Electrophysiological Characteristics of Classes of Neuron in the HVc of the Zebra Finch

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Kubota, Michinori and Ikuo Taniguchi. Electrophysiological characteristics of classes of neuron in the HVc of the zebra finch. J. Neurophysiol. 80: 914–923, 1998. Whole cell recordings were made from zebra finch HVc neurons in slice preparations. Four distinct classes of neuron were found to be based on their electrophysiological properties. The morphological characteristics of some of these neurons were also examined by intracellular injection of Lucifer yellow. Type I neurons (21 of 65 cells) had longer time-to-peak of an afterhyperpolarization following an action potential than the other classes. They exhibited both fast and time-dependent inward rectification and an initial high-frequency firing followed by a slower constant firing. Type I neurons had large somata and thick dendrites with many spines. The axons of some of the neurons in this class projected in the direction of area X of the parolfactory lobe. Type II neurons (30 of 65 cells) had a more negative resting membrane potential than the other classes. They exhibited fast inward rectification. Type II neurons could be divided into two subclasses by the absence (IIa; 22 cells) and the presence (IIb; 8 cells) of a low-threshold transient depolarization. Type IIa neurons had relatively small somata and thin, spiny dendrites. The axons of some of the neurons in this class projected in the direction of the robust nucleus of the archistriatum (RA). Type IIb neurons had relatively large somata and thick dendrites with many spines. Type III neurons (6 of 65 cells) had a shorter action-potential duration than the other classes. They exhibited prominent time-dependent inward rectification and a regular tonic firing with little or no accommodation. Type III neurons had beaded, aspiny dendrites. Type IV neurons (8 of 65 cells) had a longer action-potential duration, a much larger input resistance, and longer membrane time constant than the other classes. Type IV neurons had small somata and thin, short, sparsely spiny dendrites. The axons of some of the neurons in this class projected in the direction of the RA. These classes of neuron may play distinct roles in song production and representation in the HVc.

INTRODUCTION

Song birds, such as the zebra finch, have well-developed song control nuclei in their brains (Konishi 1985). The HVc, formerly known as the caudal nucleus of the ventral hyperstriatum or the high vocal center, is considered to play a major role in the motor control of song production (McCasland 1987; Nottebohm et al. 1976; Simpson and Vicario 1990), especially in the programming of time sequences of song components (syllables) (Vu et al. 1994; Yu and Margoliash 1996). The HVc also contains neurons that respond to sounds and songs (Katz and Gurney 1981; Lewicki 1996; Lewicki and Konishi 1995; Margoliash 1983, 1986; Margoliash and Fortune 1992; Margoliash et al. 1994; McCasland 1987; Volman 1993; Williams 1989). The HVc sends projections to two other song control nuclei: area X of the parolfactory lobe (area X) and the robust nucleus of the archistriatum (RA).

However, so far only a few intracellular recording studies of HVc neurons have been carried out (Katz and Gurney 1981; Kubota and Saito 1991; Lewicki 1996; Lewicki and Konishi 1995). These studies have concluded that HVc auditory neurons are area X-projecting neurons and RA-projecting neurons do not show auditory responses (Katz and Gurney 1981, but see below for inconsistency with these results), and that there are various Na+– and Ca2+-dependent ionic conductances in putative area X–projecting neurons (Kubota and Saito 1991), and that there are “song-specific” neurons, defined as neurons that respond significantly more to forward song than to reversed song or the song syllables presented in reverse order, which are also area X–projecting neurons (Lewicki 1996; Lewicki and Konishi 1995). Thus our physiological understanding of HVc neurons seems to be limited to and focused on area X–projecting neurons. According to a study of Golgi-impregnated neurons in the canary HVc, four types of neurons were found to be present (Nixdorf et al. 1989). However, electrophysiological properties of HVc neurons other than area X–projecting neurons are largely unknown. Recent studies have shown that auditory response activity in the RA is eliminated by lesions or inactivation of the HVc (Doupe and Konishi 1991; Vicario and Yohay 1993). These results suggest that RA-projecting neurons in the HVc may respond to auditory stimuli, consistent with the previously reported results (Katz and Gurney 1981). To further our understanding of the functions of the HVc on a cellular level, therefore, it is important to characterize the intrinsic neuronal organization of this brain region.

