6th Annual International IEEE EMBS Conference on Neural Engineering
San Diego, California, 6 - 8 November, 2013

Tetanic stimulation of cortical networks induces parallel memory

Tamar van Veenendaal, Tim Witteveen, and Joost le Feber

***Abstract*— The mechanisms behind memory have been studied mainly in artificial neural networks. Several mechanisms have been proposed, but it remains unclear yet if and how these findings can be translated to biological networks. Here we unravel part of the mechanism by showing that cultured neuronal networks develop an activity connectivity balance. External inputs disturb this balance and induce connectivity changes. The new connectivity is no longer disrupted by reapplication of the input, indicating that a network memorizes the input, analog to attractor memory networks as demonstrated in Hopfield network models. A different input again induces connectivity changes upon first application but not after repeated stimulation. Returning to the first input no longer affects connectivity, showing that memory traces are stored in parallel. A simple computer model robustly reproduces the experimental results and shows that spike timing dependent plasticity suffices to store memory traces of different inputs in parallel in neuronal networks.**

I. Introduction

Mechanisms of action behind memory have been studied mainly in artificial neural networks. In artificial recurrent excitatory networks activity patterns are dictated by attractors, local minima in the energy landscape that are associated with certain activation patterns. The continuous nature of an attractor network generally leads to random drift of a persisting activity pattern [1]. External input changes the set of attractors of a network[2]. Although artificial neural networks provide a strong indication of the working mechanism behind memory, this cannot be straightforwardly translated to biological networks, because *in vivo* simultaneous activity of multiple neurons is difficult to record and consequently, it is hard to provide accurate estimates of the synaptic coupling in a network.

Dissociated cortical neurons cultured on multi electrode arrays have received increasing attention to study network aspects of neuronal tissue, including memory. In the first week of culturing the neuronal networks are formed. After ~1 week networks become spontaneously active and reach a mature state after ~3 weeks, with relatively stable activity patterns. Activity patterns are determined by a certain connectivity, and conversely, certain patterns also affect connectivity through plasticity mechanisms like long term potentiation (LTP) or spike timing dependent plasticity (STDP). Beyond three weeks, activity patterns and connectivity stabilize, but a slow drift (on a time scale of hours to days) remains. Networks appear to develop an activity-connectivity balance, wherein occurring activity patterns support current connectivity[3]. These observations correspond to the finding in continuous attractor network models, that a self-sustained activity pattern tends to drift slowly [1].

Responses to electrical stimulation usually differ from spontaneously occurring patterns and therefore disturb the activity connectivity balance, yielding a change in connectivity. In analogy, external input in artificial neural networks changes the set of attractors. Such a change should be reflected in altered network connectivity.

Here we hypothesize that networks will develop a new activity-connectivity equilibrium, that includes the network response to the applied stimulus. To verify this hypothesis, we repeatedly applied the same stimulus to a network, separated by one hour of no stimulation, and quantified the connectivity changes that resulted from subsequent stimulation periods. If the hypothesis holds, connectivity changes should exceed spontaneous fluctuations when a certain stimulus is applied for the first time, but not upon repeated application of that same stimulus.

II. Methods

A. Cell culturing and stimulation

We obtained cortical cells from newborn Wistar rats at post natal day 1. After trypsin treatment cells were dissociated by trituration. About 400,000 dissociated neurons (400 µl suspension) were plated on a 60 electrode MEA (Multi Channel Systems, Reutlingen, Germany, see Figure 1), precoated with poly ethylene imine (PEI). This procedure resulted in an initial cell density of approximately 5000 cells per mm^2, which was in agreement with counted estimates in the first days after plating. With aging cell densities gradually decreased to ~2500 cells/mm^2. We used MEA's containing electrodes with 10 µm diameter (pitch 100 µm), or 30 µm diameter (pitch:200 µm)

Neurons were cultured in a circular chamber with inner diameter d = 20mm, glued on top of an MEA. The culture chamber was filled with ~700 µl R12 medium [4] MEAs were stored in an incubator, under standard conditions of 37°C, 100% humidity, and 5% CO_2 in air. For recording, we firmly sealed the culture chambers with watertight but CO_2

J.le Feber is with University of Twente, MIRA institute for Biomedical Engineering and Technical Medicine, PO Box 217, 7500AE, Enschede, the netherlands (+31.53.489.2786; fax: +31.53.489.2287; e-mail: j.lefeber@utwente.nl).

