Embedding Living Neurons into Simulated Neural Networks

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Introduction

The *in vitro* preparation of acute and cultured brain tissue is widely and succesfully used to study information processing at the level of single neurons and synapses. Due to the isolated condition of these neurons, the investigation of their functional interplay with an active network is strongly limited. To overcome this restriction, we present two complementary experimental approaches for embedding real cortical neurons *in vitro* into a virtual surrounding *in virtu* provided by large-scale neural network simulations.

(A) In an experimental real-time feedback setup we established a reciprocal connection between a living cortical neuron *in vitro* and a simulated neural network *in virtu*, similar to [9]. While excitatory and inhibitory projections onto the real neuron are mediated via current or conductance injection, the constant monitoring of its membrane potential allows for the detection of each generated action potential which is immediately transmitted to the postsynaptic partners within the network. The real-time requirements limit size and complexity of the simulated network.

(B) We generated excitatory and inhibitory synaptic inputs to a cortical neuron *in vitro* through off-line simulations of large scale neural networks ($\approx 10^5$ neurons). For each neuron *in virtu* we devised an 'integrate and fire' model with passive leak conductance. The network architecture features an anatomically realistic local connectivity and matches the size of a cortical column [10, 6]. This enables us to investigate neuronal output statistics in response to controlled quasi-realistic network input.

Discussion & Outlook

We have shown that a real-time interface between living neurons in vitro and a computer simulation in virtu can be easily achieved with standard equipment. The soft real-time system allows for the simulation of some hundreds of sparsly connected neurons with reasonable temporal resolution. Our experimental applications have demonstrated the usefulness of this technique for testing predictions from model studies.

Neural network simulations mostly rely on current-based models of neurons and synapses (e.g.,[2, 10]). Our results show that this typically leads to unphysiologically strong positive and negative current transients which can harm the neuron. From this we can conclude that conductance rather than current is the better signal for intracellularly interfacing with living cells and that hybrid networks should therefore rely on conductance-based neuron models [8].

Instead of interfacing via intracellular signals, other means of interacting with the living nerve tissue could widen the scope of application. Dynamic photostimulation [7, 11], for instance, could be used to stimulate brain tissue and to evoke spatio-temporal input patterns to single neurons, while network activity could be monitored extracellularly by means of multi-electrode-arrays (MEA) [5].

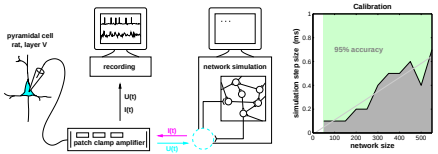
Hybrid networks can become a powerful tool to help clarify the mechanisms underling the neural computation in biological networks. As yet, applications have been limited to few examples. Thus, one future key issue will be to identify those problems and scientific questions in the neurosciences that can successfully be tackled with the hybrid approach of fusing virtual and biological systems.

Acknowledgement

We thank Armin Brandt for his help with the real-time implementation, Ellen Comes and Clemens Boucsein for assistance with the patch-clamp experiments and Uli Egert, Detlef Heck and Stefan Rotter for helpful discussions. This project was supported by the DFG (SFB 505), GIF, DAAD (V.R.) and the Heidelberg Academy of Science (M.N.).

A Propagation of synfire activity in a hybrid *in vitro* - *in virtu* network

Bidirectional Real-Time Communication

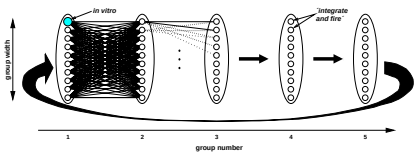


We implemented an experimental setup (left) which allows for bidirectional communication of analogue signals between real and virtual neurons in soft real-time. A standard PC (2×P III-500, 1GB) run under Linux performs the simulation of neural network models and controls the communication lines via a low cost I/O card (National Instuments, PCI 1200). For simulation we use C++ code based on the simulation environment SYNOD [3] (www.synod.uni-freiburg.de).

Here, we patched single neurons in layer V in the acute slice of rat somatosensory cortex. Membrane voltage was sampled at 1 to 10 kHz. Spike events were threshold detected before each simulation step $\Delta t'$ and processed by the postsynaptic partners in the simulated network. Excitatory and inhibtory synaptic input to the real neuron was modeled as postsynaptic current (or conductance) and injected into the soma.