The brain slice method is suitable for this kind of study because it is relatively easy to make stable intracellular recordings from small neurons. The patch-clamp recording technique has been also successfully applied to brain slices (Blanton et al. 1989; Edwards et al. 1989). We applied the technique to avian brain slices containing the HVc, to characterize its intrinsic neuronal organization. We examined the types of neuron that are present in the HVc on the basis of their electrophysiological properties and also examined their morphological characteristics.

METHODS

Slice preparations

Male zebra finches (Taeniopygia guttata), at 37- to 54-days old, were used for experiments. The birds were obtained from a breed-
Morphological examination

In some experiments, 2–2.5 mg/ml of dipotassium salt (Lucifer yellow, Sigma) was added to the recording electrodes to allow the examination of the morphological characteristics of the electrophysiologically characterized cells. Lucifer yellow was allowed to diffuse freely from the electrode for 10–20 min. After fixing with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) overnight at 4°C, slices were rinsed with 0.01 M phosphate-buffered saline (pH 7.4). Slices were then cleared by immersion in gradually increasing concentrations of glycerin (20, 40, 60, 80, and 100% for 10 min each) (Claborn et al. 1986). Stained cells were observed with a confocal laser scanning microscope (Sarastro 2000, Molecular Dynamics, Sunnyvale, CA), and optical sections were collected at 1.2-μm intervals.

Data are represented as means ± SE. The differences among the neuron classes for the various parameters were evaluated with analysis of variance (ANOVA). Multiple comparisons were done with Tukey’s honestly significant difference test.

RESULTS

Cells were selected only if they exhibited a resting membrane potential more negative than −50 mV and an overshooting action potential. Cells were classified into four distinct classes according to differences in their responses to hyperpolarizing current pulses, firing properties, action-potential durations, and AHP characteristics. Cells differed in number of parameters but could be grouped according to a hierarchical scheme. The differences among the classes were highly significant for all the parameters measured (P < 0.0001, ANOVA; Table 1), notwithstanding that all the parameters were not used to classify neurons (e.g., resting membrane potential, AHP amplitude, and sag).

We describe the characteristics of these four classes of neuron in the following sections.

Type I neurons

The key feature that makes a cell a type I neuron is a long AHP time-to-peak (>9 ms). Type I neurons represented 21 of the 65 cells characterized.

INWARD RECTIFICATION. A sag appeared in the voltage responses to hyperpolarizing current pulses at membrane potentials more negative than about −80 mV, and a rebound overshoot appeared after the termination of hyperpolarizing current pulses (Fig. 1, A and B). The sag and rebound overshoot increased as the hyperpolarizing current pulses increased, indicating the existence of a time-dependent inward rectification. This time-dependent inward rectification distinguished type I neurons from all of the other classes of neuron with the exception of type III neurons (Table 1). The peak voltage responses also exhibited inward rectification over the same range of membrane potentials as the time-dependent inward rectification (Fig. 1, A and B), indicating also the existence of a fast-activated inward rectification. These neurons had an intermediate value of resting membrane potential among the four classes of neuron (Table 1).

FIRING PROPERTIES. The AHP following an action potential in type I neurons was large in amplitude and longer in time-to-peak than in the other classes of neuron (Table 1 and Fig. 1). Small depolarizing current pulses elicited relatively regular tonic firing (Fig. 1, C, top, and D). When the current was increased to a certain level, however, the firing frequency for the first few spikes increased to produce an initial high-frequency firing followed by a much slower constant firing (Fig. 1, C, middle and bottom, and D). The duration of type I neuron action potentials was intermediate among the four classes of neuron (Table 1 and Fig. 5).

Type II neurons

The features required for type II neurons are a short AHP time-to-peak (<8 ms), an action-potential duration of >0.5
ms at the half-maximum amplitude, and a relatively short membrane time constant (<65 ms). Type II neurons represented 30 of the 65 cells characterized.

INWARD RECTIFICATION. This class of neuron had a more negative resting membrane potential and a higher action-potential threshold than all other classes of neuron (Table 1). When hyperpolarizing current pulses were applied to type II neurons, no time-dependent inward rectification was observed, but fast inward rectification was evident at membrane potentials more negative than about −80 mV (Fig. 2, A and B).