T. van Veenendaal, was with University of Twente, Enschede, the Netherlands. She is now with University of Maastricht, School for Mental Health and Neuroscience, the Netherlands (e-mail: t.vanveenendaal @maastrichtuniversity.nl).

T. Witteveenwas with University of Twente, Enschede, the Netherlands. He is now with Benchmark Electronics (e-mail: tt.witteveen@gmail.com).

permeable foil (MCS; ALA scientific), and placed the cultures in a measurement setup outside the incubator. During recording we maintained the CO_2 level of the environment around 5% and we moisturized the air. For details about the recording setup see [5] Recordings were started after an accommodation period of at least 20 minutes.

After the measurements the cultures were returned to the incubator.

We used 9 different cultures for 19 experiments, which were performed 22±6 days after plating of the dissociated cells. Cultures were electrically stimulated with biphasic current pulses, of 200µs per phase and 12-24µA amplitude. Pulses were applied either as single pulses for 10 minutes (inter pulse interval:3-5s), or as tetani (trains of 10 pulses at 100Hz, inter train interval: 5s).

B. *Connectivity analysis*

We used periods of spontaneous activity to analyze network connectivity. Long term recordings were divided into data blocks of 2^{13} spiking events. In each data block we used conditional firing probabilities to determine functional connectivity [6]. For all possible pairs of electrodes (60×59) we calculated conditional firing probabilities (CFP's) as the probability to record an action potential at electrode *j* at t=τ, given that one was recorded at electrode *i* at t=0. If a CFP curve was not flat, the two neurons were functionally connected. Functional connection were characterized by two parameters: their strength and latency [6]. These parameters may be used to follow the development of a functional connection in time [7].

In each data block, the strengths of all connections were combined into a connectivity matrix S, where S(*i,j*) contains the strength of the functional connection from *i* to *j*.

The magnitude of changes between subsequent data blocks was assessed by the Euclidean distance (*ED*) between connectivity matrices at time t and time t_0 (Eq. 1). For t_0 we chose either the last data block before stimulation, or the last data block before a series of stimulation periods at a certain electrode.

$$ED_{t_0}(t) = \sqrt{\sum_{i=1}^{n}\sum_{j=1}^{n}[S_{ij}(t) - S_{ij}(t_0)]^2} \qquad (Eq.1)$$

C. *Computer modeling*

We constructed computational models that consisted of 100 neurons, following the approach by Izhikevich [8]. The model contained a random mixture of all cell types that exist in the cortex, just like the experimental cultures did. On average, each neuron had 50 connections, 80% of all neurons were excitatory and 20% were inhibitory. These neurons were coupled by synapses showing short term depression as described by Markram et al. [9] and spike timing dependent plasticity [10]. Ongoing activity was initiated by white synaptic noise as implemented by Gritsun et al. [13].

Synaptic noise was set to a level high enough to ensure that all models displayed spontaneous activity, including network bursts. This indicated that neurons were not solely driven by noise input, but were able to trigger each other.

To simulate electrical stimulation we randomly activated a set of nine neurons (two inhibitory and seven excitatory) simultaneously, which was in general sufficient to trigger a network response. Stimulation was applied as either single stimuli or tetani, as described under cell culturing and stimulation.

III. Results

We investigated the effect of repeated stimuli on connectivity in cultured cortical networks on Multi electrode arrays (Fig. 1), and in a computer model of a small cortical network.

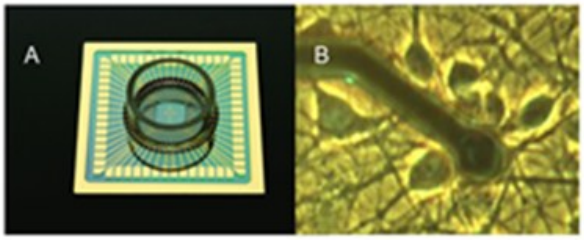

Figure 1. Multi electrode array (MEA) and close up of one of the electrodes. **A:** MEA, used to record neuronal activity in cultured cortical networks. It is based on a glass substrate with 60 embedded electrodes in the centre of the chamber, with 200 µm inter electrode distance. The glass ring glued on top was filled with glia conditioned growth medium and firmly sealed. **B**: close up of one of the electrodes and several neurons.

