Strengthening and weakening of relationships in cortical cultures: latency dependent?

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Abstract
Networks of cortical neurons were grown over multi electrode arrays to enable simultaneous measurement of signals from multiple neurons. Functional connectivity in cultured networks has been described by relationships between individual electrodes, based on conditional firing probabilities. In this study we investigated periods in which the strength of a relationship increased (strengthening) or decreased with time (weakening). We observed a slightly increased incidence of latencies around 20 ms during strengthening, while these latencies rarely coincided with weakening.

1 Introduction

Learning and memory have attained much attention over the years. The brain consists of a huge number of neurons that form networks through a multitude of synaptic connections. The formation and development of connections is assumed to be crucial in the process of learning, their conservation is assumed to be essential for memory. To demonstrate either memory or learning, one needs to monitor the connections in neuronal networks. Assuming that the specificity of connections is reflected in the patterns of electrical activity in the network, this requires simultaneous measurement of activity in a large number of neurons. In vivo this is quite a complicated procedure and several groups now use in vitro preparations of cultured neurons grown over a multi electrode array (MEA).

Several attempts have been made to induce plasticity (changes in synaptic efficacy of neuronal connections) in networks of neurons cultured on multi electrode arrays (MEA's) (Eytan et al., 2003, Jimbo et al., 1998, Shahaf and Marom, 2001, Ruaro et al., 2005). Different methods were used to induce changes in connectivity. All of these methods were based on the hypothesis that certain patterns of network activity may alter connectivity. In training sessions certain patterns of activity were imposed by electrical stimulation of the network, aiming to induce connectivity alterations. Some results appeared quite successful. However, other experiments yielded ambiguous results or were difficult to reproduce (Wagenaar et al., 2006). One of the main complicating factors is the high variability in spontaneous activity in cortical networks, which may mask induced alterations. Cortical networks become spontaneously active after ~one week in vitro. The spontaneous activity shows alternating periods of seemingly uncorrelated firing and of synchronized firing, typically growing towards network bursts (Gross et al., 1995, van Pelt et al., 2004). An example of the widely varying patterns of synchronized spontaneous activity is shown in Figure 1.

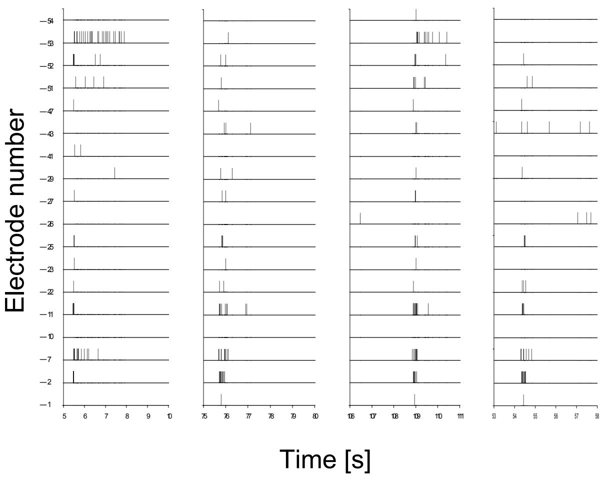

Figure 1. *Example of the diversity of patterns in spontaneous activity in a culture of cortical neurons. Vertical axis shows electrode numbers, vertical lines indicate time stamps of action potentials at that electrode. Four bursts (in 5s windows) are shown of one culture at 29 DIV, all recorded in the first 2 minutes. Although individual patterns varied widely, relationships were found in that same period that were quite stable.*

In this study we used spontaneous activity to investigate plasticity, i.e. sponataneously occurring changes in neuronal connections between pairs of electrodes. First, we calculated conditional firing probability ($CFP_{i,j}[\tau]$, see Methods section) for all electrode pairs (*i,j*). Electrode pairs with a peaked CFP curve were considered related. Then, we fitted a function to all peaked curves to describe the relationships by two parameters: strength (maximum probability above offset) and latency (time delay until maximum probability). Strengths and latencies of all relationships were calculated from blocks (typically lasting less than one hour) of data obtained from long term recordings, which lasted up to 6 weeks. This allowed us to observe the development of the strength and latency of relationships.