Type II neurons could be subdivided into type IIa (n = 22) and IIb (n = 8) neurons by the absence and the presence of the low-threshold transient depolarization, respectively (Fig. 2A).

**Type IIa Neurons.** Firing properties. In type IIa neurons, a large depolarization from the resting membrane potential was required to elicit an action potential (Fig. 2A1). Regular tonic firing was elicited by both small and large depolarizing current pulses with accommodation in the spike frequency (Fig. 2, C and D). The AHP was large in amplitude but had a short time-to-peak (Table 1). Membrane potentials in response to depolarizing current pulses gradually depolarized with time near the action-potential threshold (Fig. 2A1).

**Type IIb Neurons.** Firing properties. Type IIb neurons were distinguishable from type IIa neurons by the existence of a prominent low-threshold transient response to depolarizing current pulses. When depolarizing current pulses were applied to type IIb neurons, a transient depolarizing response appeared before the initiation of the action potential (Fig. 2A2). The depolarizing responses increased progressively in amplitude as the depolarization increased (Fig. 2A2). Firing frequency decreased during current pulses, showing spike frequency accommodation. Type IIb neurons had a significantly longer action-potential duration than type IIa neurons (Table 1 and Fig. 5). The AHP of type IIb neurons was significantly smaller in amplitude than type IIa neurons (Table 1) and had a short time-to-peak. In type IIb neurons, membrane potentials gradually depolarized to reach spike threshold after a low-threshold response (Fig. 2A2).

**Type III Neurons**

The key feature required for type III neurons is a short action-potential duration (<0.5 ms at the half-maximum amplitude). Of the 65 cells characterized, 6 were designated as type III neurons.

INWARD RECTIFICATION. A prominent sag appeared in the voltage responses to hyperpolarizing current pulses at membrane potentials more negative than about −70 mV, and a rebound overshoot appeared after the termination of hyperpolarizing current pulses (Fig. 3, A and B). The sag and rebound overshoot, which often developed into an action potential, increased as the hyperpolarizing current pulses increased. This time-dependent inward rectification was much larger than that found in type I neurons (Table 1). Type III neurons had more positive resting membrane potentials and a lower action-potential threshold than the other classes of neuron (Table 1). Type III neurons also exhibited a fast-activated inward rectification (Fig. 3, A and B) and were frequently bombarded by spontaneous excitatory and inhibitory postsynaptic potentials.

**Firing Properties.** Type III neurons were easily distinguishable from the other classes of neuron by their firing properties as well as the prominent sag in their responses to hyperpolarizing current pulses. The duration of action potential in type III neurons was shorter than in any other class of HVC neurons (Table 1 and Fig. 5). The action potential was followed by an AHP, which was larger in amplitude than in the other classes of neuron, although its time-to-peak was short (Table 1). When depolarizing current pulses were applied to type III neurons, highly regular tonic firing with little or no accommodation was elicited (Fig. 3, C and D).

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**Table 1. Electrophysiological properties of classes of HVC neurons**

