培養型プレナーパッチクランプの製作と自発的シナプス電流測定への応用

Fabrication of incubation type planer patch clamp device and its application to the spontaneous synapse current measurements

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Realization of the high-performance high-throughput screening device with the neuron network has been desired for long time for the cause analysis and drug discovery with the intractable diseases of the cranial nerve. Incubation type planar patch clamp which is potentially high throughput and can be applied for the neuron network. This technique had an important technical problems at the first proposed stage as follows; 1) how to reduce the large base line noise due to the low seal resistance, and 2) how to keep the soma of the target neuron on the micro through hole of the patch clamp chip for the long incubation time. These challenges are solved in this work as follows; 1) The base line noise has been significantly reduced by replacing the conventional Ag/AgCl electrode with the salt bridge-type Ag/AgCl electrode. and 2) the migration of the neuron has been hindered and the soma has been kept on the micro-through hole for the long incubation time by the cell cage structure, which surrounds the soma by the micro pillars. The cell cage chips have been fabricated by using the silicon on insulator(SOI) substrates and forming the micro-structures by the semiconductor micro-fabrication process (Bosch process). After forming the rat hippocampus neuron network by the three weeks of primary culture on the surface of the cell cage chip, spontaneous synapse channel currents have been successfully measured by using the Axon patch clamp amplifier as shown in Fig. 1. The observed channel currents have been analyzed and assigned clearly to the miniature excitatory post synapse current (mEPSC) mediated by AMPA receptors.

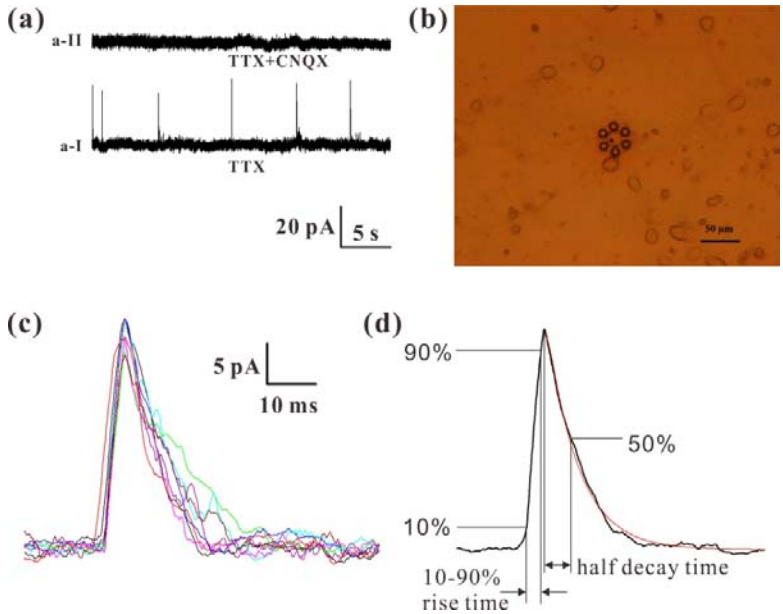

Fig. 1.(a) Observed spontaneous channel current recordings. (a-I) TTX (1μM) is added to the bath solution and the membrane potential (Vm) is 12.6 mV, (a-II) TTX (1 μM) + CNQX (25 μM) is added to the bath solution and the Vm is 16.0 mV. (b) Bright field image of neuron after 24 days culture observed before channel current measurement (a). Scale bar is 50 μm. (c) eight examples of overlaid channel current waveforms of (a-I). (d) average of channel current waveforms in (c) . A single exponential fit is overlaid. The 10-90% rise time is 3.6 ms and the half decay time is 6.2 ms.