Intracellular measurements have shown that synapses may be strengthened when postsynaptic action potentials occur within 20 ms after presynaptic firing (Zhang et al., 1998). This phenomenon, known as long term potentiation (LTP), is considered as one of the fundamental mechanisms underlying learning.

We investigated how this latency-dependent strengthening is reflected in relationships between pairs of neurons in rat cortical networks. We selected extended periods (≥ one day) in which the strength of a relationship monotonously increased or decreased, and investigated whether or not delays in the 20 ms time window tended to coincide with growing relational strength in these periods.

2 Methods

Dissociated cortical neurons were plated on MEA's with 60 electrodes (see Figure 2).

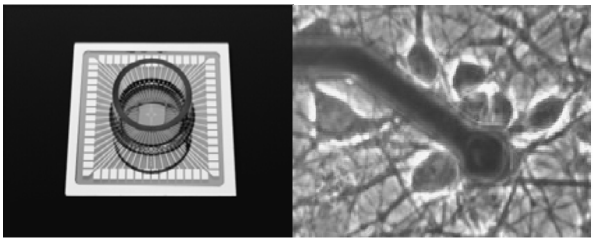

***Figure 2**. **A**: MEA used to record neuronal activity in cortical networks. 60 electrodes are integrated in the bottom at a distance of 100µm of each other. The glass ring glued on top was filled with growth medium. **B**: close up of one of the electrodes and several neurons. Electrode diameter: 10µm. Most electrodes did not pick up signal from more than one neuron.*

Action potentials were continuously recorded by their time of occurrence from 4 cultures for 5-6 weeks in vitro. Time stamps were divided into data blocks (mean: ~30 data blocks per day). In each data block we calculated the conditional firing probability (CFP) for all pairs of electrodes (*i,j*) as the probability of an action potential at electrode *j* at $t=\tau$, given that one was detected at electrode *i* at t=0. Pairs with peaked CFP were considered related. A first order approximation of a Gaussian distribution function was fitted to all CFP curves to obtain values for strength and latency. Relational strength (M) was defined as the maximum CFP above offset, latency

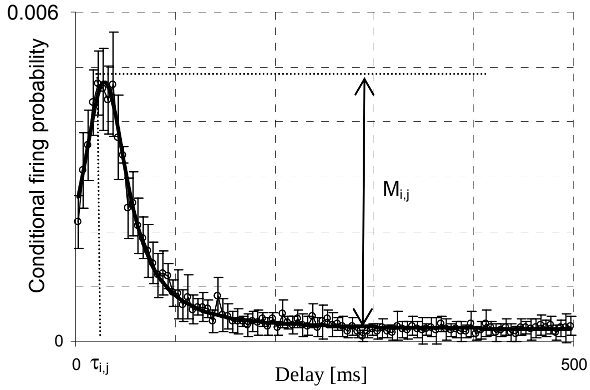

***Figure 3**. Example of conditional firing probability (○,mean ± SD of 5 consecutive bins of 0.5 ms each) and the fitted equation (line), used to obtain values for strength ($M_{i,j}$) and latency ($\tau_{i,j}$) of the relationship between a pair of electrodes (i,j)*

(τ) as the delay at $CFP^{fit}=M$. Figure 3 shows an example of measured data and the fitted curve, used to calculate M and τ.

Long term recordings were divided into data blocks. In each data block we calculated M and τ for all related electrode pairs. To study the development of M and τ we selected pairs that were related in at least 100 data blocks. The M-graph was smoothed using a moving average filter to highlight longer term trends. The filter averaged each point of the curve with its five neighbors on both sides. Figure 4 shows an example of the development of strength and latency of a relationship during more than two weeks.

We focused on periods of at least one day of monotonous decrease of M (weakening of a relationship) or increase (strengthening). Selected episodes had to comprise at least 10 data blocks. An example of such a period is indicated by the gray bar in Figure 4. We examined the latencies of relationships in these periods of monotonous weakening or strengthening.

