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Electrophysiological properties of neurons in the robust nucleus of the arcopallium of adult male zebra finches

Su-qun Liao[a,b,1], Guo-qiang Hou[a,1], Xiao-lin Liu[a,1], Cheng Long[a,*], Dong-feng Li[a,*]

[a] Key Laboratory of Ecology and Environmental Science in Higher Education of Guangdong Province, College of Life Science, South China Normal University, Guangzhou 510631, China
[b] Shaoguan University, Shaoguan 512000, China

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ABSTRACT

Nucleus robust arcopallium (RA) of the songbird is a distinct forebrain region that is essential for song production. To explore the electrophysiological properties, whole cell recordings were made from adult zebra finch RA neurons in slice preparations. Based on the electrophysiological properties, neurons in RA were classified into two distinct classes. Type I neurons were spontaneously active. They had larger input resistance, longer time constant, larger time-peak of an afterhyperpolarization (AHP), and broader action potentials than those of the other class. A slow, time-dependent inward rectification was induced by hyperpolarizing current pulses in this type of neuron, and was blocked by external CsCl (2 mM). Type II neurons had a more negative resting membrane potential than that of type I neurons. They were characterized by a steeper slope of the recovery from the peak of the AHP and frequency–current relationships, a higher firing threshold, and irregular spiking in response to depolarizing current injection.

Birdsong is a complex learned behavior consisting of sequential vocal gestures over a wide range of time scales. In songbirds, two main neural pathways are involved in song production and song learning. The motor pathway controls the vocal motor program through the hierarchical organization of several premotor nuclei. The other one is anterior forebrain pathway (AFP), a circuit homologous to the basal ganglia thalamo-cortical loops that may be involved in controlling motor behavior in mammals [8,19].

Nucleus robust arcopallium (RA) is a crucial nucleus in the song system, as has been shown with lesion studies, chronic recordings, and microstimulation experiments [20,22]. RA receives synaptic input from both the song nucleus HVC and the nucleus LMAN of AFP. The premotor nucleus HVC encodes the temporal sequence in which sounds are generated [10,27,31]. During singing, HVC neurons that project to the RA generate single bursts of spikes at precisely reproduced times within the song motif [10]. RA, in turn, projects to the hypoglossal motor nucleus and to brainstem respiratory areas [30,33]. RA projection neurons also make local connections by interneuron or the recurrent axon collaterals within RA [2]. In contrast to the extremely sparse neuronal firing patterns in HVC, syringeal and respiratory muscles are driven by continuous control signals that contain a wide range of time scales reflecting the motif, syllable, and subsyllable acoustic structure [9,34]. As an intermediary between HVC and the brainstem motor nuclei, the activity within RA, as well as the intrinsic physiological and morphological properties of RA neurons, likely play important roles in this signal transformation and integration.

Previous intracellular recording has shown that RA neurons might be classified into at least two cell types, on the basis of morphology and electrophysiology: interneuron and projection neuron [28]. But this classification is not exhaustive and the detail electrophysiological properties are unclear. Therefore, the classification of RA neurons may require further expansion. It is commonly accepted that the patch clamp technique offers many advantages over conventional intracellular recording configuration, such as the enhanced ability to control the intracellular environment, increased signal-to-noise ratio and improved voltage-clamp quality. Here, using the whole-cell patch clamp technique, we examined the types of neuron that are present in RA of slices on the basis of their intrinsic electrophysiological properties, which addressed an important issue for understanding the neural mechanisms underlying song learning and production, namely the characteristics of key song circuit neurons.

All experiments were carried out in accordance with the university and national animal guidelines. Brain slices were prepared from adult zebra finches (*Taeniopygia guttata*) as previously described [1,18,32]. The action potential (AP) was recorded under current-clamp model. The resting membrane potential (RMP) of a neuron was the voltage when steady state current = 0 [6,7]. Cell input resistance (IR) was calculated from the slope of the stable-state

* Corresponding author at: College of Life Science, South China Normal University, Guangzhou 510631, China. Tel.: +86 020 8521 1372; fax: +86 020 8521 2078.
E-mail address: dfliswx@126.com (D.-f. Li).
[1] These authors contributed equally to this research.

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Table 1
Membrane properties of neurons recorded in RA.

Parameter	Type Ia (n = 60)	Type Ib (n = 36)	Type II (n = 12)
Rest potential (mV)	Tonically active	Tonically active	−70.32 ± 6.06
Input resistant (IR, MΩ)	213.58 ± 46.73	266.12 ± 64.13#	111.07 ± 40.32*
Time constant (τ, ms)	35.27 ± 10.72	40.31 ± 8.65	17.53 ± 5.89*
Sag (%)	4.34 ± 2.41	5.16 ± 2.08	1.51 ± 0.76*
Peak amplitude (mV)	64.62 ± 7.81	64.46 ± 6.82	53.94 ± 12.28*
Half-width (ms)	0.59 ± 0.12	0.66 ± 0.16	0.50 ± 0.11*
AHP (mV)	−23.43 ± 5.07	−22.25 ± 4.43	−24.62 ± 4.57
AHP delay (ms)	13.52 ± 3.75	25.31 ± 5.56#	2.08 ± 1.19*
Rate of frequency adaptation (Hz/s)	153.96 ± 28.30	174.53 ± 30.25	102.35 ± 11.22*
Coefficient of variation for spike intervals	0.13 ± 0.05	0.11 ± 0.05	2.14 ± 1.56*

Values are means ± SD with number of neurons in parentheses and ranges in brackets. P values determined with one-way ANOVA followed by Scheffe's comparison. *or # $P < 0.05$ indicates significant difference.