A. Experiments

When cultures were isolated, without external input, functional connectivity was stable: strengths varied less than 25% of their mean value at time scales of multiple hours. Ten minutes of electrical (tetanic) stimulation significantly affected functional connectivity. Moreover, repeated

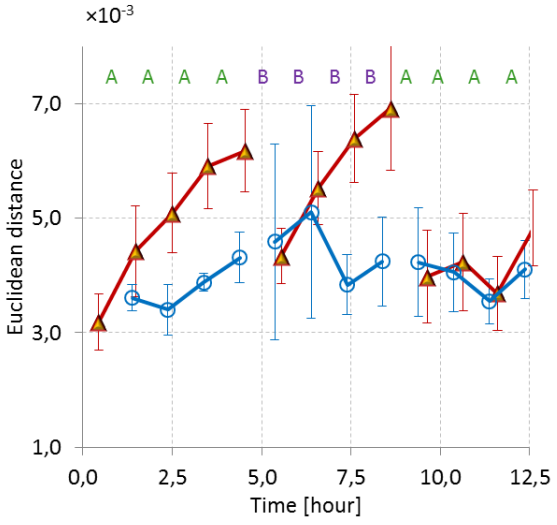

Figure 2. Euclidean distance between subsequent connectivity matrices without stimulation (○, n=4 experiments), or across periods of tetanic stimulation at electrode A or B, as indicated (Δ, n=7 experiments). For comparison, all distances are relative to state of the network before stimulation at that particular electrode (A or B).

application of the same stimulus induced much smaller or even negligible connectivity changes. Single pulse stimulation roughly yielded comparable results, but we did not. collect enough data yet to support firm conclusions.

Subsequent stimulation at another electrode again yielded large changes upon first stimulation and also smaller or no changes after successive stimuli. Finally, returning to the first stimulus did not induce connectivity changes larger than spontaneous fluctuations. As illustrated in Figure 2, differences across first stimulation periods at a certain electrode were significantly larger than those across subsequent stimulation periods, or periods of equal duration of no stimulation.

B. *Computer modeling*

A model of 100 neurons coupled by synapses with short term depression and spike timing dependent plasticity, robustly reproduced the *in vitro* finding that networks

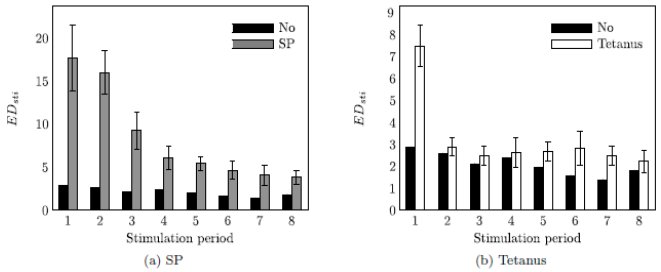

Figure 3. Connectivity changes following stimulation in a computer model. Left panel shows the effect of single pulse (SP) stimulation, right shows tetanic stimulation. Bars indicate connectivity changes across stimulation periods, averages ± standard deviations of four simulations are shown. No stimulation (No) was simulated once.

develop an activity-connectivity balance. We used several network realizations, two different implementations of STDP, and various patterns of synaptic noise and activity and connectivity stabilized in all networks. The models also reproduced the experimental finding that a first external stimulus induced large connectivity changes, but subsequent stimuli did not (tetanic stimulation), or to a lesser extent (Single pulses), as illustrated in fig 3.

