Long-term Multielectrode Registration of Neuronal Firing Activity From Rat Cerebral Cortex Tissue In-Vitro

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Abstract– Activity-dependent processes are involved in neurite outgrowth and synaptogenesis. We expect that during neural network formation neuronal morphogenesis and synaptic connectivity are reciprocally dependent on the emerging bioelectric activity in the network. We want to study whether and how bioelectric activity is involved in the formation of network structure. A multielectrode recording facility has been constructed for the long-term registration of action potentials of individual neurons during network development in both organotypic and dissociated rat cerebral cortex tissue cultures. Long-term recordings of action potentials with good signal-to-noise ratios have been obtained. Experiments to correlate these activity levels with quantitative data on neuronal morphological development are in progress. Uncorrelated periodic fluctuations at a time scale of about ten minutes have been observed.

I. Introduction

The development of neuronal networks proceeds by means of neurite outgrowth and synaptogenesis. These processes are dependent on the level of neuronal bioelectric activity (e.g. Kater *et al.*, 1988). A reciprocal relationship can therefore be expected between structural and functional development of neuronal networks (Van Ooyen *et al.*, 1995). We want to establish whether and how the outgrowth of neurons within a connected network depends on their firing activity. For this purpose a multielectrode recording facility has been constructed allowing the simultaneous and long term registration of firing activity from up to 60 single neurons in cultures of cortical neurons.

II. Methods

Multielectrode plates. Electrodes are etched in a hexagonal pattern with a mutual distance of 70 μm onto an indium tin oxide (ITO) plate (Gross *et al.*, 1982; Regehr *et al.*, 1989). A 3 μm layer of methylpolysiloxane resin (HIPEC 643, Dow Corning) was applied by spinning for insulation. At the tips of the electrode leads the insulation was etched by means of a fluor plasma leaving holes of 12 μm diameter.

Tissue culturing. The plates were treated with poly-D-lysine prior to culturing. Both dissociated and organotypic tissue from rat neocortex were cultured on the multielectrode plates. Dissociated rat cerebral cortex cultures were produced as described in Ramakers *et al.* (1991). Organotypic rat visual cortex explants were produced according to Romijn *et al.* (1988). Both types of cultures were kept succesfully in culture for up to four weeks.

Signal processing. The analog signals were amplified and amplitude discriminated for spike detection and converted to TTL pulses. These were time stamped with an accuracy of 0.1 msec by means of the SPIKE2 program using the CED 1401 data acquisition interface (Cambridge Electronic Design).

III. Results

Long-term recordings of multisite firing activity were obtained in both dissociated and organotypic rat neocortex cultures. Stability of action potential shapes over periods of weeks demonstrated the reliability of recording firing activity of individual neurons. The firing patterns obtained from the organotypic explants showed similar burst-like behavior as has been reported for single electrode extracellular recordings (Corner, 1994) with a strong synchronicity between the different electrodes. Also the preliminary recordings from the dissociated cultures revealed a complex pattern of action potential clustering at different time scales. Clustering was observed at time scales of several milliseconds, of several hundreds of milliseconds and of about ten minutes. A surprising observation was that the latter type of clustering behavior was caused by periodic fluctuations in the firing frequencies with different periods for the different electrodes. Continuous recordings from individual electrodes revealed stable firing activity for up to at least 14 hours and persistent differences in firing frequencies between different recording sites.

IV. Conclusion

The multielectrode recording facility has shown to succesfully registrate extracellular action potentials with good signal-to-noise ratios (typically about five to ten) for long periods of time (weeks) in both dissociated and organotypic cortical tissue cultures. The recorded trains of action potentials had time structures similar to those measured with convential single electrodes. A periodicity in firing rates at a time scale of about ten minutes is observed. Clear differences in the phase and period length between different sites indicate that these periodic fluctuations originate from uncorrelated mechanisms operating at the different neurons. Long-term and persistent differences in mean firing frequencies have been observed between neurons. Studies are now in progress to correlate the firing behavior of individual neurons with their morphological development by staining the neurons positioned at the electrodes and reconstructing and quantifying their dendritic and axonal branching patterns.

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References

1. S.B. Kater, M.P. Mattson, C.S. Cohan, J. Connor. Calcium regulation of the neuronal growth cone, *Trends in Neurosc.*, vol. 11, pp. 315-321, 1988.
2. A. van Ooyen, J. van Pelt and M.A. Corner, "Implications of activity-dependent neurite outgrowth for neuronal morphology and network development," J. Theor. Biol. vol. 172, pp. 63-82, 1995.
3. G.W. Gross, W.Y. Wen, and J.W. Lin, "Transparent indium-tin oxide electrode patterns for extracellular, multisite recording in neuronal cultures," *J. Neurosc. Meth.*, vol. 15, pp. 243-252, 1985.
4. W.G. Regehr, J. Pine, C.S. Cohan, M.D. Mischke, and D.W. Tank. "Sealing cultured invertebrate neurons to embedded dish electrodes facilitates long-term stimulation and recording". *J. Neurosci. Methods*, vol. 30, pp. 91-106, 1989.
5. G.J.A. Ramakers, F. Raadsheer, F. Ramaekers, and F. van Leeuwen, "Development of neurons and glial cells in cerebral cortex cultured in the presence or absence of bioelectric activity: morphological observations". *Eur. J. Neurosc.*, vol. 3, pp. 140-153, 1991.
6. H.J. Romijn, B.M. de Jong, and J.M. Ruijter, "A procedure for culturing rat neocortex explants in a serum-free nutrient medium", *J. Neurosc. Meth.*, vol. 23, pp. 75-83. 1988.
7. M.A. Corner, "Reciprocity of structure-function relations in developing neural networks: the Odyssey of a self-organizing brain through research fads, fallacies and propects", in *Progress in Brain Research, Vol. 102, The Self-Organizing Brain: From Growth Cones to Functional Networks*, J. van Pelt, M.A. Corner, H.B.M. Uylings and F.H. Lopes da Silva, (Eds.), Elsevier, Amsterdam, pp. 3-31, 1994.