Pharmacogenetic and optogenetic induction of local tonic cortical activity in anesthetized mice

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Sleep, waking and anesthesia entail distinct patterns of cortical activity, but the role of specific neuronal subpopulations in brain-state regulation remains unclear. We employed pharmacogenetic and optogenetic methods to selectively induce local, long-lasting depolarization in genetically-defined neuronal populations across behavioral states. Pharmacogenetic methods based on Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) utilize mutated M3 muscarinic receptors ("hM3Dq") that are unable to bind endogenous ligands but induce long-lasting depolarization upon binding of clozapine-N-oxide (CNO), an otherwise inert synthetic ligand. Stable Step-Function Opsins (SSFOs) are a novel variant of Channelrhodopsin 2 (ChR2-C128S/D156A) that depolarize neural membranes for up to 30 minutes following a single pulse of blue light.

Adult male CamKIIα-Cre mice (n=6) were injected intracortically with rAAV (0.5-2μL) in the right parietal / frontal lobe to induce local Cre-dependent expression of hM3Dq or ChR2-C128S/D156A in excitatory forebrain neurons, as was confirmed histologically. Microwire arrays (16-ch) were implanted bilaterally to record neuronal activity in the injected and contralateral control hemispheres. Following recovery, experiments were conducted under isoflurane (1.5%) or ketamine/xylazine (87 mg/kg and 13 mg/kg) anesthesia at steady levels of burst-suppression and slow waves, respectively. After a 10-minute baseline period, CNO (5 mg/kg) was administered i.p. or laser stimulation (473nm) was delivered intracortically. Local field potentials and neuronal activity were recorded continuously along with epidural EEG, EMG, video, and behavioral observations. Spike sorting was performed offline.

Under isoflurane anesthesia, CNO injection induced tonic spiking activity of multiple individual neurons in the injected hemisphere that began after 5-10 min and lasted throughout the experiment (~60 min), while light pulses induced immediate, long-lasting tonic firing. In both cases, the control hemisphere remained in a burst-suppression mode. Under ketamine/xylazine anesthesia, CNO injection induced tonic firing in neurons of the injected hemisphere, while robust slow wave oscillations persisted in the control hemisphere. Control experiments (saline injection instead of CNO; CNO/light pulses without prior rAAV injection) failed to induce local tonic firing under either type of anesthesia. Thus, activity of individual neurons can be decoupled from the global brain state, providing an opportunity for addressing the role of specific neuronal subtypes in state-dependent cortical network activity.

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