6]. This technique relies on bursts of bipolar square waves at kilohertz frequencies. It was shown that this type of stimulation provides several advantages over traditional monophasic or biphasic pulses, in particular lower applied voltages, and separation of stimulus and native electrical activity in the frequency domain. These two characteristics enabled a reduction of the stimulus artifacts below the amplitude of evoked action potentials (APs) in HL-1 cardiomyocyte cultures. Simultaneous stimulation and recording were also demonstrated, albeit on separate electrodes due to limitations of the amplifier used at the time.

This work extends these results to simultaneous electrical stimulation and recording of cardiac cells on the same electrode, allowing, for the first time to our knowledge, the recording of the cell activity during its own stimulation, all in the electrical domain. The system used (HF stimulator, amplifier), as well as several examples and applications are presented.

II. Methods

A. Electrophysiology Instrumentation

In order to take full advantage of the frequency separation offered by HF stimulation, a custom system was developed, consisting of a fast (>50 kHz bandwidth), high-pass current source stimulator and a band-pass amplifier capable of filtering out the large HF stimuli. The system was designed primarily for cardiac stimulation, with a recording bandwidth of 1.5 kHz and a typical HF stimulation frequency of 5 kHz.

The current source was designed around a conventional improved Howland current source [7], boosted with a fast unity-gain buffer (BUF634, Texas Instruments, Dallas, TX) to provide up to 50 mA of stimulation (±15V compliance).

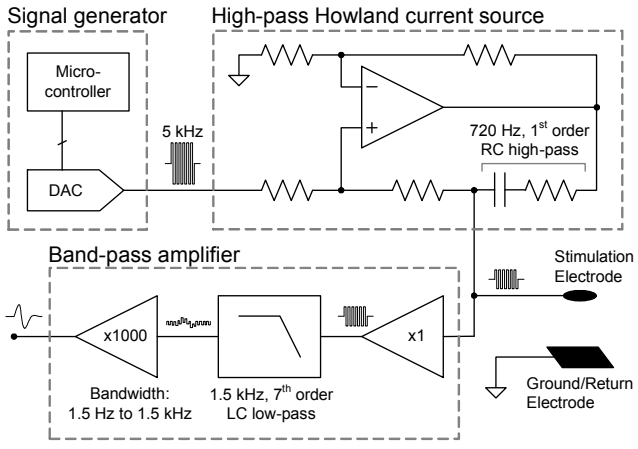

Figure 1. High-level schematics of the custom HF electrophysiology system, consisting of a high-pass HF current source stimulator (top) and a band-pass amplifier with a steep low-pass roll-off to filter out the HF stimuli without degradation of physiological signals.

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L. Giovangrandi is with the Electrical Engineering Department, Stanford University, Stanford, CA 94305, USA. (e-mail: giovan@stanford.edu)

Special considerations regarding the current noise of the source were taken, as this noise will interact with the load to generate additional voltage noise. In particular, the 1/f noise of the current source, combined with the $1/f^{\alpha}$ ($0.5 < \alpha < 1$) behavior of typical metal electrode-electrolyte impedances, generates unacceptable voltage noise levels. Therefore, a capacitor was added in the positive feedback loop of the current source (see Figure 1) to provide high-pass filtering ($f_c = 720$ Hz) of the stimulus current. This capacitor could still be bypassed to allow for DC stimulation, although all recordings in this paper were done in high-pass mode. The current source was driven by a programmable square wave generator consisting of a microcontroller (MSP430, Texas Instruments) and an external 18-bit DAC (AD5781, Analog Devices, Norwood, MA).

