Cultured cortical networks described by conditional firing probabilities

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Abstract

Networks of cortical neurons were grown over multi electrode arrays to enable simultaneous measurement of action potentials from 60 electrodes. All possible pairs of electrodes (*i,j*) were tested for synchronized activity. We calculated conditional firing probability ($CFP_{i,j}[\tau]$) as the probability of an action potential at electrode *j* at t=τ, given that a spike was detected at *i* at t=0. If a $CFP_{i,j}[\tau]$ distribution clearly deviated from flat, electrodes *i* and *j* were considered related. A function was fitted to each CFP-curve to obtain parameters for strength and delay.

In young cultures the set of identified relationships changed rather quickly. At 16 days in vitro (DIV) 50% of the set changed within one day. Beyond 25 DIV this set stabilized: during a period of a week more than 50% of the set remained intact. Most individual relationships developed rather gradually. Moreover, beyond 25 DIV relational strength appeared quite stable during periods of ≈ 10 hours, with coefficients of variation (100×SD/mean) of ≈ 25% on average.

CFP analysis provides a robust method to describe the stable underlying probabilistic structure of highly varying spontaneous activity in cultured cortical networks. It may offer a suitable basis for plasticity studies, in which induced changes should exceed spontaneous fluctuations. CFP analysis is likely to describe the network in sufficient detail to detect subtle changes in individual relationships.

Analysis of data continuously recorded for ≈ 6 weeks, showed that highest stability is reached after ≈ 25 DIV, suggesting the 4th and 5th week as a suitable period for plasticity studies.

1 Introduction

To demonstrate learning or memory in cultured neuronal networks one needs to monitor connections between neurons. Most learning studies used electrical stimulation to induce connectivity changes in networks [1, 2]. One of the problems is that spontaneous network activity may mask, or even cancel out induced alterations [3].

Networks may be characterized by 'functional connections' between pairs of electrodes: abstract representations of possibly parallel neuronal pathways between attached neurons [4, 5]. Various techniques have been developed to identify such connections, most of which are based on or related to cross-correlation analysis [4-6]. We developed a method to describe functional connections between all pairs of active electrodes in neuronal networks. The method estimates conditional firing probabilities to calculate parameters for strength and delay in relationships between electrodes. These relationships will serve to provide a stable underlying probabilistic structure in widely varying patterns of spontaneous activity, which may facilitate demonstration of learning or memory.

2 Methods

Networks of cortical neurons (cells obtained from 9 fetal or newborn Wistar rats) were grown over multi electrode arrays to enable simultaneous measurement of action potentials from 60 electrodes. All possible pairs of electrodes (*i,j*) were tested for synchronized activity. We calculated the conditional firing probability ($CFP_{i,j}[\tau]$) as the incidence of an action potential at electrode *j* at delay τ ($0 \le \tau \le 500$ ms) after a spike at electrode *i*, divided by the total number of action potentials at *i*.

To analyze the measured signals, binary arrays X_i were constructed with as many data points as the sampled signals; $X_i[n] = 1$ at a detected action potential and $X_i[n] = 0$ elsewhere.

The number of action potentials at electrode *i* that is followed by a spike at *j* with a delay τ ($N_{follow\ i,j}[\tau]$) is now calculated as:

$$N_{follow_{i,j}}[\tau] = \sum X_i[t] \cdot X_j[t+\tau] \qquad (1)$$

Equation 1 holds because it is applied to binary arrays $X_{i,j}$, with $X_{i,j}[n] \in \{0,1\}$ for all n. CFP[τ] can

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be calculated by dividing $N_{follow}[\tau]$ by the total number of action potentials at electrode i (N_i):

$$CFP_{i,j}[\tau] = \frac{N_{follow_{i,j}}}{N_i} = \frac{\sum_t X_i[t] \cdot X_j[t+\tau]}{\sum_t X_i[t]} \quad \Big| 0 < \tau \le 500 \ m\sec \quad (2)$$

It may be noted that $CFP_{i,j}[\tau]$ is a measure related to cross-correlation ($R_{i,j}[\tau]$):

$$R_{i,j}[\tau] = \frac{1}{N} \sum_{t=1}^{N} X_i[t] X_j[t+\tau] \quad (3)$$

If $CFP_{i,j}[\tau]$ showed a distribution that clearly deviated from flat, electrodes i and j were considered related. Figure 1 shows an example.