current–voltage relationship. The membrane time constant (τ) was determined by fitting standard exponential functions to the averaged electrotonic response (<10 mV) [17]. The characteristics of APs were measured from spikes as they fired spontaneously or from spike triggered by current injection. The spike amplitude was quantified as the difference between peak voltage and spike threshold. The spike width was determined at half-amplitude. The amplitude of the afterhyperpolarization (AHP) was taken as being equal to the difference between spike threshold and the most negative voltage reached during the AHP [3,4,6,7,26]. Sags were the ratio of the difference between the maximum potential and the stable potential to the stable potential induced by injecting a −100 pA hyperpolarizing current. Rate of frequency adaptation was acquired by plot of spike interval versus time elapsed since onset of spike train by 600 pA depolarizing current injection. The regression line fitted to the initial part of the curve served as a measure for frequency adaptation. Coefficients of variation for spike intervals were the SD of the mean interspike interval (ISI) during 600 pA current pulse divided by the mean ISI in that pulse. The one-way ANOVA was used for statistically significant determine.

Whole-cell patch clamp recordings were made in 108 RA neurons from 38 male zebra finches. Electrophysiological properties of RA neurons are listed in Table 1. The frequency distributions of basic membrane properties including IR, τ and AHP were significantly deviated from normality, suggesting that the expression and/or properties of a variety of ion channels differ between neuronal types. Cells were classified into at least two distinct classes according to the differences among their responses to hyperpolarizing current pulses, firing properties, AP characteristics. The parameters measured were significantly different between type I and II neurons ($P < 0.05$). A difference in AHP delay (the delay between AHP initiation and the peak of the AHP) among type I neurons was observed, which could be used to further divide type I neurons into two groups (types Ia and Ib). The AHP delay of type Ia was significantly shorter than that of type Ib.

RA neurons were observed under DIC-IR optics (Fig. 1A). The key features that sorted cells as the type I neurons were a larger IR (>180 MΩ) and relatively longer τ (>24 ms). 96 of the 108 cells characterized represented above features and displayed spontaneous activities with a peak firing rates of 7.5 Hz. A sag appeared in the voltage responses to hyperpolarizing current pulses and a rebound overshoot appeared after the termination of hyperpolarizing current pulses (Fig. 1Bi and Bii), which were distinguished from the type II neurons. The sag was increased as the hyperpolarizing current pulse was increased, indicating the existence of a time- and voltage-dependent inward rectification. CsCl (2 mM) abolished both the time- and voltage-dependent inward rectification induced by hyperpolarizing current pulses (Fig. 2). CsCl also increased the IR.

Type Ia neurons made up 60/96 of the type I neurons. The AP was followed by a monophasic AHP with the amplitude −23.43 ± 5.07 mV and delay 13.52 ± 3.75 ms. The membrane potentials were bound overshoot appeared after the termination of hyperpolarizing current pulses (Fig. 1Bi). The rebound overshoot, which often developed into an AP, increased as the hyperpolarizing current pulses increased. This time-dependent inward rectification was larger than that found in type Ib neurons.

Of the type I cells 36/96 were classed as type Ib neurons. They also exhibited a long τ of 40.31 ± 8.65 ms, consistent with the largest IR of 266.12 ± 64.13 MΩ of the RA neuron. The longest AHP delay of RA neurons was 25.31 ± 5.56 ms followed by a prolonged AHP of amplitude −22.25 ± 4.43 mV. Hyperpolarizing current stepped from the RMP elicited a transient inward rectification with a sag of 5.16 ± 2.08, which was larger than that of type Ia neurons. At the termination of hyperpolarizing current pulse, the membrane potential was rebound depolarizations and developed into an AP sometimes (Fig. 1Bii).

Both type Ia and Ib neurons spontaneously fired regular APs (Fig. 1D). Regular firing was elicited by both small and large depolarizing current pulses with accommodation in the spike frequency (Fig. 1D and E). When the current was increased to a certain level, the firing frequency for the first few spikes was increased to produce an initial high-frequency firing up to 150 Hz followed by an accommodation in the firing frequency (Fig. 1E) with the rate of frequency adaptation 153.96 ± 28.30 Hz/s and 174.53 ± 30.25 Hz/s (n = 15) in type Ia and type Ib, respectively. The membrane potential decreased and the spike were inhibited reversibly at the termination of a constant large depolarizing current injection. The slope of firing frequency injected current relationships in types Ia and Ib were 169.06 ± 27.80 and 161.46 ± 22.04, respectively (n = 15) (Fig. 1F).

The oval soma of type II neurons were smaller than that of type I neurons, the dendrites were observed sometimes (Fig. 3A). This type neurons were much rarer, only 12/108 of the RA cells tested. The key features required for type II neurons were a smaller IR and τ, shorter delay of AHP.

This class of neurons had a more negative RMP and a higher AP threshold than that of type I neurons. Significant membrane characteristics of type II neurons included their activity which was either silent (83.3%) or fired highly irregular spontaneous APs at RMP of −70.32 ± 6.06 mV (Fig. 3Biii). To quantify the propensity of neurons to engage in firing, we defined a "burst index": the mean ISI within a current pulse divided by the minimum ISI within that pulse, averaged over all current pulses that evoked more than two spikes. Neurons that fired with perfect regularity would have a burst index of one, while any deviation from uniform ISI duration yields a burst index greater than one. The means of burst index of type I and II neurons at RMP were 1.27 ± 0.12 and 10.20 ± 3.78 ($P < 0.001$), respectively. Their IR was 111.07 ± 40.32 MΩ and τ was