On the amplification side, the stimulation/recording electrode was coupled to the amplification stage through a 7th-order LC Butterworth filter ($f_c = 1.5$ kHz) in order to block the HF stimuli before amplification and prevent saturation. A unity-gain buffer (LT1793, Linear Technologies, Milpitas, CA) was added to isolate the electrode from the passive filter and maintain a high input impedance. The amplification (60 dB), band-limited from 1.5 Hz to 1.5 kHz, was provided in two stages (LT1125, Linear Technologies), followed by a 4th-order active Butterworth low-pass filter ($f_c = 1.5$ kHz). Taking into account the in-band attenuation of the LC filter, the total gain of the amplification channel was 54 dB (500). A second amplification channel was built to allow for simultaneous recording of a second electrode. All signals (stimulation, two amplified channels) were digitized at 12-bit resolution at 20 ksps (DAQCard-6062E, National Instruments, Austin, TX), and analyzed on PC using MATLAB® (The MathWorks, Natick, MA). Cells signals were subsequently filtered with a 3rd-order, 51-point Savitzky-Golay filter.

The microelectrode arrays (MEAs) used for this study have been described in detail earlier [8]. They contained a plurality of platinum electrodes of various sizes on glass substrates: a 6 x 6 array of 22 μm-diameter platinum electrodes (further coated with platinum black) for recording, and additional larger electrodes located on each side of the recording array and used for stimulation (Figure 2). Large annular electrodes were used for simultaneous stimulation/recording. Two sizes were used, with area of 0.1 mm^2 and 0.2 mm^2, and average impedance magnitudes at 5 kHz of 14.6 ± 3.6 kΩ and 7.9 ± 1.0 kΩ, respectively (n = 6). All recordings and stimulations were performed in unipolar configurations, using a coiled platinum wire in contact with the media as ground/return electrode.

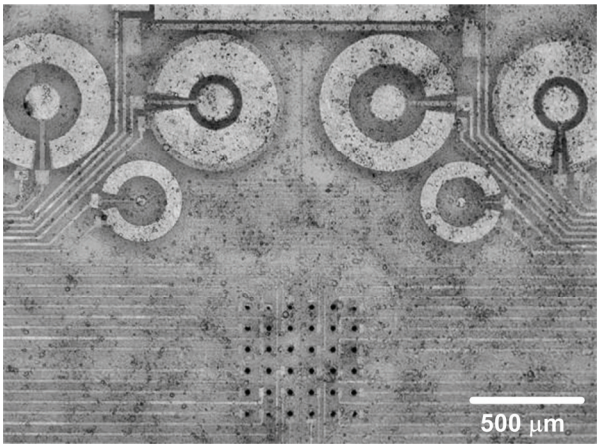

Figure 2. Microphotograph of HL-1 cardiomyocytes cultured on a microelectrode array. The large annular electrodes on top were used as primary stimulation/recording electrodes, while electrodes from the 6x6 array were used as distal recording electrodes.

B. Cell Culture

The HL-1 cardiomyocyte cell line [9] was used in all experiments. Culture protocol and MEA seeding were identical to [6]. Briefly, cells were cultured in Claycomb media (Sigma, St. Louis, MO), supplemented with fetal bovine serum, norepinephrine, penicillin-streptomycin, and L-glutamine. Cells were seeded on MEAs previously coated with fibronectin, and kept at 37°C. Experiments were performed after the cells reached 100% confluency, at room temperature.

III. Results and Discussion

A. Hardware Characterization

The frequency responses of the stimulation and amplifier channels are shown in Figure 3. Also shown is the power spectrum density and corresponding time trace at the

