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PHARMACOLOGICAL STUDY OF THE ONE SPIKE SPHERICAL NEURON PHENOTYPE IN *GYMNOTUS OMARORUM*

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Abstract—The intrinsic properties of spherical neurons play a fundamental role in the sensory processing of self-generated signals along a fast electrosensory pathway in electric fish. Previous results indicate that the spherical neuron's intrinsic properties depend mainly on the presence of two resonant currents that tend to clamp the voltage near the resting potential. Here we show that these are: a low-threshold potassium current blocked by 4-aminopyridine and a mixed cationic current blocked by cesium chloride. We also show that the low-threshold potassium current also causes the long refractory period, explaining the necessary properties that implement the dynamic filtering of the self-generated signals previously described. Comparative data from other fish and from the auditory system indicate that other single spiking onset neurons might differ in the channel repertoire observed in the spherical neurons of *Gymnotus omarorum*.

Key words: intrinsic properties, electroreception, time coding, low responsiveness window, onset neuron, single spiking.

INTRODUCTION

Pulse gymnotiforms generate brief and complex electric fields by the activation of specialized electric organs (Lissmann, 1958). The presence of objects of conductance different from water modifies such fields determining changes in the pattern of self-generated transcutaneous electric currents (Lissmann and Machin, 1958). These object-dependent changes of the pattern, so called electric images, inform the fish about the intrinsic properties of such objects as conductance, shape and size and also about its position relative to the fish's body (Caputi et al., 2008). Different types of electroreceptors are specifically tuned to the local waveform of the self-generated electric organ discharge, originating to two parallel electrosensory pathways (fast and slow respectively, Szabo, 1974) which encode electric images differently.

The fast electrosensory pathway begins with a type of electroreceptors known as ''pulse markers'' as they fire a single spike per electric organ discharge. The latency of this single spike encodes the amplitude of the local field (Szabo, 1974; Bastian, 1977; Watson and Bastian, 1979). Pulse markers trifurcate and each branch projects through mixed synapses on second-order neurons (spherical neurons) contained in the three external sensory maps present at the electrosensory lobe (referred to as centro-medial, centro-lateral and lateral, Szabo, 1974; Maler, 1979; Castello et al., 1998). The axons of spherical cells arising from these three maps converge, through the lateral lemniscus, on a single map in the mesencephalic magnocellular nucleus, where coincidence detection is proposed to occur.

The fast electrosensory path can be electrophysiologically traced, in the freely discharging fish, as a sharp compound action potential running from peripheral nerves to the magnocellularis nucleus at the mesencephalon (Castello et al., 1998). Although this compound action potential is precisely phase locked with the EOD, its amplitude and duration show variations reflecting the degree of firing synchrony of the fiber population (Castello et al., 1998). At the mandibular nerve the amplitude of the compound action potential is independent of the interval between sensory stimuli, but at the lateral lemniscus it decreases as the interval between sensory stimuli shortens. This phenomenon was called post activation low responsiveness window (Castello et al., 1998). At short delays (5–25 ms dependent on intensity) paired-pulsed stimulation of the skin shows an absolute absence of response to the second stimulus. Beyond this limit the amplitude of the response to the second stimulus increases as a function of the delay (Castello et al., 1998). This low responsiveness window is not due to a corollary discharge (Castello et al., 1998) but depends on the intrinsic properties of the spherical neurons itself (Nogueira et al., 2006). The interplay of the central regulation of the pacemaker firing rate and this post activation change in path responsiveness behaves as a dynamic filter, favoring the self-generated signals to the detriment of interfering events (Nogueira and Caputi, 2011; Caputi, 2012).

The second-order neurons of the fast electrosensory pathway are pausi-dendritic spherical cells that characteristically show an asymmetric voltage response

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Abbreviations: 4AP, 4-aminopyridine; TEA, tetraethylammonium; DTX, dendrotoxin.

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to intracellular rectangular current pulses of equal intensity and opposite polarity. For above-threshold depolarizing pulses the neuron fires a single action potential, precisely phase locked with the stimulus onset. Subthreshold pulses produce an early voltage hump peaking at about 2 ms (Nogueira et al., 2006; Fig. 1A). This response is similar to that observed in the fast auditory pathways of birds and mammals (Manis and Marx, 1991; Bal and Oertel 2000, 2001).

This article shows that the K^+ channel blocker 4-aminopyridine (4AP) turned the onset profile of the spherical neuron into a repetitive firing pattern. In addition, 4AP reduces the long refractory period that is responsible for the functional role of these neurons. This indicates that the intrinsic properties of spherical neurons are dominated by a low-threshold potassium current (I_{KT}). The cell also expresses an hyperpolarization sag blocked by CsCl suggesting the presence of a mixed hyperpolarizing activated cationic current (I_h). These pharmacological findings are similar to those reported for fast auditory pathway of birds and mammals (Manis and Marx, 1991; Bal and Oertel 2000, 2001) suggesting a convergent evolution in these fast sensory pathways.