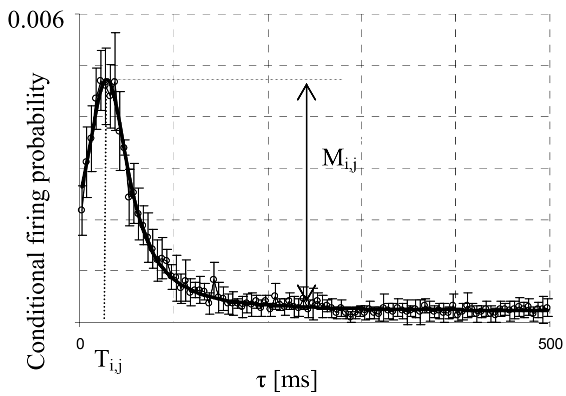

Fig. 1. Example of a conditional firing probability curve, calculated using Equation 2. Fitting Equation 4 yields maximum CFP ($M_{i,j}=4.5 \cdot 10^{-3}$) and delay until this maximum ($T_{i,j}$=29ms). Means ± SD of 10 consecutive values from 0.5 ms bins are shown, data was recorded at *10 DIV*. The maximum probability may seem extremely small. However, this is the probability to record a spike in a 0.5 ms interval. In the above figure, the probability to record a spike at electrode j within 50 ms after an action potential at electrode i can be estimated as $\approx 2 \times 50 \times 0.004 = 0.4$.

Equation 4 was fitted to the shape of the CFP curve.

$$CFP_{i,j}(\tau) = \frac{M_{i,j}}{1 + (\frac{\tau - T_{i,j}}{w_{i,j}})^2} + offset_{i,j} \quad (4)$$

$M_{i,j}$ and $T_{i,j}$ were interpreted as measures for strength and delay of the relationships. We constructed 2 matrices M and T, containing all parameters $M_{i,j}$ and $T_{i,j}$ to describe the whole network. We investigated the stability of the set of relationships in a network and the stability of strength and delay of individual relationships.

To asses the stability of the set of relationships, we divided long term recordings into data blocks with a fixed number of action potentials (33000) and we calculated M matrices for each data block. All nonzero elements in M represented an identified relationship. Similarity between two data blocks (Si) was calculated as the number of relationships that were found in both blocks, divided by the product of the number of relationships in data block A and data block B. To obtain a balanced expression the denominator was squared. Finally, we took the square root of this fraction:

$$Si = \sqrt{\frac{\left|\{M_A \neq 0\} \cap \{M_B \neq 0\}\right|^2}{\left|\{M_A \neq 0\}\right| \cdot \left|\{M_B \neq 0\}\right|}} \quad (5)$$

Consecutively, we calculated similarity indices between each possible reference block and all data blocks. Three examples of thus obtained curves are shown in Figure 2. All curves showed a maximum at the location of the reference block, and decreased in both directions. We defined int50 as the interval around a reference block with >50% of the set of relations intact. Figure 2B shows the development over time of the length of this interval.

Additionally, we grouped the data blocks into series of 15 consecutive blocks. For all relationships that were found in more than 50% of the blocks in a series, we calculated the coefficients of variation (100×SD/mean) of $M_{i,j}$ and $T_{i,j}$.