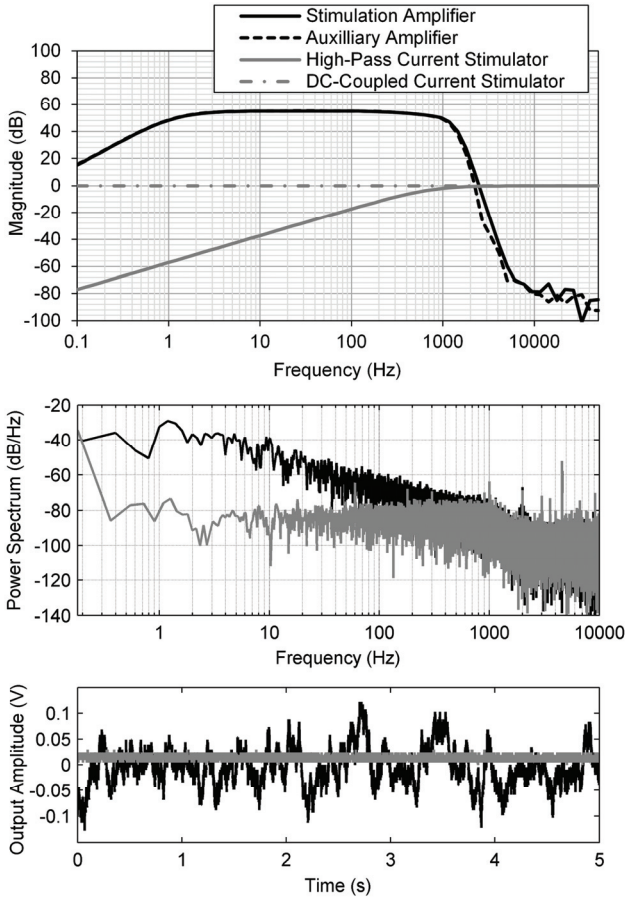

Figure 3. Top: frequency response of amplifiers and current source, in both DC-coupled and high-pass configurations. Middle and bottom: noise spectrum and corresponding time trace for DC and high-pass configurations, when connected to a microelectrode (in saline solution).

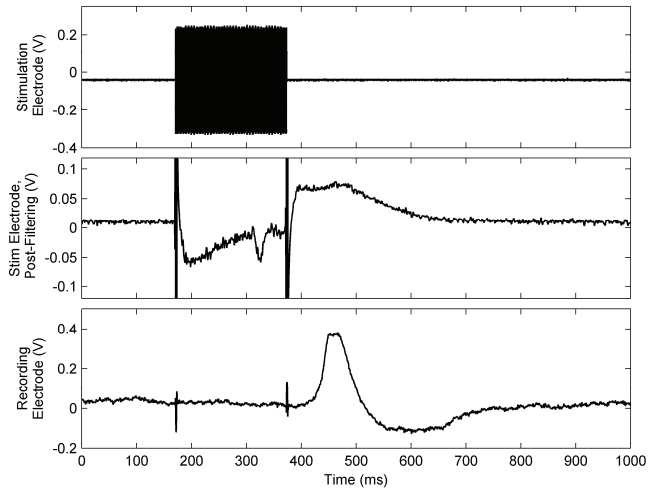

Figure 4. Simultaneous recording of depolarization during a burst of HF stimulation (5 kHz, ±20 μA, 200 ms). Top: resulting voltage on the stimulation electrode. Middle: signal from the stimulation electrode, after filtering and amplification, revealing the depolarization (action potential) triggered by the HF stimulus. Bottom: action potential recorded on a separate electrode of the recording array (see Figure 2), about 1 mm from the stimulation electrode.

amplifier output, with the amplifier/stimulator connected to a microelectrode in phosphate buffer saline (PBS). Noise measurements were performed on a realistic load, as opposed to a purely resistive load or even input-grounded, since the current noise of the stimulator will interact with the load to produce a voltage noise to the amplifier. Note the strongly attenuated noise in the amplifier output when switching from DC-coupled to high-pass current source, thanks to the reduction in the 1/f noise component of the current source. The typical noise (input-referred) in DC-coupled mode was 70 μV_{rms}, dropping to less than 15 μV_{rms} in high-pass mode.

The current source had a measured output impedance of 1.1 MΩ at 1 kHz, and a slew rate of 360 μA/μs for the 100 μA/V gain used in this work.