<table>
<thead>
<tr>
<th>Property</th>
<th>Type I (Mean ± SE)</th>
<th>Type II (Mean ± SE)</th>
<th>Type III (Mean ± SE)</th>
<th>Type IV (Mean ± SE)</th>
<th>Significant Differences*</th>
<th>Type II Subclass</th>
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<tbody>
<tr>
<td>Resting potential, mV</td>
<td>−73.8 ± 1.3</td>
<td>−83.2 ± 1.0</td>
<td>−63.0 ± 2.6</td>
<td>−71.9 ± 1.4</td>
<td>Type III &gt; type I, IV &gt; type II</td>
<td>−83.0 ± 1.2</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>219 ± 18</td>
<td>242 ± 23</td>
<td>165 ± 19</td>
<td>749 ± 123</td>
<td>Type IV &gt; type II, III</td>
<td>232 ± 29</td>
</tr>
<tr>
<td>Time constant, ms</td>
<td>47.4 ± 3.0</td>
<td>34.5 ± 2.6</td>
<td>20.0 ± 2.7</td>
<td>86.5 ± 5.1</td>
<td>Type IV &gt; type I &gt; type II, III</td>
<td>31.4 ± 2.9</td>
</tr>
<tr>
<td>Sag, %</td>
<td>10.3 ± 1.5</td>
<td>1.4 ± 0.2</td>
<td>30.4 ± 3.6</td>
<td>1.6 ± 0.5</td>
<td>Type III &gt; type I &gt; type II, IV</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Spike duration at half-amplitude, ms</td>
<td>0.89 ± 0.03</td>
<td>0.90 ± 0.04</td>
<td>0.38 ± 0.03</td>
<td>1.16 ± 0.04</td>
<td>Type IV &gt; type I &gt; type II, IV</td>
<td>0.84 ± 0.05†</td>
</tr>
<tr>
<td>Spike threshold, mV</td>
<td>31.2 ± 1.3</td>
<td>43.9 ± 0.9</td>
<td>18.6 ± 2.5</td>
<td>32.1 ± 1.6</td>
<td>Type II &gt; type III</td>
<td>44.4 ± 1.1</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>−21.2 ± 0.6</td>
<td>−20.1 ± 0.7</td>
<td>−25.8 ± 1.5</td>
<td>−13.7 ± 1.1</td>
<td>Type IV &gt; type I &gt; type II, IV</td>
<td>−21.8 ± 0.7†</td>
</tr>
<tr>
<td>AHP time-to-peak, ms</td>
<td>18.1 ± 1.6</td>
<td>1.9 ± 0.2</td>
<td>2.7 ± 0.5</td>
<td>3.8 ± 0.8</td>
<td>Type I &gt; type II, III, IV</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of neurons were as follows: type I, n = 21; type II, n = 30; type III, n = 6; type IV, n = 8; type IIa, n = 22; type IIb, n = 8. AHP, afterhyperpolarization. * Significant differences indicated by the use of Tukey’s honestly significant difference test for multiple comparisons after analysis of variance (P < 0.05). † Significant difference between type IIa and type IIb (P < 0.05, Mann-Whitney U test).
**Type IV neurons**

The key feature required for type IV neurons is a long membrane time constant (\(>65\) ms). Of the 65 cells characterized, 8 were designated as type IV neurons.

**INWARD RECTIFICATION.** They had a much higher input resistance and a much longer membrane time constant than the other classes of neuron (Table 1). Type IV neurons exhibited a fast inward rectification at membrane potentials more negative than about −80 mV, but no time-dependent inward rectification was observed (Fig. 4, A and B). These neurons had a relatively positive resting membrane potential among the four classes of neuron (Table 1).

**FIRING PROPERTIES.** Type IV neurons had a longer action-potential duration than the other classes of neuron (Table 1 and Fig. 5). The AHP of this neuron type was small in amplitude and had a short time-to-peak (Table 1). When relatively large depolarizing current pulses were applied to type IV neurons, action potentials often ceased to be elicited in the middle of the pulse, and the firing frequency decreased during the pulse, showing strong spike-frequency accommodation (Fig. 4, C and D).

**Morphological characteristics of HVC neurons**

Some of the neurons that were identified electrophysiologically were also labeled by injection of Lucifer yellow to examine their morphological characteristics. Thirty-four cells were well filled and reconstructed successfully via the confocal system. Axons were identified by the thin, nontapering, and smooth-surfaced appearance. The results are summarized in Table 2.

Type I neurons (13 of 34 cells) had large somata (20–31 \(\mu m\) diam), large dendritic arborizations (220–290 \(\mu m\) diam), and thick, long, spiny multipolar dendrites (Fig. 6A). Type I neurons had spherical dendritic arborizations or elongated arborizations. Although the axons of neurons in this class were rarely observed beyond the border of the HVC, due to slicing procedures, the axons of two type I neurons did project beyond the rostral border of the HVC in the direction of area X.

