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Intrinsic Membrane Properties of Laryngeal Motoneurons that Control Sexually Differentiated Vocal Behavior in African Clawed Frogs, *Xenopus laevis*

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Males and females behave differently during reproduction. Although sexually differentiated patterns of behavior in vertebrates are clearly regulated by the action of gonadal steroids, the neural mechanisms underlying the expression of sex-specific behavior are largely unknown.

Male and female African clawed frogs (*Xenopus laevis*) produce sexually distinct vocalizations composed of a series of clicks. The fundamental difference between male and female calls is the rate at which the clicks are repeated (reviewed in 1); male calls cover a wide range of click repetition rates (8 to 80 Hz), whereas female calls contain only slow repetition rates (2 to 20 Hz). This behavioral difference can conveniently be reduced to the sexual difference in contraction rate of laryngeal muscle (2), which, in turn, is determined by the sexually distinct firing patterns of laryngeal motoneurons (3). Thus, there is a direct correspondence between the sexually dimorphic patterns of vocalization and the activity of motoneurons.

How, then, do male and female laryngeal motoneurons produce sex-specific patterned activity? While the overall pattern is probably produced by a pattern generator upstream of the motoneurons, the motoneurons themselves may have intrinsic membrane properties that differ between male and female *Xenopus.* Testing this possibility was the goal of this study.

Whole-cell patch clamp recordings were used to characterize the membrane properties of laryngeal motoneurons in n.IX-X of adult male and female *Xenopus.* A thick brain slice preparation of *Xenopus* hindbrain was developed, and the neurons were visualized by IR/DIC microscopy. To facilitate identification, the motoneurons were retrogradely labeled with fluorescent dye (AlexaFluor 594 biocytin, Molecular Probes, Eugene, OR) before the brain was sliced. Responses to hyperpolarizing and depolarizing current steps (200 ms long) by 6 male and 10 female motoneurons (3 male and 6 female frogs), were recorded in current clamp mode. The resting membrane potential, threshold, spike amplitude, and spike half-width were directly measured from voltage traces. The membrane time constant was determined by fitting single exponential curves to hyperpolarizing voltage responses. Input resistance was calculated from the steady-state membrane potential in response to different hyperpolarizing current pulses. The capacitance of the cell was calculated from input resistance and time constant. The peak firing rate was determined by measuring the interval between the first two action potentials in response to the largest depolarizing current (1.5–2nA) applied to each neuron.

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Membrane properties of male and female motoneurons are summarized in Table 1. All the properties measured are statistically similar in the two sexes except for the input resistance and the cell capacitance; input resistance is significantly lower, and the cell capacitance is significantly higher in male motoneurons than in female motoneurons. These differences predict that male motoneurons are larger than female motoneurons. A previous study has shown that the dendrites of male n.IX-X neurons are longer than those of female n.IX-X neurons, although the somal size is similar in the two sexes (4). The difference in the dendritic arborization may account for the differences in input resistance and cell capacitance in the two sexes. Functionally, sexual differences in the input resistance imply that the male and female motoneurons exhibit different responsiveness to synaptic input.

At depolarized membrane potentials, all the motoneurons showed repeated action potential firing that accommodated over a time course of 100 ms. The peak firing rates, determined by the first two spikes in response to depolarizing currents, were well over 100 Hz in both sexes. To determine whether the neurons could maintain this rapid firing frequency for more than two spikes, four female and two male motoneurons were stimulated with trains of depolarizing pulses at various rates (0.5 ms, 7–10 V, 10 to 300 Hz). Both male and female motoneurons could follow the depolarizing pulses at frequencies of at least 90 Hz. Although the maximum click rate of female vocalizations is 20 Hz, and that of male calls is 80 Hz, the motoneurons of both sexes can fire at a much higher frequency than is required for call production.

Taken together, the results suggest that the laryngeal motoneurons of *Xenopus* do not limit the click repetition rate, and that the motoneurons may be sexually differentiated in their responsiveness to synaptic input.

Table 1

Basic membrane properties of male and female laryngeal motoneurons of adult Xenopus laevis

Membrane properties	Male ($n = 6$)	Female ($n = 10$)	P-value
Resting membrane potential (mV)	−49.1 ± 1.2	−48.1 ± 2.5	n.s.
Time constant (ms)	9.15 ± 0.91	11.46 ± 1.44	n.s.
Input resistance (MΩ)	69.4 ± 3.9	182.4 ± 25.7	$P < 0.01$
Cell capacitance (nF)	0.148 ± 0.017	0.066 ± 0.009	$P < 0.01$
Spike amplitude (mV)	35.56 ± 3.81	35.94 ± 4.30	n.s.
Spike half-width (ms)	0.69 ± 0.10	0.67 ± 0.11	n.s.
Threshold (mV)	−29.11 ± 10.25	−36.14 ± 3.12	n.s.
Highest firing frequency (Hz)	192.17 ± 29.47	257.40 ± 25.86	n.s.

