Proceedings of the 1st International IEEE EMBS
Conference on Neural Engineering
Capri Island, Italy • March 20-22, 2003

Embedding Living Neurons into Simulated Neural Networks

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Abstract- **We present a novel technique for interfacing between a neural network simulation and living neurons. In two experiments we demonstrate how such hybrid *in vitro – in virtu* networks can be used to investigate neuronal function and to test model predictions.**

***Keywords* – Cortical networks, dynamic current clamp, Fano factor, hybrid neural networks, integrate-and-fire, synfire chain**

I. Introduction

The *in vitro* preparation of acute and cultured brain tissue is widely and successfully used to study neuronal processing at the level of single neurons and synapses. Due to their isolated condition, however, the investigation of their functional interplay with an active network is strongly limited. At the same time, *in virtu* neural network simulations have reached a state where it is now possible to investigate moderately realistic network models of high complexity. Such model studies lead to predictions which, in turn, can be tested experimentally. Here, we present two complementary approaches for embedding real cortical neurons into the virtual surrounding of a simulated cortical network. We show that a cortical cell that receives input from a large scale network model exhibits spiking statistics which are consistent with the model prediction. In a real-time application, similar to [1], we incorporate a living neuron into a hybrid synfire network model to restore stable propagation of synchronous activity. For the technical realization we developed and calibrated a setup for interfacing between computer simulation and electrophysiology in soft real-time.

II. Methodology

A. Electrophysiology

Acute slices from rat (P15-18) somatosensory cortex were prepared following standard procedures. In vitro whole-cell patch recordings (pipette resistance 2-6 MΩ) were obtained from visually identified layer V pyramidal neurons. Intracellular membrane potential was monitored in bridge mode while synthetic synaptic currents were injected through the same electrode using an Axoclamp 2B amplifier (Axon Instruments). Both signals were sampled at 10-20kHz using a CED 1401 with Spike 2 data acquisition software (Cambridge Electronic Devices, UK).

B Simulation

Simulations were performed using the simulation software NEST/SYNOD[1] which provides a convenient environment for performing medium to large size neural network simulations. In the real-time application we directly used C code based on NEST/SYNOD.

Neurons were modeled as leaky integrate-and-fire (I&F) neuron with a resting potential of –70mV and a voltage threshold for spike initiation set to –50mV. The membrane time constant was 10ms. Each spike event resets the membrane potential to its resting value, and is followed by a refractory period of 2ms. Postsynaptic currents (PSCs) were modeled as α-functions with a rise time of 0.3ms. If not stated otherwise, the peak amplitude of the excitatory PSC was set to yield a peak amplitude of +0.14mV for a single postsynaptic potential (PSP), the inhibitory PSP amplitude was scaled by a factor *g* equal to or larger than 1. For numeric integration we used a time grid with constant spacing [2] of 0.1ms in the offline application and 0.5ms in the online application.

C. Timing and Interfacing

We developed a low-cost interface (Fig.1) between a typical electrophysiological *in vitro* setup and a computer simulation in real time[2]. We use a single standard PC with a dual processor board (2xPIII-500), 1GB of RAM and Linux as operating system for both, numeric network simulation and control of the communication lines via the I/O card PCI-1200 (National Instruments) and open source device drivers for Linux (www.comedi.org).

We used a timing algorithm which was adequate to meet the soft real-time condition. This means that every time step must only approximate the desired temporal resolution Δt with a predefined precision. In our case we required a short term average accuracy of 95% for an average across the past 100 time steps.

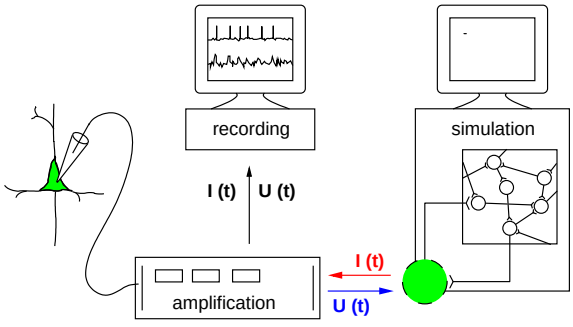

Fig. 1. Setup for real-time interfacing.