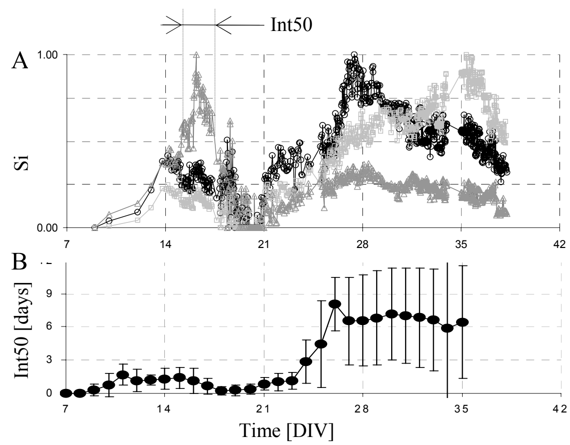

Fig. 2. Development of similarity indices. **A**: typical example of a long term recording, divided into 772 data blocks. Successively, similarity indices (Si, Eq. 5) were calculated for all blocks, using three reference blocks: 16 DIV (Δ), 27 DIV (○), and 35 DIV (□). Similar graphs were constructed using all other data blocks as a reference. Curves were smoothed using a 5th order moving average filter (not shown). Then, we determined Int50: the duration of the interval around each reference point, in which 50% of the set of relations remained intact (Si remained above 0.5). For each culture Int50 was averaged per day. **B**: Averaged graph for all cultures. Standard deviations refer to differences between cultures. Beyond 35 DIV, in two or more cultures the 50% intact interval could not be determined because the end of the long term recording was reached before Si dropped below 0.5. The increase beyond 25 DIV was significant (ANOVA, p<0.01).

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In each series these coefficients of variation were averaged to obtain a measure for stability in that series: CV_M and CV_T.

3 Results

CFP curves

The vast majority of the non-flat relationships in all 9 cultures could be adequately described by Equation 4. Relationships differed widely in both strength and delay. $M_{i,j}$ ranged from $6 \cdot 10^{-6}$ to $6.8 \cdot 10^{-2}$ (approximately following a negative exponential distribution and averaging $(1.0 \pm 1.1) \cdot 10^{-3}$). We found $T_{i,j}$ values between 0 and 250 ms, more than 98% of which were below 100ms. Figure 1 shows an example.

Stability

Long term recordings from four cultures were investigated for stability. In young cultures the set of identified relationships changed rather quickly. At 16 days in vitro (DIV) 50% of the set changed within one day on average. Beyond 25 DIV this set stabilized: during a period of a week more than 50% of the set remained unchanged (See Figure 2B).

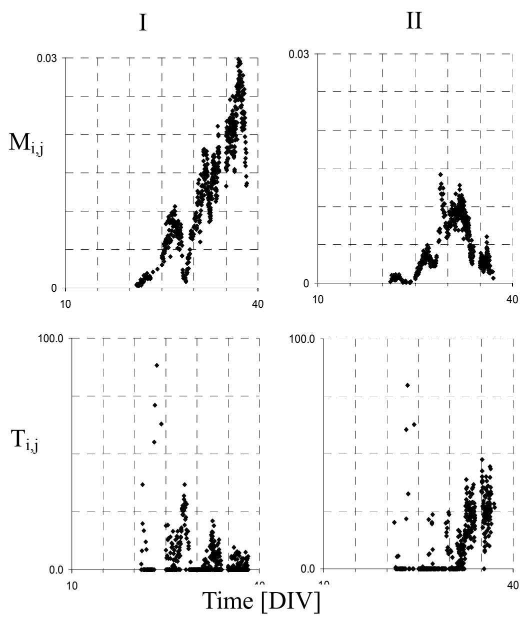

Fig. 3. Examples of development of strength and delay of relations throughout long term recordings. 61 relations that were found in the last 50 data blocks of long term recordings from 4 cultures were selected. The development of $M_{i,j}$ (upper panels) and $T_{i,j}$ (lower panels) of these relations between pairs of electrodes (i,j) was traced back throughout the long term recording. The figure shows examples of two basic types of development: I) shows a relation with increasing $M_{i,j}$ (39% of the relations); in II) $M_{i,j}$ increased first and then decreased (28%). In the other 33%, $M_{i,j}$ fluctuated around a horizontal line.