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Optic Nerve Responses of *Limulus* in its Natural Habitat at Night

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What information does the eye send to the brain when an animal sees? We are exploring this question with the relatively simple visual system of the horseshoe crab, *Limulus polyphemus.* By combining cell-based computational models of the retina with single-cell electrophysiology, we have examined the optic nerve code underlying *Limulus* vision during the day in the animal's natural habitat (1).

Field studies during the animals' mating season show that male horseshoe crabs use vision to find mates and do so about equally well day and night (2). We attribute their remarkable nighttime vision to a circadian modulation of the sensitivity of their lateral eyes (3). At night, efferent optic nerve fibers carry signals from the circadian clock in the animal's brain to its eyes, increasing nighttime retinal sensitivity as much as 1,000,000 times. The increased sensitivity nearly compensates for the average 1,000,000-fold decrease in ambient light intensity after sundown. Here we investigate optic nerve activity recorded from the animal in its habitat, both day and night, with emphasis on signals that convey information about potential mates at night.

A convenient method for recording what the horseshoe crab sees underwater is to mount a miniature video camera, "CrabCam," on the animal (1). This documents the crab's eye view during the day, but not at night when light levels fall below the camera's sensitivity. To investigate the optic nerve responses of an animal in its natural habitat at night, we used a repetitive, artificial stimulus that simulates the movement of a potential mate within the animal's visual field. The stimulus is a rotating grey cylinder (30 cm in diameter, 15 cm in height) with a black sector (30 cm in width) that simulates the size of a typical female horseshoe crab. The cylinder was placed 1 m from the crab and rotated by hand (4–8 rpm), moving the black sector horizontally at 7–13 cm/s, which simulates the average speed of a horseshoe crab. Our strategy is as follows: first, during the day and under water, we record the optic nerve response of a stationary crab to the rotating cylinder while simultaneously videotaping the eye's input with the shell-mounted CrabCam. This allows us to document the visual input when ambient light levels are sufficient for CrabCam operation. We then leave the animal and cylinder in place underwater until after sundown, when we repeat the experiment carried out during the day, but of course without the Crabcam. This method allows us to record the response of the eye to a known visual stimulus at night in the animal's natural habitat.

We recorded the response of single optic nerve fibers following a procedure developed in this laboratory (1). In brief, we trephine a hole in the carapace about 2 cm anterior to the right lateral eye, expose the optic nerve trunk, and draw it into a chamber that is then attached to the carapace. We tease away a single active optic nerve fiber corresponding to a single ommatidium and pull it into a micro-suction electrode. The chamber is sealed to make it water tight, and then a small point light source is used to locate the optic axis of the recorded ommatidium. We then mount the CrabCam on the carapace and align it in the direction of view of the recorded ommatidium. The CrabCam (72° by 54° field of view) encompasses about a quarter of the hemispheric view of the lateral eye, which is seen by about 250 ommatidia, each viewing about a 6° region during the day and about a 12° at night (4). The animal is firmly attached to a weighted platform that is placed on the sandy bottom of the animal's habitat and oriented so that the optic axis of the recorded ommatidium intersects the axis of rotation of the cylinder located about 1 m away. Experiments were carried out at depths of 0.5–1 m in estuaries near the Marine Biological Laboratory in Massachusetts. Signals from the microsuction electrode and the CrabCam are led *via* shielded cables (13 m in length) to a portable camcorder on shore or in a nearby boat.

Figure 1 (right) shows two video frames taken with the CrabCam during the day. These frames show the rotating cylinder at two distances (.87 m and 1 m) from the horseshoe crab. On the left are 14 s samples ("Day") of the responses of a single optic nerve fiber to rotations of the cylinder at the two distances from the crab. The video frames were taken 6 s after the beginning of the response records (arrows), when the grey-black edge of the cylinder began to enter the field of view of the recorded ommatidium from the right. In both cases, the black sector evoked clear decreases in response, with the larger decrease recorded when the cylinder was closer to the animal and water turbidity was minimal. The top "Day" response was recorded at 1630 h and the second was recorded at 1800 h. In both cases, the setup was bathed in direct sunlight. After the second record was recorded, the animal and cylinder were left underwater as nightfall approached.

The experiment was then repeated several hours later after sundown (2030–2100 h) but without CrabCam recordings because of insufficient lighting. Figure 1 ("Night") displays the responses of the same single optic nerve fiber to nine sequential rotations of the cylinder (thin black traces). The heavy black trace gives the average of the nine responses. Note that the individual responses

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