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Protocol Article

A protocol for preparation and transfection of rat entorhinal cortex organotypic cultures for electrophysiological whole-cell recordings

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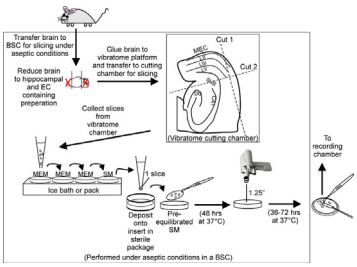
GRAPHICAL ABSTRACT



ABSTRACT

Understanding how neuromodulators influence synaptic transmission and intrinsic excitability within the entorhinal cortex (EC) is critical to furthering our understanding of the molecular and cellular aspects of this region. Organotypic cultures can provide a cost-effective means to employ selective molecular biological strategies in elucidating cellular mechanisms of neuromodulation in the EC. We therefore adapted our acute slice model for organotypic culture applications and optimized a protocol for the preparation and biolistic transfection of cultured horizontal EC slices. Here, we present our detailed protocol for culturing EC slices. Using an *n*-methyl-D-glucamine (NMDG)-containing cutting solution, we obtain healthy EC slice cultures for electrophysiological recordings. We also present our protocol for the preparation of "bullets" carrying one or more constructs and demonstrate successful transfection of EC slices. We build upon previous methods and highlight specific aspects in our method that greatly improved the quality of our results. We validate our methods using immunohistochemical, imaging, and electrophysiological techniques. The novelty of this method is that it provides a description of culturing and transfection of EC neurons for specifically addressing their functionality. This method will enable researchers interested in entorhinal function to quickly adopt a similar slice culture transfection system for their own investigations.

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Method details

Animal Welfare Declaration

Animal procedures described here conformed to guidelines approved by the University of North Dakota Animal Care and Use Committee.

Materials

General resources

- Chemical fume hood
- Isoflurane
- Bell jar
- Ice bucket
- 50 mL and 15 mL conical tubes
- Microcentrifuge tubes
- Biological safety cabinet (BSC)
- Vibratome (VT1000, Leica)
- Cyanoacrylate glue
- Millicell culture inserts (#picmorg50, Millicell)
- 40 × 11 mm petri dishes or 6-well plates
- 100 × 20 mm petri dish
- Ice packs
- 1 mL transfer pipette
- Sterile transfer pipette tips
- Sterile package of 18 in. × 26 in. surgical draping (4410-imc, IMCO products)
- 1 250 mL beaker
- 1 30 mL beaker
- 1 20 mL beaker
- 37 °C incubator
- Biorad Gene-Gun Low-Pressure System (#1652451, Biorad)
- Polyvinylpyrrolidone (PVP, #PVP360-100G, Sigma)
- Spermidine (#S2501-1G, Sigma)
- 1.6 μm gold microcarriers (#1652264, Biorad)
- Tefzel Tubing (#1652441, Biorad)
- Molecular biology grade ethanol
- Mini centrifuge (*e.g.* Daigger Sprout Mini-Centrifuge)
- Nitrogen tank
- Helium tank
- Serological pipettes
- Pipette aid
- Vacuum and syringe filters (0.22 μm)