See mouse stellate isolation protocol

Isolated mouse stellate pinned to clean Sylgard perti dish

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

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

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

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.

Graded stimulus shocks (100 µs) delivered from the concentric bipolar electrodes to identified nerves in 50-100µA steps, from 800µA 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.