This also applied for a second (different) stimulus, provided that synaptic strengths did not reach extreme values

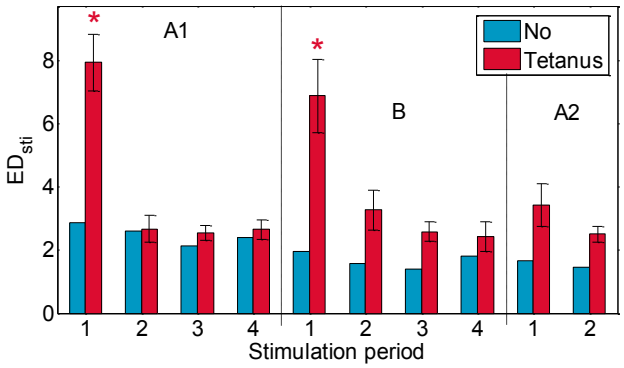

Figure 4. Euclidean distances across subsequent stimulation periods at electrode A or B (n=4 simulations), or periods of no stimulation (n=1). Significant differences (multiple comparisons test, $p<0.05$) are indicated by an asterisk.

(maximum strength or zero). In general this was the case when tetanic stimulation was applied, but not after single pulse stimulation. Return to the first stimulus did not induce changes larger than spontaneous fluctuations, see Figure 4.

IV. Discussion

When left in isolation, cortical networks develop rather stable firing patterns [5], as well as a stable connectivity [6], both showing only minor changes on a timescale of several hours. This means that the occurring patterns do not affect connectivity, and therefore, activity and connectivity are in balance. Similar findings of self-stabilizing activity were obtained in artificial neural networks (see e.g. [14]).

An external input usually induces deviating activity patterns, driving the network out of the existing balance. Networks will develop a new balance, probably including the response pattern to that stimulus. This hypothesis is supported by the finding that subsequent application of the same input had no further effect on connectivity. Investigation of artificial neural networks showed that external input usually changes the set of attractors in a network [2]. The connectivity matrix S probably reflects such a change in the set of attractors. From the waning effect of a stimulus on the connectivity matrix S, we concluded that cortical networks memorize inputs.

A similar pattern occurred upon stimulation at a second, different electrode. Again, large connectivity changes occurred across the first stimulation period, and much smaller changes upon subsequent periods, indicating that the network also memorized the second input. Moreover, this confirmed that the network was still able to adapt to external inputs, and that the unaffected connectivity after several repetitions of the first stimulus was not caused by impeded network plasticity. In other words: the network was still able to adapt connectivity in response to the first stimulus, but there was no longer an attracting force.

Returning to the first stimulus did not affect network connectivity, indicating that the first memory trace was not erased by application of the second stimulus. Instead, both memory traces existed in parallel, at least for the four hour periods in our experiment.

Our results show that inputs are stored in network connectivity, but not how they are encoded. We hypothesize that connectivity and accordingly spontaneous firing patterns will change to include the stimulus response pattern. Consequently, also the stimulus response will change due to connectivity changes. Probably stimulus response and spontaneous patterns will develop towards each other, but this remains subject to further study.

Computational modeling robustly reproduced the experimental findings. To realize sufficient ongoing network activity and network bursts, the degree of connectivity (the relative number of connections per neuron) and the level of synaptic noise were higher than biologically plausible. This was a direct consequence of the very small network size of

100 neurons. Larger network models imposed a very high computational load and were not necessary here to reproduce the experimental findings. However, it has been shown that the storage capacity, or the number of possible stable states (atractors), scales linearly with the number of synapses per neuron [2] and, therefore, parallel storage of memory traces would even be better facilitated in larger networks.

Spike timing dependent plasticity in combination with short term synaptic depression was sufficient to enable parallel storage of memory traces. Short term depression has been shown to destabilize attractors in artificial neural networks [11], but it is required to end network bursts in a natural way and thus to avoid endless bursts of activity [12]. Spike timing dependent plasticity enabled network connectivity to adapt to different inputs. However, occasionally stimulation lead to connectivity close to the borders of the connectivity space, with many synapses at their maximum strength or at zero. In such cases more extensive stimulation was required to pull the network out of this state towards another equilibrium. It appeared that tetanic stimulation was more suitable than single pulse stimulation for retaining connectivity away from these borders. Apparently, in the computer model network activation tended to strengthen strong synapses and to reduce weaker synapses. Second and subsequent pulses of a tetanus coinciding with an induced network response, frequently forced synapses away from their extreme values, and kept networks in a state more susceptible to memorizing new inputs. This observation may be a modeling artifact, our experimental results do not provide evidence to extrapolate this observation to biological networks. Possibly, addition of a third, homeostatic plasticity mechanism that has been demonstrated in *in vitro* networks,, synaptic scaling [15], to the computer model could avoid these artifacts. This might expand the usability of the model to single pulse stimulation.