Type IIa neurons (7 of 34 cells) exhibited small or medium-sized, round or oval somata (11–21 \(\mu m\) diam), and short, thin, spiny dendrites (Fig. 6B). Type IIa neurons had small spherical or oval dendritic arborizations (140–220 \(\mu m\) diam). The axons of neurons in this class were sometimes...
FIG. 2. Inward rectification and firing properties of type II neurons. A1: voltage responses of a type IIa neuron to hyperpolarizing and depolarizing current pulses. The inward rectification exhibits no sag in the voltage responses to hyperpolarizing current pulses. Resting potential was −88 mV. A2: voltage responses of a type IIb neuron to hyperpolarizing and depolarizing current pulses. There is an inward rectification without sag in the voltage responses to hyperpolarizing current pulses, and transient low-threshold depolarizing responses at depolarized potentials. Resting potential was −80 mV. B: current-voltage relationship of the cell shown in A1. C: repetitive firing properties of another type IIa cell. Top, middle, and bottom: tonic firing elicited by 0.09, 0.16, and 0.28 nA current pulses, respectively. Resting potential was −84 mV. D: relationship between the instantaneous spike frequency and time for different applied currents in the cell shown in C.

(3 of 7 cells) seen to project beyond the caudal border of the HVc in the direction of the RA.

Type IIb neurons (6 of 34 cells) had relatively large fusiform somata (18–22 μm diam) and thick, densely spined, multipolar dendrites with relatively large dendritic arborizations (180–260 μm diam; Fig. 6C). The soma size and the total dendritic length were significantly larger than those of type IIa neurons (Table 2). Axons of type IIb neurons could not be followed beyond the border of the HVc.

Type III neurons (3 of 34 cells) were also clearly distinguishable, morphologically, from the other classes of neuron. They had medium-sized somata (17–19 μm diam), relatively large dendritic arborizations (200–270 μm diam), and multipolar, beaded dendrites without spines and extensively arborized axons around their dendritic fields (Fig. 6D). Axons of type III neurons could not be followed beyond the border of the HVc.

Type IV (5 of 34 cells) neurons had small round or fusiform somata (15–18 μm diam), small dendritic arborizations (140–200 μm diam), and thin, short, sparsely spiny multipolar dendrites (4 of 5 cells; Fig. 6E). The remaining cell had thin, short, spiny multipolar dendrites. These axons were sometimes seen projecting beyond the ventrocaudal border of the HVc, in the direction of the RA (2 of 5 cells).

DISCUSSION

The present study shows that at least four electrophysiologically distinct classes of neuron exist in the HVc of the zebra finch. We did not observe neurons that did not fit any of these four classes, although we cannot exclude the possibility of the existence of other classes of neuron in the HVc. Juvenile zebra finches were used in this study because classes of neuron similar to those found in adult birds are already present in the HVc of young birds (Kubota and Saito 1991). However, there is a caveat that detailed characteristics described here may be different from those in the adult birds and those in the normal in vivo situation. We did not observe any systematic trends of the properties of HVc neurons as a function of location within the HVc. Although the “paraHVc” has been shown to be present along the ventricle in the medial caudal neostriatum ventral to the HVc (Nordeen et al. 1987), it is unlikely that the results in the present study were affected by the presence of the paraHVc.
because the properties of the recorded neurons did not depend on where they were recorded from and all classes of neuron were observed in lateral slices. Each class of HVc neuron will be discussed in sequence below.

Type I neurons

Type I neurons exhibited electrophysiological and morphological characteristics that were similar to those reported previously (Kubota and Saito 1991). The morphological characteristics of type I neurons were similar to those of area X–projecting neurons of the adult zebra finch HVc that have been studied in vivo (Fortune and Margoliash 1995; Katz and Gurney 1981); i.e., large soma and thick, spiny dendrites. The axons of some type I neurons were followed beyond the HVc in the direction of area X. It appears likely, therefore, that type I neurons in the HVc project to area X. An initial high-frequency firing and a relatively low action-potential threshold may indicate that the firing of type I neurons is readily elicited by weak excitatory inputs. Type I neurons are likely to have the time-dependent inward rectifier as well as the persistent Na⁺ conductance (Kubota and Saito 1991). It has been shown that a time-dependent inward rectifier produces membrane resonance and a persistent Na⁺ conductance enhances the resonance (Hutcheon et al. 1996). When membrane resonance is present, neurons fire action potentials preferentially to rhythmic inputs at specific frequencies. Therefore type I neurons may play an important role in rhythmic activity in the HVc.