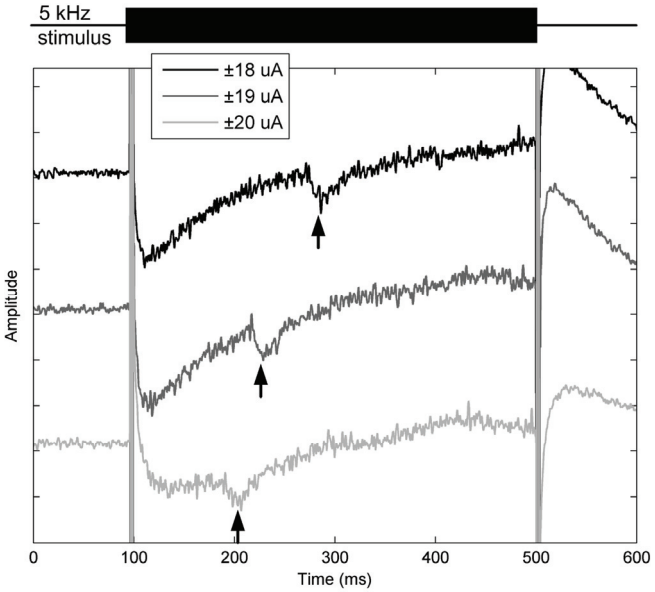

Figure 5. Monitoring of onset of depolarization (arrows) with increasing stimulus amplitudes. Larger amplitudes ('strength') require fewer pulses ('duration') to achieve capture, and result in earlier depolarizations. Simultaneous stimulation/recording allows the direct observation (and quantification) of this strength-duration (S-D) relationship, greatly facilitating the generation of S-D curves.

B. HL-1 Stimulation

Pacing of spontaneously- and non-spontaneously-beating cultures was performed under various conditions, and evoked action potentials were recorded, from the same stimulating electrode and from another distal electrode. Figure 4 shows a typical paced beat. While short artifacts are still visible at the onset and offset of the burst, the artifact during the HF burst is kept small enough (thanks to the HF rejection capability of the amplifier) that the evoked AP is clearly visible. Burst shaping strategies may help reduce the onset/offset artifacts, as shown in [6], but have not been applied in this work. Note the relatively low voltage required (500 mV_{p-p}) to achieve capture.

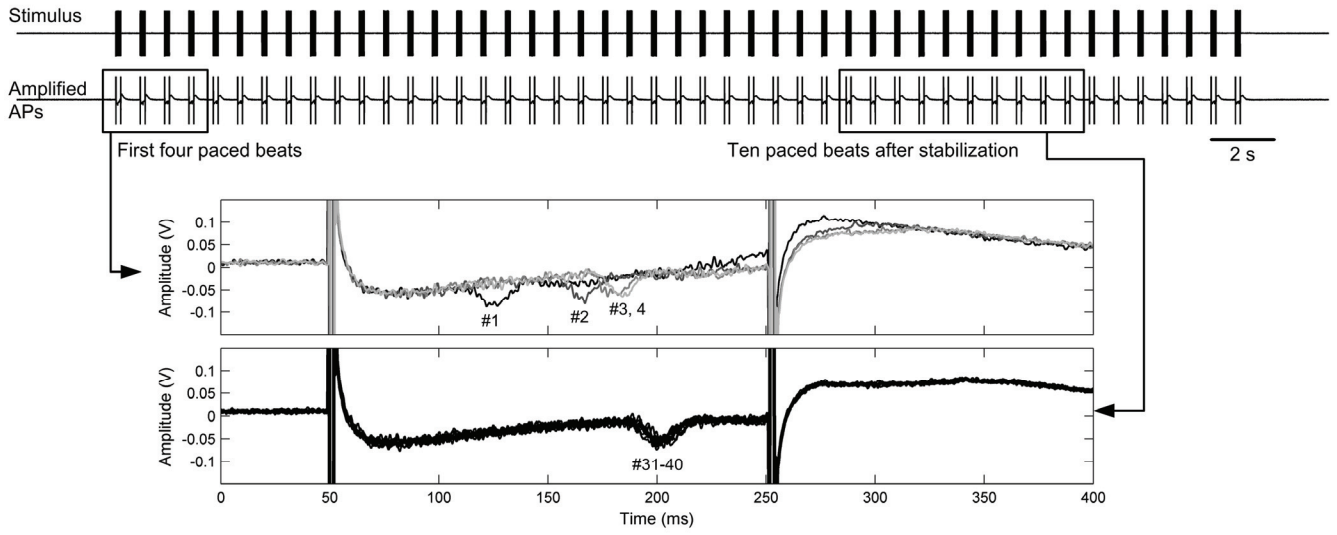

Figure 6. Illustration of beat-to-beat monitoring of stimulation threshold. The beat-level resolution easily reveals an initial threshold increase (shown by the rapidly increasing depolarization delay) after the start of a pacing train in a non-spontaneously beating cardiomyocyte culture. The threshold then stabilizes after a few tens of beats.

