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

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Abstract

Transient 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 (114/137) showed "persistent firing"—defined as repetitive spiking that greatly outlasts the spike-initiating current. Persistent firing (PF) was initiated by an outward current step or an excitatory synaptic input. It lasted tens of seconds to several minutes and could be reliably terminated by an inward current step. PF was relatively insensitive to injected 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 levels remained elevated throughout the PF period. In some neurons, the rate of PF was a monotonically increasing function of the amplitude of the spike-initiating current. This graded PF could theoretically maintain analog information about the size 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 mechanisms. 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 "sample-and-hold" memory circuit 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 pherirhinal cortex (PR). This poster 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 (PN 13-30 days) were deeply anesthetized with halothane and decapitated. The brain was quickly removed and placed in ice-cold oxygenated (95% O2/ 5% CO2) sucrose-aCSF containing (in mM): 206 sucrose, 2.8 KCl, 1 CaCl2, 1 MgCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3 and 10 D-glucose for about 3 minutes. The brain was blocked and glued to the tray of a vibratome. Coronal slices (300 mm) containing perirhinal cortex were cut at ~1°C using a temperature-controlled vibratome (Vibratome 3000). Slices were immediately moved into a 24-well slice incubation chamber maintained at room temperature (22 – 26°C) for at least 1 hour. The oxygenated incubation aCSF contained (in mM): 124 NaCl, 2.8 KCl, 2 CaCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3 and 10 D-glucose, pH 7.4, 295 mOsmol. Individual slices were then transferred to a submerged-type recording chamber and were perfused with oxygenated aCSF maintained at 31°C using an automatic temperature controller (Warner Instruments).

Electrophysiological Recordings

PR was visualized using an upright microscope (Olympus BX51) equipped with infrared-filtered light, differential interference contrast (IR-DIC) optics, and a Hamamatsu C2400 video camera and video enhancement device. Slices were matched to sections corresponding to plates 34-45 of a rat stereotaxic atlas (Paxinos & Watson, 1998). In some experiments, a bipolar stimulating electrode, positioned using a 4X objective, was placed in layer I of PR. Whole-cell recordings were obtained from visually selected PR neurons restricted to area 36 (dorsal of the rhinal sulcus). Recordings were unambiguously not from EC. Neurophysiological responses were examined to 0.5 - 4 sec current steps. All drugs were bath applied at the desired concentrations from stock solutions prepared in distilled water (carbachol; atropine; pirenzipine; 4-DAMP; gallamine) or DMSO (AFDX-116; PD102807). Voltages were corrected for a +13 mV liquid junction potential between the bath and patch pipette solution (Moyer et al., 2002).

Calcium Imaging

Calcium-dye 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 a calcium-insensitive 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. Dyes were excited at 810 nm and fluorescence was detected using one of two external photomultiplier tubes. Electrophysiological and optical data were synchronously acquired. Fluorescent images were acquired by using a line scan (~2ms/scan) of the soma or proximal dendrite (~20μm from the soma). Changes in calcium fluorescence were quantified as percent DF/F where DF is the change in fluorescence during neuronal activity, and F is resting fluorescence levels before synaptic stimulation.

Noise generation

Noise was generated by RP2.1 Real-Time Processor (Tucker-Davis Technologies, Inc. Alachua, FL). A virtual circuit controlled by a custom software written in Delphi (Borland Software Corporation, Cupertino, CA) was used to produce waveform containing hyperpolarizing and depolarizing current steps combined with noise. Noise was produced by a Gaussian noise generator component and was digitally filtered with a series of two biquad filters: high-pass 1Hz and low-pass 20 Hz, 50 Hz, or 500 Hz. The generator was tuned to obtain a signal with amplitude SD = 20 pA, or 50 pA. Noise was added to depolarizing (2 s 100pA) and hyperpolarizing (5 s 150pA) current steps. This complex waveform was used as a command voltage for Axopatch 200B. Noise was introduced to a cell 60 – 120 s before the first current pulse and was present during the whole recording window.

Figure 1. Location of recording and stimulating electrodes

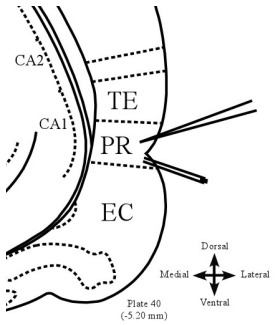


Figure 2. Whole-cell recording and calcium fluorescence changes.

