STIMULATING NEWS FOR MEA ENTHUSIASTS

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Micro-electrode arrays (MEAs) have been used to record from a variety of neural preparations, as well as for stimulating extracellularly. In stimulation experiments reported to date, a large variety of different stimulation waveforms have been employed, but no systematic study has been published about the different effects of different waveforms.

We are interested in identifying a class of stimuli that can be used for long term stimulation experiments on dissociated rat (E18) cortical cultures grown on MEAs [1]. Our goals are:

- To influence the morphology and functional connectivity of developing neuronal networks by patterned stimulation;
- To find reproducible response patterns that can be used to control an animat [2].

This study investigates:

- The relative impact of stimulus amplitude and duration for voltage controlled stimuli as well as for current controlled stimuli;
- Which temporal aspect of the stimulus waveform is most responsible for eliciting responses;
- The difference in responses to high-frequency stimuli relative to single pulses.

So far, we primarily used voltage-controlled stimuli of varying strength and duration. Experiments with current-controlled pulses are underway. It is worth noting that while modellers prefer current-controlled pulses because they are easier to interpret biophysically, some of the best results in the field have been achieved using voltage-controlled stimuli. Real-world stimulus waveforms are never pure square waves in either voltage or current space, and we discuss the impact of this observation on voltage-controlled stimulation.

For the purpose of describing the effect of varying stimulus parameters, it is useful to distinguish three parts of the stimulus response:

- Direct, axonally transmitted responses
- Post-synaptic evoked responses
- Evoked barrages

Direct responses are typically observed in the first 10 ms after stimulation [3]. They have very high temporal precision (100 μs jitter is common), and are highly reliable, some of them occurring in 80% of trials. Post-synaptic responses have less precise timing, and lower reliability. Evoked barrages (culture-wide bursts) occur in a small fraction of trials. They may last several hundred milliseconds, span across most of the array and consist of thousands of spikes. When stimuli are applied every few seconds, these evoked barrages may partly replace the spontaneously occurring barrages the culture exhibits when it is not stimulated [4].

We have found that in mature cultures, the patterns of direct and post-synaptic responses can be stable for several days, with very little variation in latencies and reliabilities.

To test which temporal aspect of the square wave voltage stimulus pulses was responsible for eliciting the responses, we varied the width of the pulses. We found that above a certain minimum, further increases in pulse duration had little or no impact on efficacy. We determined that the first cathodic current pulse produced by the voltage controlled waveform determined the timing of most responses. We also tested the effect of different voltages on direct responses, and found that each of them reached maximal reliability in its own well-defined voltage range. Both smaller and higher voltages were less effective at eliciting the response. Thus stimuli from a single electrode can evoke different response patterns depending on the stimulation voltage. We are currently studying the effects of varying the amplitude-duration ratio of cathodic current pulses to determine whether we can thus differentially stimulate different parts of neurons in the culture, in order to establish an even richer ensemble of possible stimuli.

To study short-term plasticity effects, we varied the stimulation frequency between 0.2 Hz and 100 Hz. We hypothesized that at high frequencies, responses might be weaker because neurons near the electrode become fatigued of firing at high rates. We found that below 1 Hz, direct responses were independent of frequency, as expected, while at higher stimulation rates, the number of spikes evoked per stimulus drops. Stimulating for several minutes at (fixed) rates between 20 and 100 Hz, we observed a gradual and reversible reduction in response strength. The direct spikes first shift to longer latency, then become less precisely timed, and subsequently less reliable across trials. After a sufficient number of high frequency stimuli, all responses are suppressed. This loss of stimulation efficacy does not make the culture quiet though. Indeed, shortly after stimuli ceased to evoke responses, the spontaneous pattern of barrages re-appeared, indicating that the stimulation fatigue extends only to neurons immediately affected by the stimulus pulses.

A better understanding of the importance of various stimulation parameters will guide future stimulator design and experimental choices. A parametrized set of stimuli that remain effective for long term stimulation will allow us to design stimulation paradigms that maximize the impact of stimuli on long-term network activity, and thus increase our ability to influence the structure of the developing network by imposed activity patterns. It may also help for harnessing the computational power of the network for controlling an animat [2].

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References

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