Persistent-Firing Neurons in Layer II/III of Rat Perirhinal Cortex

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Abstract

Persistent memory is essential for adaptive behavior and cognition. One theory of transient memory is based on single-neuron bistability. Here we describe such a phenomenon in neurons recorded in layer II/III of rat perirhinal cortex. In brain slices bathed in solutions containing a muscarinic agonist, 83% of perirhinal neurons showed persistent firing—defined as repetitive spiking that lasted tens of seconds to several minutes and could be reliably terminated by an inward current step. PF was relatively insensitive to injected current noise, was strictly dependent on calcium-activated non-specific cation channels, and only occurred following activation of muscarinic cholinergic receptors (m1 – m3). PF was not abolished by blocking excitatory and inhibitory synaptic transmission. Two-photon imaging revealed that intracellular calcium ion concentrations remained elevated throughout the PF period. In some neurons, the level of PF was a monotonically increasing function of the amplitude of the initiating synaptic excitation. PF may contribute to a transient memory system that is enabled and sustained by cholinergic input.

Introduction

The phenomenon of persistent firing (PF, Egorov et al, 2002, 2006; Leung et al, 2006, Frank & Brown, 2003; Major & Tank, 2004) has important theoretical implications for understanding working or short-term memory systems. This continuous activity persists long after the offset of a depolarizing current step and shows an increase in firing frequency with increasing depolarizing current steps. PF is a plausible cellular mechanism for constructing a temporary “hold memory” that does not depend on synaptic modifications. PF has been studied in layer V neurons of the entorhinal cortex (EC) and lateral nucleus of amygdala following exposure to a cholinergic agonist. Here we report PF in neurons of layer II/III of perirhinal cortex (PR). This paper addresses the neurophysiology, neuropharmacology, and noise sensitivity of PF in layer II/III PR neurons.

Methods

Slice Preparation

Methods are described in detail elsewhere (Moyer & Brown, 2007). Briefly, male Sprague-Dawley rats (P 17–21 days) were deeply anesthetized with sodium thiopental and decapitated. The brains were quickly removed and placed in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 5.4, MgSO4 1.25, CaCl2 2.5, NaHCO3 25, NaH2PO4 1.25, and D-glucose 10 (for about 3 minutes). The brain was sliced (300 microns) and glued to the interface of a vibratome (Vibratome Instruments, St. Louis, MO). Slices were immediately immersed in a 24-well chamber maintained at room temperature (22 – 25°C) for 2 hours. The exposed surface of the slice was covered with physiological saline (22 – 25°C) at a rate of 1 ml/min. The ACSF contained in multiwell plates (1.24 NaCl, 2.5 KCl, 2.5 NaHCO3, 1.25 NaH2PO4, 26 NaH2SO4 and 10 D-glucose). pH 7.4, 28 °C. Individual slices were then transferred to a submersible-type recording chamber and were perfused with physiological ACSF maintained at 37°C using an automatic temperature controller (Water Instruments).

Electrophysiological Recordings

PF was visualized using an upright microscope (Olympus BX51) equipped with infrared-filtered light and differential interference contrast (DIC) optics, and a Hamamatsu C4080 video-camera and color video monitor. The image was transferred to a computer where it was displayed in video-mode to observe the spontaneous activity or responses to various types of stimuli. Slices were placed in a standard slice chamber consisting of two chambers (one for ACSF-containing and the other for ACSF-free ACSF) separated by a thin filter. The ACSF was maintained at 34°C. PF was observed in layers II/III of PR neurons contralateral to site 56 (center of the tissue). Recordings were simultaneously taken from EC. Non-pyramidal responses were classified as 0.5 – 4 sec current steps. All images seen both applied to the dorsal surface from ACSF solution (defined as calcium-sensitive; pyramidal, granular, or dentate). All images seen both applied to the ventral surface from ACSF solution (defined as calcium-insensitive; pyramidal, granular, or dentate). Voltage was recorded with a < 1 nS liquid junction potential between the bath and patch pipette solution (Moyer et al, 2008).

Calcium Imaging

Calcium imaging was performed using a custom built two-photon laser scanning microscope (TPLSM). Both a calcium-sensitive dye (100 µM Fluo-4 pentapotassium salt, Molecular Probes, Eugene, OR) and an Alexa 594 dye (20 µM Alexa Fluor-594 hydrazide (AF-594), Molecular Probes, Eugene, OR) were added to the recording pipette solution. Neurons were allowed to fill with dye for 5 – 10 min before imaging. Images were acquired at 800 nm and fluorescence was detected using two of these two-photon fluorescent tubes. Electrophysiological and optical data were simultaneously acquired. Fluorescence images were acquired by using a line scan (~2µm/scan) of the soma or proximal dendrite (~20µm from the soma). Changes in calcium fluorescence were quantified as percent ΔF/ΔF0 using the ratio of fluorescence intensity in the absence and presence of the depolarizing current.

Noise generation

Noise was generated by DSP 2, Real-Time Processor (Tucker-Davis Technologies, Inc. Alachua, FL). The program can be downloaded for free from the authors website (http://www.smallbrainsoft.com). The program was used to produce time-varying light signals by modulating the frequency of a sinusoidal input signal. Noise was then added to a continuous light signal and was digitally filtered with a series of two infinite impulse response filters using a root mean square error criterion. Noise was digitally filtered with a series of two infinite impulse response filters using a root mean square error criterion. Noise was then added to a continuous light signal. The filtered noise was used as a command voltage for Axopatch 200B. Noise was introduced to the cell 60 – 120 s before the first threshold crossing and was present during the whole recording session.

Results

• Perirhinal neurons in layer II/III exhibit cholinergic-dependent PF—similar to that found in EC layer V and LA.
• PF is an intrinsic property of neurons and can be evoked despite synaptic input blockade. Injected noise failed to disrupt persistent firing.
• The results suggest that PR neurons, similar to EC neurons, can exhibit a transient and non-synaptic “memory” of excitation. PF in PR neurons could be important for traces of excitation following conditioning of the temporal gap between the offset of the conditional stimulus and the onset of the unconditional stimulus.

Discussion

Perirhinal neurons in layer II/III exhibit cholinergic-dependent PF—similar to that found in EC layer V and LA.

References