

Electrophysiology course

Yuko Sekino (associate prof., Univ. Tokyo)

Hiroki Yasuda (lecturer, Gunma Univ.)

[Purpose]

The central nervous system has wide range of function including learning, thinking etc. and these are all based on synaptic transmission in the brain. To investigate synaptic transmission, we record electrical activity in the CNS not only from the whole brain of live animals, but also from brain slice preparations. In this course, you practice recording synaptic responses and synaptic plasticity in hippocampus using the MED (Multi Electrode Device?) 64 System and an optical system with voltage sensitive dyes.

[Methods]

slice preparation

① making extracellular solutions

The extracellular solution (ACSF) we usually use is, 119 mM NaCl, 2.5 mM KCl, 4.0 mM CaCl₂, 4.0 mM MgSO₄, 1.0 mM NaH₂PO₄, 26.2 mM NaHCO₃, 11 mM glucose. Before use, the solution should be bubbled well with 95% O₂/ 5% CO₂ gas.

② making slices

1. before dissecting

Cool down ACSF to less than 4°C, bubble with 95% O₂/ 5% CO₂ gas. Fill an incubating chamber with ACSF (at room temperature) and keep bubbling.

2. dissecting hippocampus (rapidly and gently!!)

i) Anesthetize a rat with isoflurane in a bell jar

ii) When the rat become unconscious, decapitate the head, remove skin and skull over the brain. Take the brain and put it in cold ACSF immediately.

iii) After keeping the whole brain in cold ACSF for several minutes, put it on a wet paper on a cold Petri dish.

iv) Cut the brain in half and remove brainstem and thalamus, because these tissues are covering hippocampus.

v) Slide a spatula between hippocampus and cortex and turn hippocampus over with the spatula. Remove tissues covering hippocampus, then you are done with dissecting hippocampus!! Keep dissected hippocampal tissue in cold, bubbled ACSF.

3. Cutting slices

- i) Put hippocampus from both hemispheres in an agar holder. Glue the agar holder to a cutting chamber, fill the chamber with cold ACSF and keep bubbling.
- ii) Place the chamber in a slicer and start cutting hippocampal slices.
- iii) Keep slices in an incubating chamber.
- iv) After you are done with cutting all the slices, keep the incubating chamber in a water bus at 30°C for a while. After that, you can keep the incubating chamber at room temperature.

Recording synaptic responses.

- ① electrophysiological recording with the MED64 system
- ② optical electrophysiological recording with a voltage sensitive dye.