Type II neurons

Type II neurons seem to have an ionic conductance that is responsible for delaying the initiation of action potentials, because the membrane potential depolarized less in response to depolarizing current pulses than would be expected from the ohmic responses close to the action-potential threshold. This property, together with the high action-potential thresholds, makes it probable that temporally and/or spatially integrated, strong excitatory inputs are required for type II neurons to fire.

Type IIa neurons are likely to project to the RA, because occasional projections of the axons beyond the HVc, in the direction of the RA, were observed. This corresponds to the observation that axons projecting from the HVc to the RA are aligned almost in the parasagittal plane, resulting in the
FIG. 4. Inward rectification and firing properties of a type IV neuron. A: voltage responses to current pulses. Note long membrane time constant. Resting potential was −78 mV. B: current-voltage relationship of the cell shown in A. Input resistance is high. C: repetitive firing properties of the cell shown in A. Top, middle, and bottom: firing elicited by 0.04, 0.1, and 0.16 nA current pulses, respectively. Firing ceased during the current pulses. D: relationship between the instantaneous spike frequency and time for different applied currents in the cell shown in C.

preservation of the axons of RA-projecting neurons from slices cut in that plane. Recent studies have shown that neurons in the RA can respond to auditory stimuli (Williams 1989), in particular, to the bird’s own song (Doupe and Konishi 1991; Vicario and Yohay 1993). In addition, the auditory responses of RA neurons are reversibly eliminated following lesions or inactivation of the HVc, but not following lesions or inactivation of the lateral magnocellular nucleus of the anterior neostriatum (Doupe and Konishi 1991; Vicario and Yohay 1993), which is the other input nucleus of the RA. These results suggest strongly that RA-projecting neurons in the HVc themselves respond to auditory stimuli and can carry auditory information, especially information about the bird’s own song, although some area X–projecting neurons have been reported to respond to the bird’s own song (Lewicki 1996). Temporal and harmonic combination-

FIG. 5. Shapes of the action potentials of the classes of neuron. Type I, action potential of a type I neuron. Type IIa, action potential of a type IIa neuron. Type IIb, action potential of a type IIb neuron. Type III, action potential of a type III neuron. Duration of the action potential is short. Type IV, action potential of a type IV neuron. The action potential has a long duration. Scale bars in type IV also apply to type I–III.
sensitive neurons have been found in the zebra finch HVc (Lewicki and Konishi 1995; Margoliash and Fortune 1992). The fact that type IIa neurons require large depolarizations to elicit action potentials is consistent with the idea that they may be temporal combination-sensitive neurons that integrate over long periods, or perhaps harmonic combination-sensitive neurons that require two harmonics to be excited (Margoliash and Fortune 1992).

Type IIb neurons were distinguished from type IIa neurons by the presence of the low-threshold transient depolarization. This transient depolarization seems to be not so strong to elicit burst firing as seen in the mammalian olivary and thalamic neurons (Jahnsen and Llinás 1984; Llinás and Yarom 1981), which are thought to be implicated in the oscillatory behavior. However, the transient depolarization in type IIb neurons may be suitable for coordinating the timing of firing of the other classes, and possibly of other type IIa neurons. The axons of type IIb neurons could not be followed beyond the border of the HVc. This may be due to the orientation of the slicing procedure. Therefore whether type IIb neurons are projection neurons or interneurons remains to be determined.

**Type III neurons**

Type III neurons were the easiest to distinguish from the other classes. The electrophysiological and morphological characteristics of type III neurons are similar to those of the γ-aminobutyric acid–containing inhibitory interneurons found in the mammalian hippocampus (Schwartzkroin and Mathers 1978), the putative inhibitory interneurons of the neocortex (McCormick et al. 1985), and the basolateral amygdala (Washburn and Moises 1992); i.e., the short-duration of action potential, little or no accommodation of repetitive firing, and beaded, aspiny dendrites. We suggest, therefore, that type III neurons observed in the present study are inhibitory interneurons within the HVc. Because type III neurons also have the time-dependent inward rectifier, type III neurons may also play an important role in rhythmic activity in the HVc together with type I neurons.