V. Conclusion

We conclude that cultured cortical networks memorize external inputs. A second input does not erase the traces of the first one, but both memory traces are stored in parallel. Two synaptic plasticity mechanisms, spike timing dependent plasticity and short term depression are sufficient to enable parallel memorization of different inputs. Our observations in *in vitro* networks support earlier findings in artificial attractor memory network models, and will facilitate the translation of memory theory to biological networks.

References

[1] E. Carter and X.-J. Wang, "Cannabinoid-mediated disinhibition and working memory: dynamical interplay of multiple feedback mechanismsin a continous attractor model of prefrontal cortex", *Cereb. Cortex,* vol. 17, pp. i16-i26, 2007.

[2] D. J. Amit, "Modeling brain function, the world of attractor networks", Cambridge, UK: Cambridge University Press, 1989.

[3] J. le Feber, Stegenga J, Rutten WLC, "The Effect of Slow Electrical Stimuli to Achieve Learning in Cultured Networks of Rat Cortical Neurons", *PLoS ONE,* vol. 5, p. e8871, 2010.

[4] H. J. Romijn, van Huizen F, Wolters PS, "Towards an improved serum-free, chemically defined medium for long-term culturing of cerebral cortex tissue", *Neurosc Biobehav Rev,* vol. 8, pp. 301-334, 1984.

[5] J. Stegenga, le Feber J, Marani E, Rutten WLC, "Analysis of cultured neuronal networks using intra-burst firing characteristics", *IEEE Trans Biomed Eng.,* vol. 55, pp. 1382-1390, 2008.

[6] J. le Feber, Rutten WLC, Stegenga J, Wolters PS, Ramakers GJ, Van Pelt J, "Conditional firing probabilities in cultured neuronal networks: a stable underlying structure in widely varying spontaneous activity patterns", *J. Neural Eng.,* vol. 4, pp. 54-67, 2007.

[7] J. le Feber, Van Pelt J, Rutten WLC, "Latency related development of functional connections in cultured cortical networks", *Biophys. J.,* vol. 96, pp. 3443-3450, april 2009 2009.

[8] E. Izhikevich, "Simple model of spiking neurons", *IEEE trans Neural networks,* vol. 14, pp. 1569-1572, 2003.

[9] H. Markram, Wang Y, Tsodyks M, "Differential signaling via the same axon of neocortical pyramidal neurons", *Proc Natl Acad Sci USA* vol. 95, pp. 5323-5328., 1998.

[10] S. Song, Miller KD, Abott LF, "Competitive Hebbian learning through spike-timing-dependent synaptic plasticity", *Nature Neurosci,* vol. 3, pp. 919-926, 2000.

[11] A. Sandberg, Tegner J, Lansner A, "A working memory model based on fast Hebbian learning", *Network: Comput. Neural Syst.,* vol. 14, pp. 789-802, 2003.

[12] D. Eytan and S. Marom, "Dynamics and effective topology underlying synchronization in networks of cortical neurons", *J Neurosci,* vol. 26, pp. 8465-8476, 2006.

[13] T. A. Gritsun, le Feber J, Rutten WLC., "Growth Dynamics Explain the Development of Spatiotemporal Burst Activity of Young Cultured Neuronal Networks in Detail", *PLoS One,* vol. 7, p. e43352, 2012.

[14] D. J. Amit and N. Brunel, "Model of global spontaneous activity and local structured activity during delay periods in the cerebral cortex", *Cereb. Cortex,* vol. 7, pp. 237-252, 1997.

[15] G. G. Turrigiano, Leslie KR, Desai NS, Rutherford LC, Nelson SB, "Activity-dependent scaling of quantal amplitude in neocortical neurons", *Nature,* vol. 391, pp. 892-896, 1998.