[1] http://www.synod.uni-freiburg.de

[2] A detailed documentation is provided on http://www.biologie.uni-freiburg.de/research/realtime/

Within each experimental time step, the timing algorithm reads the system clock twice, before and after the current simulation step, and waits until the time interval Δt is completed before initializing the next step. If the actual time interval between two read outs is larger than the desired interval, the timing process compensates with less waiting time during the following steps.

Each experimental step involves the read out of the current value for the membrane voltage of the real neuron, followed by a threshold spike detection. The momentary current value to be injected into the neuron is translated into a current command voltage for the Axoclamp amplifier which reliably controls the current drive. Both analogue signals are low-pass filtered at 2-5kHz, depending on the temporal resolution Δt. This setup may readily be used for dynamic current clamp since the actual membrane voltage is acquired in each single time step.

D. Experimental Application

We designed two different experiments that combined a computer simulation of a cortical network model with a living neuron in the cortical slice preparation.

1) Our first example comprised the simulation of a large-scale cortical network of about 100,000 model neurons adopting the architecture of a locally connected random network (LCRN) as described in [3]. In contrast to sparsely connected random networks [4,5], and based on statistical neuroanatomy, this 2-dimensional network topology incorporates a statistical rule for local connectivity [6] with a connectivity space constant of 0.3mm. The total network size matches a monolayer of about 2 x 2mm rat neocortex with a ratio of one inhibitory neuron per four excitatory neurons. All neurons received additional Poisson input, the rate of which serves as a control parameter for the inherent dynamics of the network, the second parameter being the inhibition weight *g* [3].

In a first step, we simulated several minutes of activity and recorded membrane voltage and somatic current from a sample of nine model neurons positioned on a regularly spaced grid within the network. Subsequent to the simulation and after appropriate scaling we injected the currents recorded from the single model cells into the soma of a pyramidal cell in vitro.

2) For a demonstration of the real-time capabilities of our setup in a true hybrid network we chose to integrate a living cell into a synfire chain model [7,8]. As illustrated in Fig. 2, the chain consisted of 5 groups, each with a group width of 10 neurons. The excitatory feedforward projection from each group to the next was divergent-convergent and complete; the fifth group projected back onto the first group, thereby creating a cyclic chain. Each neuron independently received additional Poissonian input from 10,000 neurons, of which 88% were excitatory, firing at a rate of 1Hz, and 12% inhibitory at 12.5Hz.

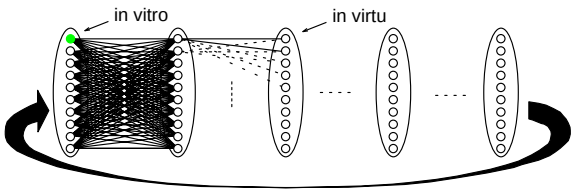

Fig. 2. Synfire chain model. The single gray neuron in the left group is replaced by a pyramidal neuron in vitro.

The number of neurons per synfire group was chosen to be rather small compared to the typical group width of about 70-100 which ensured stable propagation in earlier simulation studies [8]. We compensated for this size reduction by a tenfold increase in synaptic efficacy for all inter-group projections, resulting in a tenfold PSP amplitude of 1.4mV per single PSC in comparison to 0.14mV for the Poissonian background input. This is equivalent to assuming a learned synfire chain with strongly potentiated synapses.

The ring design of our chain allowed us to keep the number of groups small enough to ensure a satisfactory temporal resolution of 0.5ms in real-time. Random numbers for the Poissonian background had been gained and stored in working memory before the start of the experiment. For typical synaptic delays of approximately 2-3ms, a pulse packet [8,9], i.e. the synchronous volley of in our case up to ten spike events per neuron group, could travel the loop within less than 20ms. We therefore artificially increased each single synaptic delay to 20ms to allow the real neuron to reach a state more close to the resting condition before it again participated in the transmission of the travelling pulse packet.

