Original Paper

Brain, Behavior and Evolution

Brain Behav Evol 2000;55:152–162

Temperature Dependence of Electrocommunication Signals and Their Underlying Neural Rhythms in the Weakly Electric Fish, *Apteronotus leptorhynchus*

K.D. Dunlap[a, b] G.T. Smith[b] A. Yekta[a]

[a] Department of Biology, Trinity College, Hartford, Conn., and [b] Section of Neurobiology, School of Biological Sciences, University of Texas, Austin, Tex., USA

Key Words

Electric fish · Q_{10} · Temperature · *Apteronotus* · Harmonics · Chirping · Electric organ discharge

Abstract

Weakly electric fish emit an electric communication signal that is controlled by a highly specialized neural circuit. In *Apteronotus,* the continuous electric organ discharge (EOD) is generated by electrotonically coupled neurons in the hindbrain pacemaker nucleus, and transient EOD modulations involve chemical synapses from descending midbrain and thalamic prepacemaker nuclei. We characterized the effects of temperature change (18–32 °C) on both the continuous EOD and EOD modulations, chirps, in *A. leptorhynchus*. EOD frequency was linearly related to temperature (Q_{10} = 1.62). By contrast, the temperature dependence of EOD amplitude changed with temperature. Amplitude increased steeply with temperature below 25 °C (Q_{10} = 2.0), but increased only gradually above 25 °C (Q_{10} = 1.15). EOD waveform, and consequently harmonic content, was also affected by temperature. The amplitude of the second harmonic was relatively high at both low and high temperature and relatively low at intermediate temperatures. The amplitude of the third harmonic increased monotonically with temperature. Thus, temperature has qualitative as well as quantitative effects on the production of the EOD. Chirp rate (Q_{10} = 3.2) had a higher temperature dependence than that of the continuous EOD, which likely reflects its reliance on chemical rather than electrotonic synapses. In vitro pacemaker firing frequency had a similar, but slightly higher Q_{10} (1.82) than that of the EOD frequency.

Introduction

Virtually all animal behaviors are influenced by body temperature, and this is due in part to the temperature sensitivity of the nervous tissues that initiate behavior [Montgomery and MacDonald, 1990]. Many neural processes ranging from molecular to system levels respond in different ways to temperature change. In their pioneering work on neuronal excitation in squid, Hodgkin and Huxley [1952] reasoned on theoretical grounds that the temperature dependence of action potential propagation was determined primarily by axoplasmic resistance and maximum conductance values, and accordingly, they predicted that the temperature coefficient (Q_{10}) of conduction velocity should be 1.7. (Q_{10} is the relative change in a parameter for a 10 °C increase in temperature.) Subsequent empirical work on many differ-

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/journals/bbe

Kent Dunlap
Department of Biology, Trinity College
Hartford, CT 06106 (USA)
Tel: 860-297-2232, Fax: 860-297-2538
E-Mail: kent.dunlap@mail.trincoll.edu

ent preparations (e.g., toad, horseshoe crab, guinea pig) produced results that agreed closely with Hodgkin and Huxley's prediction for the temperature dependence of activity in a single neuron [reviewed in Joyner, 1981].

The activity of neural circuits typically has a greater temperature dependence than that of single neurons because of qualitatively different processes occurring at synapses. For example, one important synaptic event, neurotransmitter release, relies on calcium currents, and consequently synaptic transmission typically occurs with high Q_{10}s (~3–5) that characterize calcium channel kinetics [Nobile et al., 1990; Janssen, 1992]. Again, studies of diverse preparations (e.g., rat, horseshoe crab, frog) have consistently shown that transsynaptic transmission is more sensitive to temperature changes than axonal transmission [reviewed in Janssen, 1992].

These previous studies used invasive procedures to characterize the temperature-dependence of neural processes. Furthermore, the behavioral relevance of the neural circuits examined by these studies was often not clear. The electrocommunication system of weakly electric fish provides an unusual opportunity to examine non-invasively the temperature-dependence of a neural circuit whose activity can be related directly to behavior. The weakly electric fish *Apteronotus leptorhynchus* continuously emits an electrical discharge into the water from a specialized, neurally-derived electric organ. The electric organ discharge (EOD) creates an electric field around the fish that has at least three functions [Bullock and Heiligenberg, 1986]. First, by sensing distortions to this field caused by external objects, it can use this signal to localize prey and navigate through its environment. Second, the discharge rate of the electric organ is sexually dimorphic, and thereby it is used in gender recognition. Finally, brief modulations of the EOD known as chirps serve as communication signals in both courtship and aggression [Hagedorn and Heiligenberg, 1985; Dunlap et al., 1998]. All of these signals can be recorded non-invasively by placing electrodes in the water surrounding the fish.