Most individual relationships showed a rather gradual development on a time scale of days, Figure 3 shows two examples. Moreover, beyond 25 DIV relational strength appeared quite stable during measurement series of 15 data blocks (≈ 10 hours), with coefficients of variation around 25% on average (see Figure 4).

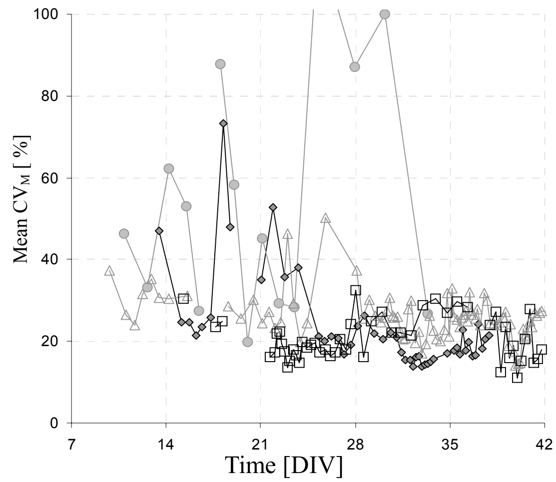

Fig. 4. Development of the average coefficient of variation of relational strength in 4 long term recordings. A long term recording was divided into data blocks. In all data blocks M matrices were determined. Next, data blocks were grouped into series of 15 consecutive blocks. For the set of relationships that were found in at least 50% of the 15 blocks in a series, means and standard deviations were calculated for all $M_{i,j}$. Then coefficients of variation (100×SD/mean) were calculated for each relation in the selected set. The figure shows mean coefficients of variation (CV_M). ♦: culture I, ●: culture II, Δ: culture III, and □: culture IV. Pooled data of all cultures yielded a correlationcoefficient ρ=-0.32. This correlation was significant ($p<0.01$).

CV_M was calculated from the set of relations that were found in at least 50% of the data blocks in a series. The size of this set averaged 54% of the total number of relationships that could be analysed.

CV_T showed much larger values than CV_M, often more than 100%. CV_T also tended to decrease with aging of the culture, but this decrease was not significant.

4 Discussion

CFP analysis provides a robust method to describe the stable underlying probabilistic structure of highly varying spontaneous activity in cultured networks of cortical neurons. The set of identified relationships appeared quite stable. Furthermore, these relationships were quite stable in terms of strength and delay on a timescale of several hours to several days, while development in strength and delay on this time scale were rather gradual. Stability increased with aging of the culture. The high values of CV_T were caused by relationships that had (almost) zero delay in the major part of a series, with one or a few outliers that led to a relatively large standard deviation and thus to a high CV_T.

It is probable that relationships are single abstract representations of multiple pathways between electrodes. This was illustrated by an occasional CFP curve that showed two distinct peaks (<<1%). Our fit algorithm reduced these to a representation with a single peak and thus one strength and delay.

Besides the pathways between electrodes, relationships were also influenced by the surrounding network. Many of the recorded neurons were in excitatory loops, leading to autocorrelations with extra peaks at certain (non-zero) delays. In a linear approach, it has been suggested to deconvolve the autocorrelation out of the CFP curve to obtain a 'synaptic response function' [7]. In this study we did not perform such a deconvolution. However, if a relationship is identified, this does indicate the existence of a neuronal pathway between a pair of electrodes.

CFP analysis may offer a suitable basis for plasticity studies, in which induced changes should exceed spontaneous fluctuations. Furthermore, the analysis is likely to describe the network in sufficient detail to detect subtle changes in individual relationships.

Analysis of data continuously recorded for ≈ 6 weeks, showed that highest stability is reached after ≈ 25 DIV, suggesting the 4th and 5th week as a suitable period for plasticity studies.

5 References

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