3 Results

Two cultures (2267 data blocks) showed relationships with long enough periods of strengthening (N=47) or weakening (N=41) that contained at least 10 data blocks.

Figure 5 shows the normalized latency distributions during strengthening and weakening, compared to

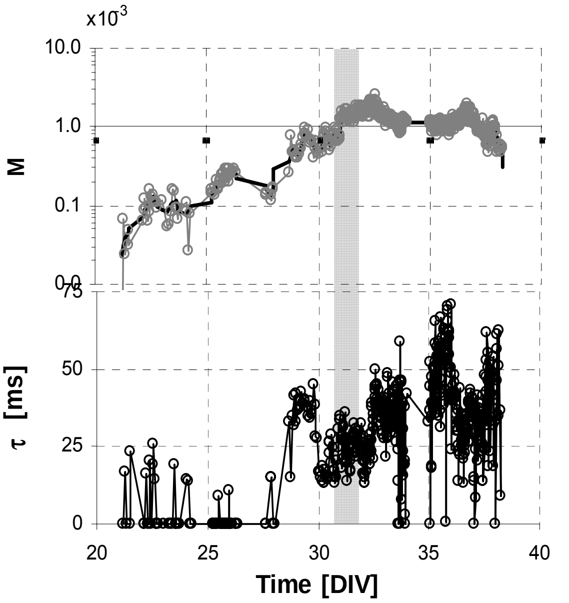

Figure 4. *Example of the development of strength (M) and latency (τ) of a relationship. A long term relationship was divided into data blocks. In each data block strengths and latencies of relationships were determined. Thus, the development of relationships between pairs of electrodes that were related in at least 100 data blocks were observed.*

the overall latency distribution in these cultures. The latency distribution during strengthening slightly exceeded the average distribution around 20 ms. More convincingly, the latency distribution during weakening clearly dropped below average at latencies between 10 and 25 ms. Furthermore, latencies around 40 ms tended to coincide with weakening relationships.

4 Discussion

At 4 weeks in vitro latencies in non-synaptically propagated responses (axonal delay) may have reached ~ 10 ms (Wagenaar et al., 2005). With a synaptic delay estimated at approximately 2 ms (Nakanishi and Kukita, 1998), delays between 10 and 20 ms may well arise from a monosynaptic pathway. The high incidence of low-latency relationships in cortical cultures (see Figure 5) suggests a large contribution of monosynaptic connections.

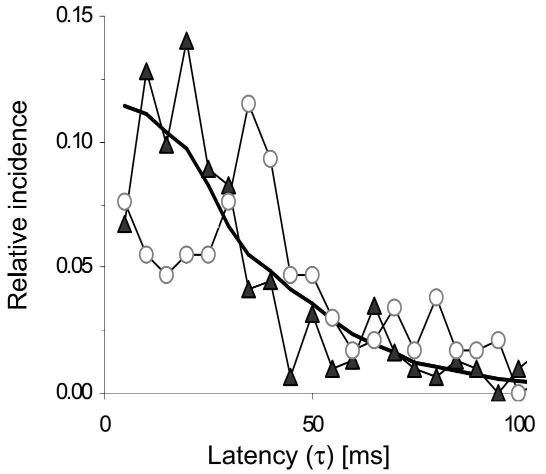

Figure 5*: Distribution of latencies during strengthening (▲) and weakening (○) of relationships. The solid line indicates the general distribution of latencies in these cultures. Bin width: 5 ms.*

Intracellular measurements showed induction of long term potentiation upon activation of the post-synaptic neuron within a ~20 ms time window after presynaptic firing (Zhang et al., 1998). Our results agree with these findings and suggest latency-dependent strengthening of relationships in cortical cultures. Thus, plasticity mechanisms appear, which are driven by the patterns of spontaneous activity, and which on the other hand do influence these patterns. This leads to either never ending changes, or to a kind of balance between activity and connectivity. Generally, this latter option appears more likely as activity patterns in cortical networks reach some kind of stable stage after ~4 weeks in vitro (Kamioka et al., 1996, Chiappalone et al., 2006, van Pelt et al., 2004).

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