The neural circuit generating the continuous EOD is extraordinarily simple. It consists of only five cell types, four of which are electrotonically coupled. The EOD originates in the pacemaker cells of the medullary pacemaker nucleus [Ellis and Szabo, 1980; Tokunaga et al., 1980; Elekes and Szabo, 1985]. These cells make electrical synapses onto relay cells which, in turn, travel down the spinal cord to synapse onto electromotor neurons [Bennett et al., 1978]. The axons of the electromotor neurons extend into the tail to form the electric organ, and their simultaneous excitation generates the EOD [Waxman et al., 1972]. The rate at which the electric organ discharges is termed the EOD frequency, and, at any given temperature, it is extraordinarily constant over a time span of hours when the fish is isolated [Moortgat et al., 1998].

Chirps are produced in response to conspecific EOD signals and regulated primarily by a portion of the prepacemaker nucleus (PPn-C) in the diencephalon. Prepacemaker neurons that activate chirps make glutamatergic synapses (via AMPA receptors) onto relay cells, causing transient (~10 ms) increases in EOD frequency and decreases in EOD amplitude [Heiligenberg et al., 1996; reviewed in Zupanc and Maler, 1997]. Prepacemaker neurons receive input from several sensory and other brain regions, and the rate of chirping is likely regulated through these other transmitter or neuromodulator-mediated inputs. Thus, in an intact animal we can examine the temperature dependence of both a continuous, electrically coupled system and a transient modulation mediated through chemical synapses. In addition, we can excise the pacemaker nucleus from the brain and determine its temperature-dependent activity in vitro.

Here, we expand upon the previous work by Enger and Szabo [1968] and Feng [1976] to characterize more fully the temperature sensitivity of the electrocommunication signals of a wave-type weakly electric fish, *Apteronotus leptorhynchus*, and to compare the activity of the pacemaker neurons in vivo and in vitro. Our additional analysis enables us to report three new findings on the temperature sensitivity of wave-type species. First, temporal and amplitude components of the wave are affected differentially by temperature change. Secondly, the electrotonically-coupled circuitry underlying the continuous EOD and the chemically-mediated circuitry controlling chirping respond to temperature change differently. Finally, in vitro activity of the pacemaker neurons and the in vivo electromotor output which they control differ in their thermal dependence.

Materials and Methods

Apteronotus leptorhynchus were obtained from commercial dealers and housed in tanks that were part of a 400 gallon circulating system. Water conditions were held at a constant temperature (28.0 °C), light cycle (12L:12D) and water conductivity (800 µS/cm). Fish were fed frozen brine shrimp every 2 d. Our research was performed under the guidelines outlined in 'Principles of laboratory care' (NIH publication No. 85-23).

Five male and three female fish were tested for the effects of temperature on EOD frequency and amplitude. (EOD frequency was defined as the rate at which the electric organ discharges in Hertz, and EOD amplitude was defined as the peak to peak voltage change in millivolts.) Gender was identified by the sexual dimorphism in EOD

frequency. Three of these fish were also used to determine the effects of temperature on in vitro firing rate of pacemaker neurons. An additional twelve male fish were tested for temperature effects on evoked chirping. Only males were used because they are much more likely to exhibit chirping than females [Dulka and Maler, 1994; Dunlap et al., 1998].

In vivo Testing Apparatus

To measure EOD parameters and evoked chirping responses, we used an apparatus similar to that described earlier [Dunlap et al., 1998]. In brief, fish were placed in a clear plexiglass tube (8 cm diameter × 24 cm length), with two 5 × 7 cm sections cut from the mid-point of each side. Each of these windows and one end of the tube were covered with nylon mesh; the other end was fitted with a removable mesh door. The apparatus was submerged in a 20 L testing aquarium (64 × 64 × 50 cm), and the temperature of the water within 2 cm of the fish was measured continuously by a digital thermometer within 0.1 °C.

The fish's EOD was recorded through Ag-AgCl electrodes (1 cm in length) attached to the ends of the tube (parallel to the fish's body axis). The signal of the fish was amplified and monitored continuously on an oscilloscope (Tektronix, Beaverton, Oreg., USA) and frequency counter (Fluke model 7260A, Everett, Wash., USA) and recorded on video tape. To evoke chirps from the test fish, sinusoidal stimulus signals generated by a pulse generator (Wavetek, San Diego, Calif., USA) were presented to the fish through two carbon electrodes (0.5 cm diameter × 7 cm length) attached to the apparatus at the mid-point of the windows and positioned orthogonal to the axis of the fish's body. The field intensity created by this stimulus was 0.85–1.10 mV/cm.

Previous studies indicate that placing *A. leptorhynchus* in a tube can distort the EOD waveform [Rasnow et al., 1993]. To determine the effect of the tube on waveform, we also recorded EODs from fish placed in fine nylon mesh netting with the recording electrodes in a position identical to that of the plexiglass tube.