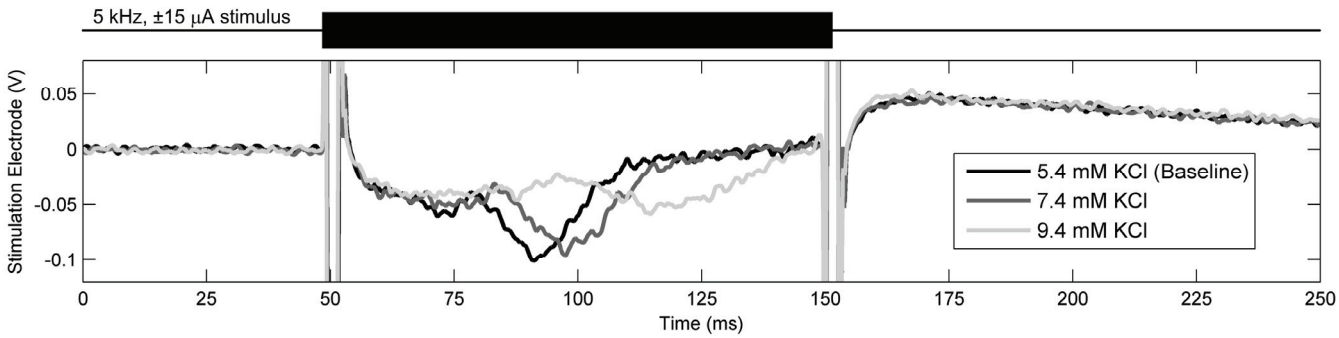

Figure 7. Pharmacological modulation of stimulation threshold by potassium chloride (KCl). The increased threshold resulting from high extracellular K^+ concentration (hyperkalemia) is visible in the delayed triggering of the depolarization (stimulation with ±15 μA at 5 kHz for 100 ms, 2 Hz pacing rate). In this particular case, five consecutive beats were averaged for each concentration to reduce noise.

Figure 5 illustrates the stimulus strength-duration relationship, in which larger stimuli need to be applied for lesser time to achieve capture. In the case of HF stimulation, based on a summation mechanism (*Gildemeister effect*) [6, 10], the duration is effectively the minimum number of pulses required to capture the tissue. Simultaneous stimulation and recording provides a direct, beat-to-beat measurement of this duration, as measured by the time-to-depolarization (or pulse count) from the onset of the burst.

The ability to monitor the stimulation threshold on a beat-to-beat basis is also exemplified in Figure 6, which shows the first paced beats in a non-spontaneously-beating culture after the onset of pacing. A rapid adaptation of the stimulation threshold, as measured again by the time from burst onset to depolarization, occurs in the first few beats, until it stabilizes at a final, higher level. Such beat-by-beat monitoring of stimulation threshold are only possible thanks to the ability to record the tissue activity *during* the stimulation.

Lastly, stimulation threshold monitoring during pharmacological modulation was demonstrated with the addition of KCl to the extracellular space (hyperkalemia). Figure 7 shows the increase in threshold (longer time-to-depolarization) following the addition of 2 and 4 mM KCl to the medium (final concentrations of 7.4 and 9.4 mM $[K^+]_o$). This increase is consistent with clinical [11] and *in vitro* studies [12] for moderate to high-concentration of $[K^+]_o$.

IV. Conclusion

HF stimulation is a promising alternative to current modalities, both for research and clinical applications. This work demonstrates that with appropriate hardware, it is possible to fully leverage the frequency separation between stimulation and recording inherent to this mode of stimulation, and to provide novel opportunities in electrical stimulation. Clinical applications could include closed-loop pacemakers, where the device terminates the burst once depolarization has been detected, effectively delivering an optimal charge. The automatic measurement of the stimulation threshold (time-to-depolarization) could also be used to assess the electrophysiological impact of compounds, either *in vivo* or *in vitro* for drug screening.

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