A constant illumination optical transmission method for freespace biological networks

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Abstract: Development of a biological internet requires the development of transmission and spatial information protocols. We present a free-space, constant illumination light-based signaling protocol and its optigenetic neuron receiver. The receiver functions as a simple processor which outputs information to its connected network. The transmission protocol maintains a constant energy and visually apparent constant illumination while encoding spike trains to be output from the neuron. The constant illumination makes the signal compatible with the built environment.
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1. Neuron based free space light signal receiver-processor paradigm

We present a light-based signaling protocol and a neuron-based signal processor. The goal is to facilitate development of biological networked spatial location awareness, computation, and information storage capability to move toward the possibility of biological networking over light.

Our light transmission protocol matches the wavelength and processing speed of ChR2 transfected optigenetic neurons, which allows some simple signal processing to be completed in a single cell. Spatially addressing neurons via (free space) targeted laser stimulation, or by area illumination from omni-directional LED transmission, in conjunction with direct electrical recording allows input and readout of information transmission. Where spatially addressing cells is not possible, serial signal input via LED room lights to a single optigenetic neuron can provide a signal source for a neural processing circuit.

2. Results

Free-space optical signal transmission of information determining spike trains to an optigenetic neuron signal processor was implemented. Information encoded in the signal was decoded by the receiver (neuron) at its maximum oscillation speed, which approaches 100 Hz in tests with mouse hippocampus neurons. It has been shown previously that action potential information transfer peaks at 200 Hz [1] but we find that this speed varies widely and 100 Hz was more appropriate in our tests.

An LED based transmission system was able to reliably transmit (8 successful of 8 symbols transmitted) information to transfected neurons acting as our optical signal receiver at distances over 20cm. This distance shows that a neuron based receiver can be used in an interior environment where the information transmission occurs through a free space optical transmitter, for example through room lights.

We further developed a free space laser transmitter for longer distances and a mobile phone based transmission controller, allowing a mobile device to define the information received at the neuron receiver.

3. Optical transmission system

We developed a 470nm 100watt LED light signal source and adopted a square wave signal encoding which is a 100 Hz / 200 Hz binary symbol set, where 100 Hz produces a neural activation sequence in the receiver while 200 Hz does not. We also constructed a steerable free space laser transmitter which employed the same encoding method.

This signal encoding method was chosen because it can function within common indoor environments. As the signal is encoded in light, it can easily be integrated with common indoor designs and architectures. The lowest frequency of the signal at 100 Hz is at the edge human visual response flicker threshold, fast enough to be

unobtrusive for many environments. The signal can then be transmitted from room or area lights, which can encode data in the transition between 100 and 200 Hz. This realizes a spatial information transmission system.

3. Receiver

Acquisition of time based information from oscillation can be found naturally in some single cell systems [2] and is clearly present in single neurons and networks. We use the characteristics of a single cell to delimit information by the volume limited electrical potential of the cell. The receiver we describe is a light sensitive optigenetically transfected neuron, which in our tests has a maximum oscillation frequency of about 100 Hz. The receiver is a single cell which has a capacitance which in conjunction with membrane located ion pumps determines the cell's electrical oscillation frequency. The maximum oscillation frequency of the cell was measured via whole cell patch clamp recording while under illumination from the 100 Watt transmitter at 20 cm distance and for comparison, also through current injection.

To create the receiver, primary cell cultures of mouse hippocampus neurons were grown at a low density of approximately 1 cell per square mm, and transfected via a ChR2-GFP or ChR2-mCherry viral vector [3]. Two different viral vectors were used, a lentivirus and an adenovirus vector each resulting in successful transfection rates of 10 to 20 percent of all cells. A small percentage of neurons were destroyed by transfection. Transfected neurons were identified by fluorescence microscopy.

Of successfully transfected neurons, patchclamp recording (Axon Instruments) of action potentials and electrical oscillation was made of cells plated in culture wells (wells, n=10; cells tested per well n=3). A 470nm 100 Watt square wave in the frequency range from 200 Hz to 50 Hz was transmitted to the cells from a distance of 20 cm, and maximum frequency of oscillation was tested. We found that square wave transmission caused large (>50mV) electrical response in hippocampal cells at frequencies below 100 Hz and response tapered to a <10mV response at frequencies above 100 Hz.

4. Signal reception details

The structure of the signal is two symbols, a low frequency and a high frequency symbol. Reception of the low frequency signal causes a spike train at the transmission frequency. Above a frequency threshold *Q* the cell is unable to produce a spike rain. This threshold frequency varies by cell and environmental conditions, and is between 50 and 100 Hz for mouse hippocampal neurons.

At low frequencies (≤100 Hz) the ion flow was sufficient to cause action potential [4]. The low energy period of the square wave is long enough to allow the cell to recover, after which the next high period in the transmission can cause another action potential. This results is a spike train.

At high frequencies (≥100 Hz) optigenetically operated ion channels were kept open by incoming photon energy, action potential occurs in a pattern of a single activation after which the refractory period is extended indefinitely as long as the signal frequency is high. This results in a single action potential and then a quiet state.