III. RESULTS

A. Calibration of the Soft Real-Time System

With the simulation of sparsly connected random networks we calibrated our soft real-time system for two parameters that were varied independently: the network size, i.e. the number of simulated neurons, and the temporal resolution Δt of the experiment. The light gray patch in the calibration plot of Fig. 3 signifies the region for which the error in short term average temporal precision was below 5%. This allows for a rough estimate of the maximum network size for a required time resolution, and vice-versa, based on our hardware configuration. Generally, the limiting parameters proved to be the number of explicitly simulated elements (e.g. neurons or synapses) and the degree of connectivity rather than, for instance, the number of communication lines for interfacing with the *in vitro* experiment.

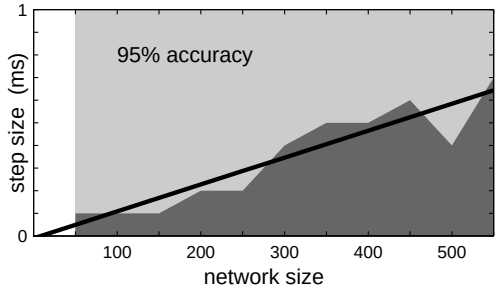

Fig. 3. Calibration of the soft real-time system. For the parameter range indicated by the light gray region a soft real-time accuracy of 95% is achieved. Black line: linear fit to the boundary values.

B. Consistent Output Statistics in Model and Real Neuron

Similarly to sparsely connected random networks [10], and depending on the inhibition weight *g* and the strength of the external input, the LCRN exhibited four dynamical network states, which are discerned by either global asynchrony or synchrony of the network activity and by the degree of firing irregularity [3].

Here, we were particularly interested in the regime where the neurons in the network exhibited irregular firing patterns with a high coefficient of variation (CV) of the inter-spike interval in the range of 0.5-1, similar to what has been observed in *in vivo* spike-train recordings from awake animals (e.g. [11]).

Our question was whether the spiking statistics of real cortical neurons were consistent to those of their simulated counterparts. We therefore simulated the asynchronous irregular and the synchronous irregular states, and recorded the membrane potentials and somatic currents from a sample of 9 neurons in each state. Since we used current-based model neurons in our simulations, somatic currents for synchronized inputs could easily become very large. In particular, we observed unphysiologically strong inhibitory events leading to current amplitudes of up to –15nA, which only marginally. influenced the model neurons, but may destroy a living cell. We therefore applied a linear downscaling by a factor of 10 prior to the injection. The temporal statistics and dynamics of the input, however, were preserved.

As shown in Figs. 4b and 4c for two neurons recorded in either condition, the values of CV around unity agreed well for model and real neurons. This means that the output of a cortical pyramidal cell *in vitro* driven with a realistic stationary background input from a large cortical network is highly irregular. By contrast, for Poissonian input, i.e. for shot noise injection, the same cell type exhibited a more regular firing, as demonstrated in Fig. 4a.

In analogy to earlier studies [11] we also measured the Fano factor (FF), i.e. the ratio of count variance and mean count across consecutive intervals of equal length. The theory of renewal processes predicts that, on average, it should hold that $FF=CV^2$. As shown in Fig. 4, this is indeed the case for all three conditions, i.e. the cortical neuron´s spiking showed a good agreement with a renewal process at this level of second order statistics, independent of the values of either FF or CV.

C. Cortical Neuron Stabilizes Synfire Propagation

The synfire experiment as explained in the method section was carried out in three different settings, each for a total duration of 5 minutes.

First, we repeatedly simulated the full chain with ten I&F neurons per group (Fig. 2), each time with a different initial condition for the Poissonian background. At the start of each experiment, all neurons in the first group simultaneously received a strong excitatory input to `ignite´ the synfire chain. In all of in total 500 repetitions we observed stable transmission of coincident spiking for the full duration of five minutes. Within this time, the pulse packet cycled about 3,000 times through the ring-chain.