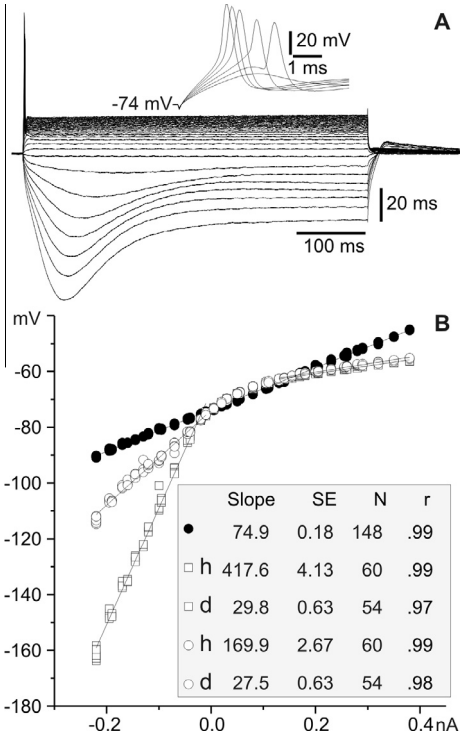

Fig. 1. Spherical neuron phenotype is determined by two main resonant currents: (A) membrane voltage response to square current pulses of different intensities and polarities (in this case the difference in amplitude between current steps was 66 pA). Typically the neuron responds with a single spike at the stimulus onset in all cells (n = 37). Expanded traces showing these spikes are represented at the inset. Note the reduction in latency and the increase in amplitude with stimulus intensity. Subthreshold responses to depolarizing pulses are characterized by an initial hump followed by a constant voltage value. Hyperpolarizing pulses cause larger voltage changes. Beyond a threshold intensity hyperpolarization peaks between 50 and 100 ms after pulse onset and is followed by a depolarizing sag. (B) Voltage vs. current plots obtained pooling data from 6 series of current steps. Voltage was measured at different delays from the onset (arrow at 1.5 ms, dotted lines at 100 ms and just before the offset). The linearity observed at the peak of the hump of subthreshold responses (black dots, voltage = current * 74.9–73.7, r = 0.99, p < 0.01, N = 148) is lost at intermediate delays as a consequence of the dynamics of a low-threshold outward rectification current. For depolarizing pulses the asymptotic slope decreases after the hump or the spike and do not change significantly along the pulse (compare square vs. open circles). For hyperpolarization the asymptotic slope increases along the pulse (compare square vs. open circles). This is due to the activation of an inward rectification current. We obtained similar data in the 37 cells and made a similar analysis before and after the pharmacological treatment.

EXPERIMENTAL PROCEDURES

General

All experiments were performed in brain slices of *Gymnotus omarorum* (12–18 cm long). This species is easily gathered in lakes and creeks close to Montevideo (lat. 35.5, long. 55). Fish capture, transportation, care and experiments were performed in accordance with institutional and national guidelines and regulations for animal welfare (Protocol 001/03/2011 Animal Care Committee of the IIBCE, and the International Guiding Principles for Biomedical Research Involving Animals).

Whole cell patch recordings of spherical neurons in brain slices

Animals were ventilated with aerated water at 4 °C while under deep anesthesia (MS 222 100 mg l^{-1}). After few minutes of cold-water perfusion, the skull was opened and the brain extracted to a Petri dish filled with low sodium Ringer solution at 4 °C (ion concentration in mM: KCl = 2, $CaCl_2$ = 2.6, $KHPO_4$ = 1.25, $NaHCO_3$ = 24, $MgSO_4$ = 1.6, glucose = 20, and sucrose = 201; pH 7.4). 200 µm coronal slices were obtained using a vibratome (Leica VT1000S) within less than 10 min after skull opening. Slices were incubated in low-sodium solution for 30–60 min. When low-sodium Ringer reached room temperature, slices were transferred to the normal sodium recording solution (i.e. the same composition but with NaCl 120 mM instead of sucrose).

Neurons were identified using Nomarski optics under infrared illumination and on line video-microscopy. In a previous study we had shown the correlation between Nomarski images and intracellular labeled cells (Nogueira et al., 2006). With this technique we were not able to identify the innervation pattern on each recorded cell. Most of the recorded cells (34) belonged to the centro-medial and centro-lateral maps. The limit between these two regions is not clearly identifiable in transverse slices of *G. omarorum* with the optical technique used. This uncertainty and the low number of cells obtained from the lateral map, impeded the comparison of cell properties across maps.

Whole cell patch recordings were obtained using 8–12 MΩ tip-polished borosilicate micropipettes filled with the following solution (mM): Kgluconate = 122; $MgCl_2$ = 2.5; MgGluconate = 5.6; $CaCl_2$ = 0.3;