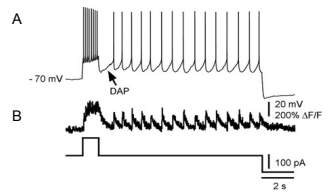


Figure 5. Synaptic stimulation elicits PF

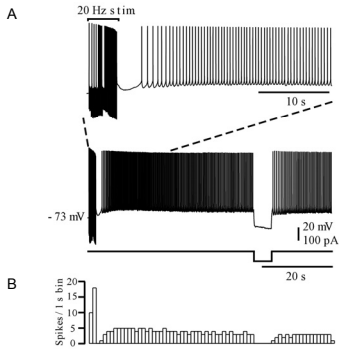


Figure 3. Stimulus intensity-dependent PF

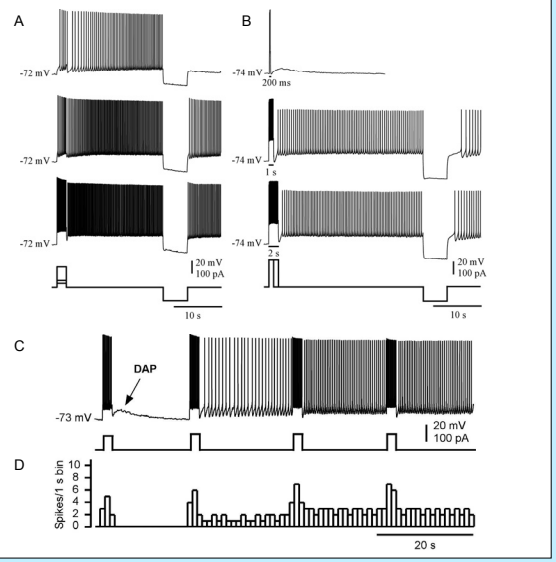


Figure 4. Muscarinic antagonists (M1-M3) and flufenamic acid inhibit PF

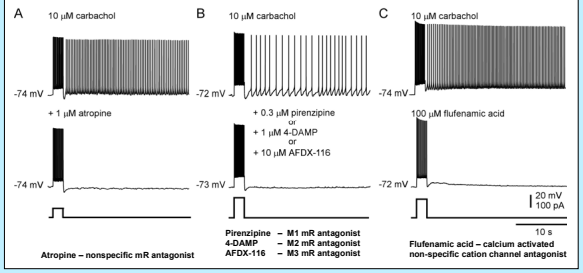


Figure 6. GLU and GABA antagonists effect on synaptic responses and PF.

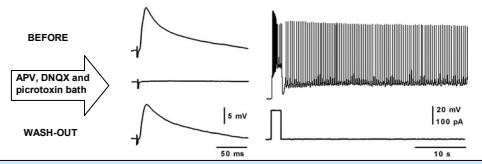
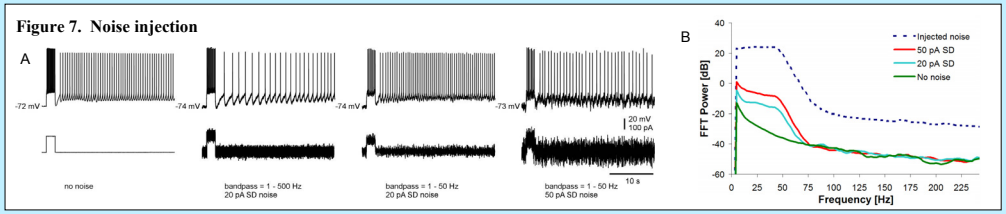


Figure 7. Noise injection



Results

- **In the presence of carbachol 83% (114/137) of perirhinal neurons exhibited "persistent firing" (Figures 2-5).**
- **Dual-photon calcium imaging revealed that intracellular Ca^{2+} levels remained elevated throughout the persistent-firing period (Figure 2A, B).**
- **Activity persisted for tens of seconds to several minutes after the offset of the depolarizing current. The level of persistent firing depended on the intensity and duration (Figure 3A, B) of the current step. Repetitive depolarizing current steps increased the rate of persistent firing (Figure 3C). Hyperpolarizing current steps terminated persistent firing or decreased the firing rate.**
- **Persistent firing is dependent on activation of muscarinic receptors. Atropine (1 μM), pirenzipine (0.3 μM), AFDX-116 (10 μM), and 4-DAMP (1 μM) blocked persistent firing (Figure 4A-B)..**
- **Flufenamic acid (100 μM), the calcium-activated non-specific cation (CAN) channel antagonist, blocked persistent firing in PR neurons (Fig. 4F).**
- **Synaptic stimulation of PR layer I is sufficient to elicit persistent firing in PR layer II/III neurons (Figure 5).**
- **Synaptic input is not necessary to maintain a persistent firing. Persistent firing was elicited in the presence of glutamatergic antagonists (50 μM APV , 10 μM DNQX) and GABAergic receptor antagonist (100 μM picrotoxin, Figure 6).**
- **PF was relatively insensitive to injected current noise (Figure 7).**

Discussion

- **Perirhinal neurons in layer II/III exhibit cholinergic-dependent PF—similar to that found in EC layer V and LA.**
- **PF in PR 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 in trace fear conditioning by filling the temporal gap between the offset of the conditional stimulus and the onset of the unconditional stimulus.**

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