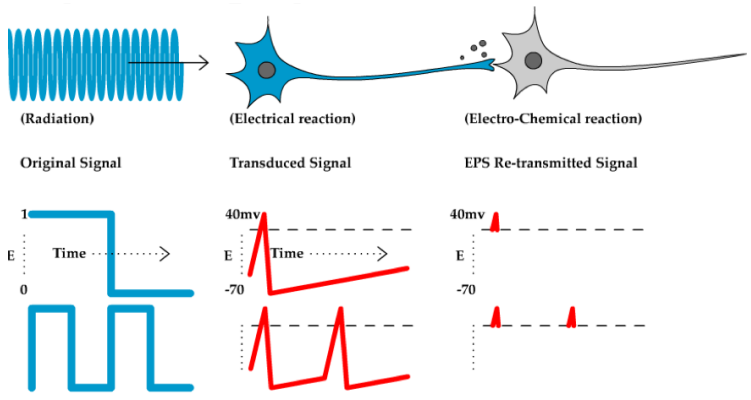

Figure 1. *Signal and response. Two frequencies are shown, a high frequency 200 Hz signal and a low frequency 100 Hz signal. The low frequency signal allows the neuron to rest and become capable of producing a spike train between rest periods. The high frequency signal does not allow the neuron to rest and it stops producing action potentials.*

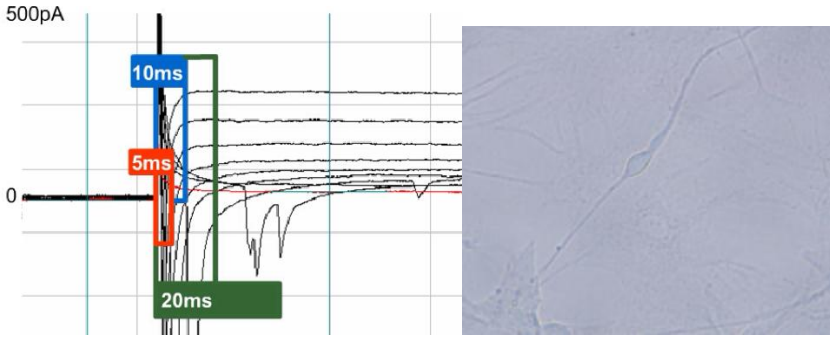

Figure 2. *Recovery time for repolarization (whole cell patchclamp). Near 100% probability of recovery after 20ms, 50% probability of recovery after 10ms, and 50% probability of recovery after 5ms. Stimulation by current injection -100mv at 10 Hz, 1ms pulse (Axon Instruments).*

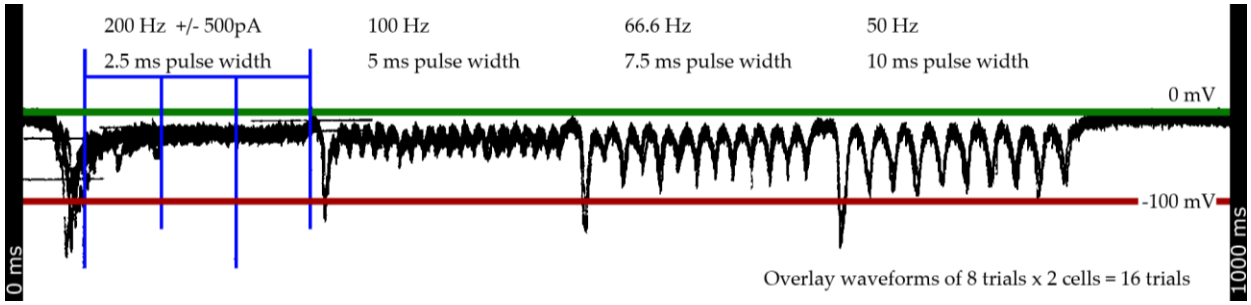

Figure 3. *Stimulation by current injection via patch clamp showing maximum oscillation frequency.*

6. Conclusion

The transmission method described here integrates a constant illumination light signal, and a biological receiver and is a step toward free space bio-optical networks. The constant illumination characteristic of the signal makes it appropriate for integration with the built environment, including room interior lights, display screens, outdoor illumination. The biological construction of the receiver opens up new possibilities for what types of systems can receive information, and bridges the divide between technological systems and biological systems. This simple transmission reception paradigm allows spatially determined (by the illuminated area) information transfer to biological systems at biologically relevant speeds that can approach 100 Hz. Specialized cells and modified ion channels can increase this speed as was shown in 2010 by Gunaydin et al. [5].

The biological internet may not yet exist, but from the example of previous networks that developed at a rapid pace, we might expect that the human environment may be built with networks of many types. Electrical, optical, and water networks are ubiquitous, radio networks have reached capacity in many regions, and optical networks are well developed. Free space optical, and biological networks are currently sparse and have great development potential.

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