If we, however, eliminated a single neuron from one group, indicated by the gray neuron in Fig. 2, we observed unstable propagation. In more than 90% of the total 500 runs, synchronous activity eventually died out before the end of the experiment (Fig. 5a). In 90 (18%) of these cases, the transmission failed to complete the first cycle. Thus, for a single missing I&F neuron, the propagation of synfire activity under these conditions was vulnerable and did not reach the attractor where propagation is stable [8].

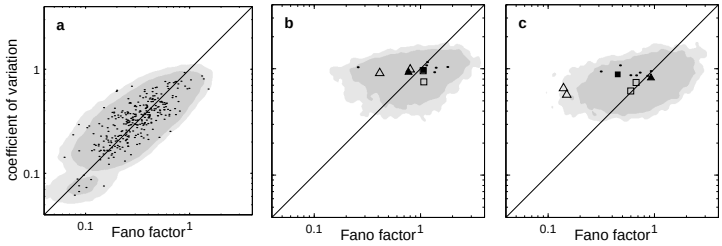

Fig. 4. Spike count variability (FF) and spike-train irregularity (CV^2) for (a) shotnoise injection in pyramidal neurons in vitro (N=19 cells), (b) network input as generated in the asynchronous irregular state and (c) in the synchronous irregular state. The open symbols in (b,c) indicate results from the *in vitro* injection in 2 different neurons. The filled symbols mark the results for the corresponding model cell. Black dots indicate different model neurons recorded simultaneously. Gray shadings indicate 95% and 99% confidence region for the equality of FF and CV^2 obtained from gamma-simulations [11].

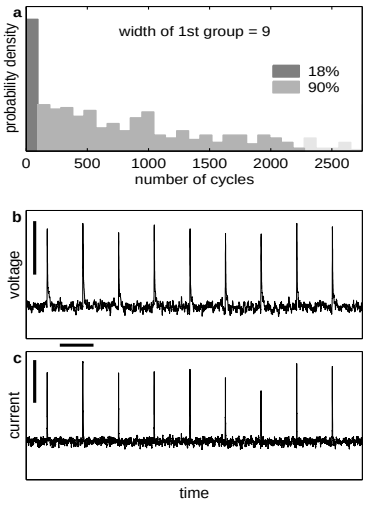

Fig. 5. Stability of pulse packet propagation through the synfire chain. Elimination of 1 out of 10 neurons in the first group results in an unstable propagation (a). Inclusion of a cortical neuron in the first group reestablishes a stable condition where each input volley (c) triggers one output spike (b). Vertical scale bars: 50mV, 1nA; horizontal scale: 100ms.

In a third setting, we replaced the missing 10th model neuron by a real neuron in the acute slice preparation. This again led to a stable propagation of the pulse packet activity throughout the total duration of 5 minutes. Fig. 5 shows one second of membrane potential (b) and injected current (c) for a cortical neuron as monitored in whole-cell patch configuration during the course of the experiment. In the current trace, the synchronized input in form of large compound EPSCs can be clearly distinguished from the background. It varied slightly in size and shape, mainly due to a varying number of presynaptic neurons contributing to the input volley, whereas there was only little change due to the jittering of input spike times which remained well within ±1ms. Within the 1s shown in Fig. 5b, each single input volley immediately lead to the generation of an output spike, and propagation prolonged for full 5 minutes, while for identical initial conditions but in the configuration with only 9 neurons in the first group, propagation terminated after 66 cycles.

IV. Discussion and Conclusions

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. Further improvement in terms of temporal resolution and accuracy can be achieved by implementing a real-time kernel for Linux. Our experimental applications have demonstrated the usefulness of this technique for testing predictions from theoretical model studies. Thus, hybrid networks of this kind have the potential to become a powerful tool to help clarify the mechanisms underlying the computational process in biological neural networks.

Instead of interfacing via intracellular current and voltage signals, other means of interaction with living nerve tissue could widen the scope of application. Dynamic photostimulation [12], for instance, could be used to stimulate a slice preparation and to evoke spatio-temporal input patterns to single neurons, while network activity could be monitored extracellularly by means of multi-electrode-arrays (MEA) [13].

Acknowledgment

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 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.

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