**Type IV neurons**

Type IV neurons had a high-input resistance, long membrane time constant, and long duration of action potential. These electrophysiological properties are similar to those of developing neurons from various brain areas (Kasper et al. 1994; Kriegstein et al. 1987; McCormick and Prince 1987; Ramoa and McCormick 1994). It has been shown, even in the adult zebra finch, that some neurons continue to be generated and migrate into the HVc, and that many of these are destined to become RA-projecting neurons (Alvarez-Buylla et al. 1990). Type IV neurons are likely to project to the RA, because occasional projections of the axons beyond the HVc, in the direction of the RA, were observed. Thus we suggest that type IV neurons may be immature neurons that have only recently migrated into the HVc. Although they tend to respond inadequately to stimuli due to the premature inactivation of their firing, type IV neurons appear to be readily excited by even weak, and low-frequency stimuli. This is probably due to their high-input resistance and long membrane time constant.

**Comparison with other studies**

A morphological classification of neurons has been made previously in a study of the canary HVc, i.e., furry dendrite, thick dendrite, short dendrite, and aspiny neurons (Nixdorf et al. 1989). Corresponding neuron types, except aspiny neurons, were also found in the zebra finch HVc where neurons were retrogradely labeled (Fortune and Margoliash 1995). It is of interest to compare the classes of neuron observed in the present study and those in the previous studies, although comparisons of absolute values between the present and the previous results are difficult due to the differences of methods and species used. The morphological characteristics of type I and type IIa neurons are similar to those of the thick dendrite and the short dendrite neurons, respectively. The characteristics that type I neurons have the largest cell body, dendritic length, and total dendritic arborization among all classes of neuron match those of the thick dendrite neurons. Also, the characteristics that type IIa neurons have
the smallest cell body, dendritic length, and total dendritic arborization, except type IV neurons, which do not appear to have been described in the canary HVc, match those of the short dendrite neurons. Type IIb neurons appear to correspond to the furry dendrite neurons. The characteristics that type IIb neurons have intermediate values of cell body size, dendritic length, and total dendritic arborization match those of the furry dendrite neurons. Type III neurons appear to correspond to the aspinous neurons, although we do not know to which extent type III neurons are similar to the aspinous neurons because of the unavailability of their data in the canary study. The neurons corresponding to type IV neurons do not appear to have been described in the canary study. Type IV neurons may have been classified as the short dendrite or the aspinous neurons in the canary study. Therefore the electrophysiological classification of neurons seems to correspond well with the morphological classification.

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REFERENCES


edwards, f. a., konneth, a., sakmann, b., and takahashi, t. a thin slice preparation for patch clamp recordings from neurons of the mammalian central nervous system. pflugers arch. 414: 606–612, 1989.


hutcheon, b., miura, r. m., and pil. e. subthreshold membrane resonance in neocortical neurons. j. neurophysiol. 76: 683–697, 1996.


katz, l. c. and gurney, m. e. auditory responses in the zebra finch’s motor system for song. brain res. 211: 192–197, 1981.


lewicki, m. s. intracellular characterization of song-specific neurons in the zebra finch auditory forebrain. j. neurosci. 16: 5854–5863, 1996.

lewicki, m. s. and konishi, m. mechanisms underlying the sensitivity of songbird forebrain neurons to temporal order. proc. natl. acad. sci. usa 92: 5582–5586, 1995.


margoliash, d. preference for autogenous song by auditory neurons in a song system nucleus of the white-crowned sparrow. j. neurosci. 6: 1643–1661, 1986.


mccormick, d. a., connors, b. w., lighthall, j. w., and prince, d. a. comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. j. neurophysiol. 54: 782–806, 1985.


nottebohm, f., stokes, t. m., and leonard, c. m. central control of song in the canary, serinus canarius. j. comp. neur. 165: 457–486, 1976.


simpson, h. b. and vicario, d. s. brain pathways for learned and unlearned vocalizations differ in zebra finches. j. neurosci. 10: 1541–1556, 1990.

vicario, d. s. and yohay, k. h. song-selective auditory input to a forebrain vocal control nucleus in the zebra finch. j. neurobiol. 24: 488–505, 1993.


washburn, m. s. and moses, h. c. electrophysiological and morphological properties of rat basolateral amygdaloid neurons in vitro. j. neurosci. 12: 4066–4079, 1992.
