

30. Methods

3001 Static and perfusion culture of adult neocortex

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When 400 microns-thick adult neocortical slices were incubated in a commercially-available CO₂ incubator with D-MEM/F-12, field potentials evoked by underlying white matter stimulation disappeared within 4 hours. On the other hand, a recording over long hours was possible by continuous culture medium perfusion. We found that several hours were needed until humidity and gas composition in the dish reached an equilibrium with the atmosphere inside the incubator by the previous method. By improving the incubating system so that adequate humidity and pH was obtained, field responses was obtained even in static medium.

3002 GENE TRANSFER SPECIFIC FOR BRAIN USING MICROGLIAL CELL LINES

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We found that microglia had a specific affinity and migrating ability to the brain. When microglial line cells labeled with a fluorescent dye were injected into the aorta of an adult male Fisher rat, many fluorescent cells were observed in the brain and few were seen in the liver. These cells also migrated into the brain of E13 embryo when injected via exo utero. However, labeled macrophages were observed in the liver but few or no such cells in the brain. To transfer a gene into brain using our novel technique, microglial line cells were transfected with a lacZ gene expression vector and were injected into aorta of rats. When the brain sections were stained with X-gal, many blue cells were observed throughout the brains prepared from rats injected with the cells carrying a lacZ gene expression vector. Therefore, this system can be applied for a specific transfer of biologically valuable substances such as enzymes and genes into the brain.