Jul 12, 2019

Mouse Stellate- Intracellular recording

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1 Works for me dx.doi.org/10.17504/protocols.io.2qfgdtn

scott john

1 See mouse stellate iosolation protocol

2 Isolated mouse stellate pinned to clean Sylgard perti dish

3 Continuously superfuse isolated stellate (6–7 ml/min) with PSS (32–35°C).

4 Impale individual neurons using microelectrodes filled with either 2M KCl (60–120 MΩ) or 2M KCl + 2% Neurobiotin (80-160 MΩ; Vector Labs).

5 Record intracellular membrane voltage using a Multiclamp 700B amplifier coupled with Digidata 1550B data acquisition system and pCLAMP 10 software (Molecular Devices, Sunnyvale, CA).

6 Characterize responsiveness of membrane potential to membrane physiology. Depolarizing current steps (0.1–0.5 nA amplitude, 500-ms or 1-s duration) are used to assess neuronal excitability.Cells are classified as either phasic (<2 APs), bursting (2-5 spikes in short burst), or tonic (>5 spikes continuously) based on the number of APs elicited by the intracellular current. Hyperpolarizing current steps (500 ms) of increasing amplitude are used to test for rectification in the current-induced hyperpolarization.Action potential (AP) amplitude and duration are measured from spontaneous or nerve evoked APs.After-hyperpolarization amplitude and duration were measured from brief intracellular current injections (100-500pA, 5ms), spontaneous APs, or nerve evoked APs.

7 Graded stimulus shocks (100 µs) delivered from the concentric bipolar electrodes to identified nerves in 50-100µA steps, from 800uA to 0µA were used to generate a stimulus recruitment curves (AMPI Master 8 and IsoFlex optical Isolation unit). 5-20 stimuli were delivered at each stimulus intensity, with an interval of 3 sec between stimuli. Analysis of synaptic events focused on EPSP latency and jitter (SD of latency) as indices of conduction, path and release.