In this configuration we calibrated the soft real-time process by simulating sparsely connected random networks for network size (number of neurons) and time resolution of simulation (right). We required the soft real-time condition ($\Delta t' \approx \Delta t_{\text{real}}$) to yield a short term average temporal accuracy of 95%.

Network Model With Embedded Synfire Chain

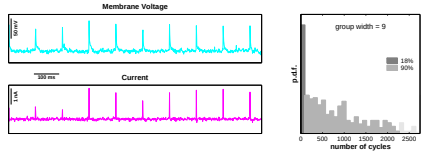


The network model initially comprised a synfire chain [1] of 5 groups, each consisting of 10 neurons. The projection from each group to the next was full divergent-convergent, with the fifth group projecting back onto the first group, thereby creating a loop. Synfire activity was initiated by strong simultaneous input to all neurons of group 1.

One arbitrarily chosen I&F neuron in group 1 was then either removed from the network or replaced by a cortical neuron (●) *in vitro*. Parameters of group size and synaptic strength were adjusted such that with only 9 neurons in the first group propagation of synchronous spiking was unreliable.

Each neuron *in virtu* was modeled as a leaky-integrator (voltage-threshold -55 mV, membrane time constant 10 ms) and received Poissonian background from 10,000 presynaptic neurons (88% excitatory at 1 Hz, 12% inhibitory at 12.5 Hz) [4, 13]. PSCs were modeled by an α-function. To compensate for the relatively small group size, we increased the synaptic weights of feed-forward connections between the synfire groups, relative to the strength of background synapses.

Cortical Neuron Ensures Stable Propagation



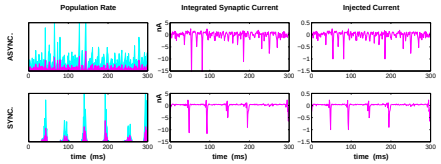
Repeated simulations with a group size of 10 I&F neurons leads to an infinite propagation of synchronous spiking (stable atractor) upon simultaneous ignition of the very first group.

When eliminating one single I&F neuron in the first group, however, we observed an unstable behaviour. The synchronous activity eventually dies out after a duration which varies across individual simulations (right) due to the random realisations of Poissonian background input. In 18% of the cases propagation terminated within the first loop.

Replacement of the missing 10th neuron in the first group (see connection scheme) by a cortical neuron *in vitro* re-establishes stable propagation throughout the duration of repeated simulations. Using identical Poissonian background input without embedding the real neuron lead to a propagation failure after 66 cycles.

B Neuronal output statistics depending on dynamic network state

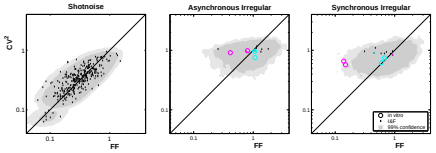
Locally Connected Random Networks *in virtu*



Here, we compare the spiking statistics of I&F neurons and that of real cortical neurons receiving input from a network of about 100,000 neurons which is modeled using a locally random connection scheme based on anatomical studies [10, 6].

Different dynamic network states can be adjusted by control of external input drive and relative synaptic weight of inhibition versus excitation [2, 10]. We concentrated on two states, characterised by *asynchronous irregular* (upper) and slow *synchronous irregular* (lower) spiking of the total population (left). Synaptic input to several I&F neurons was monitored (middle), clipped and scaled (right), and subsequently injected into the soma of a layer V pyramidal neuron.

Irregularity versus count variability



We quantified trial-by-trial variability by the Fano factor FF and spiking irregularity by the squared Coefficient of Variation CV^2 of the ISI distribution

$$\mathbf{FF} = \frac{\textbf{variance of count}}{\textbf{mean count}} \qquad \mathbf{CV^2} = \frac{\textbf{variance of inter-spike intervals}}{\textbf{(mean inter-spike interval)}^2}$$

The theory of renewal processes predicts that both measures are, on average, equal: $\mathbf{FF} = \mathbf{CV^2}$. For both types of network input, asynchronous (middle) and synchronous (right), the spiking statistics of the neurons *in vitro* showed good agreement with the renewal assumption. Irregularity of firing is equal for both, model and real neurons, and yielded rather high values compared to the case of balanced shotnoise input (left) [12].

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