In vivo Testing Procedures

Each fish was removed from its home aquarium and placed in the testing apparatus. The water in the test aquarium was aerated and circulated, and the initial temperature (28.0 °C) and conductivity (800 µS) were kept identical to those of the fish's home tank. Fish were allowed to acclimate to these conditions for at least 15 min before testing.

Fish were tested for their response to warming, cooling or both. The test aquarium was warmed by two aquarium heaters, and cooled by placing glass jars of ice water in the aquarium. For both warming and cooling, the rate of temperature change was 0.23–0.26 °C/min (mean = 0.25 ± 0.007 °C/min), and the range of temperatures was 18.0–32.0 °C. To be certain that the fish's temperature had equilibrated to the water temperature, the water temperature at each extreme (18 and 32 °C) was held constant for 5 min before the temperature was manipulated. The order of the temperature manipulations (warming vs. cooling) was chosen randomly. The water temperature, EOD frequency and EOD amplitude were recorded every 3–4 min.

To quantify the effect of the test apparatus on EOD waveform, five fish were placed alternately in nylon mesh netting and plexiglass tube and tested identically to the procedure above. Recordings were made at three temperatures (18, 25 and 32 °C) and only during cooling.

Twelve different fish were tested for chirping in response to sinusoidal stimulus signals at five temperatures (18, 22, 25, 28 and 32 °C). Chirping was recorded during either warming or cooling. The stimulus was adjusted to 3–5 Hz below the fish's EOD frequency and presented once for 30 s at each temperature. Chirping rate was quantified as the total number of chirps/min.

Harmonic Analysis of EOD Waveform

The harmonic content of the EOD was determined from the Fast Fourier Transformation (FFT) of 500 ms segments of signal recorded on video tape using a procedure published previously [Dunlap and Zakon, 1998]. Recordings were converted from analog to digital, sampled at 22 kHz and analyzed using Canary software (Cornell Laboratory of Ornithology, Ithaca, N.Y., USA). For the analysis, frame length was 371.5 ms, overlap was 98.44% and the FFT size was 8192 points. The amplitudes (dB) of the first three peaks (F1, F2, F3) were subtracted from the baseline amplitude, and the amplitude of the second (F2) and third (F3) harmonic (relative to the fundamental frequency) was calculated as F2/F1 and F3/F1, respectively.

In vitro Testing

Fish were deeply anesthetized with 0.75% 2-phenoxyethanol (Sigma, St. Louis, Mo., USA) and placed on ice. Their brains were quickly removed and placed in cold artificial cerebrospinal fluid (ACSF, in mM: 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 1.1 $MgSO_4$, 1.1 $CaCl_2$, 16 $NaHCO_3$, 10 D-glucose; pH 7.4). The pacemaker nucleus, which is visible as a protrusion on the ventral surface of the medulla, was removed with iridectomy scissors and placed in a recording chamber, where it was continuously superfused with ACSF bubbled with 95% O_2/5% CO_2.

Intra- and extra-cellular recordings of neurons in the pacemaker nucleus were made with glass microelectrodes (borosilicate, o.d. 1.2 mm, i.d. 0.7 mm, resistance ~30–60 MΩ) filled with 3M KCl. Recordings were made from 2–4 cells from 3 individuals. For 6 of the 9 cells, recordings were made during both warming and cooling; the remaining three were recording during warming only. The signal from these electrodes was amplified with an Intra 767 amplifier (World Precision Instruments, Sarasota, FL), filtered at ~10 kHz, and recorded on a data recorder (Vetter model 400, Rebersburg, Pa., USA).

The temperature of the chamber was measured with a thermister placed within 2 mm of the tissue in the bath. This thermister was connected to a digital thermometer (resolution = 0.1 °C) and a TCU-2 temperature controller unit (Fine Science Tools, Foster City, CA) that controlled a 7.7 Ω heating element in a water bath through which the superfusing ACSF passed. After 3 min of baseline recording, the temperature of the recording chamber was gradually raised by increasing the setting of the temperature controller. Each preparation was exposed to a range of temperatures (average minimum temperature – 23.3 °C; average maximum temperature – 26.9 °C). When temperatures approached 27 °C, temperatures were lowered by decreasing the setting of the temperature controller and adding a small amount of ice to the water bath through which the superfusing ACSF passed.

The firing frequency of neurons in the pacemaker nucleus was measured off-line from the data recordings. Signals from the data recorder were digitized with a signal manifold (USM-100, World Precision Instruments), A-D converter (Data Translation model DT2821), and a 386-based microcomputer running the Spike analysis program (Hillal Associates, Englewood, N.J., USA). Signals were sampled at 24 kHz. Frequency was determined by measuring the time between the peak of the first and last spike in the 25 ms bin and dividing the number of spikes between those peaks by the time interval between the peaks. With the 24 kHz sampling rate, this resulted in a frequency estimate with a resolution of approximately ± 0.16%. The start of the 25 ms bins

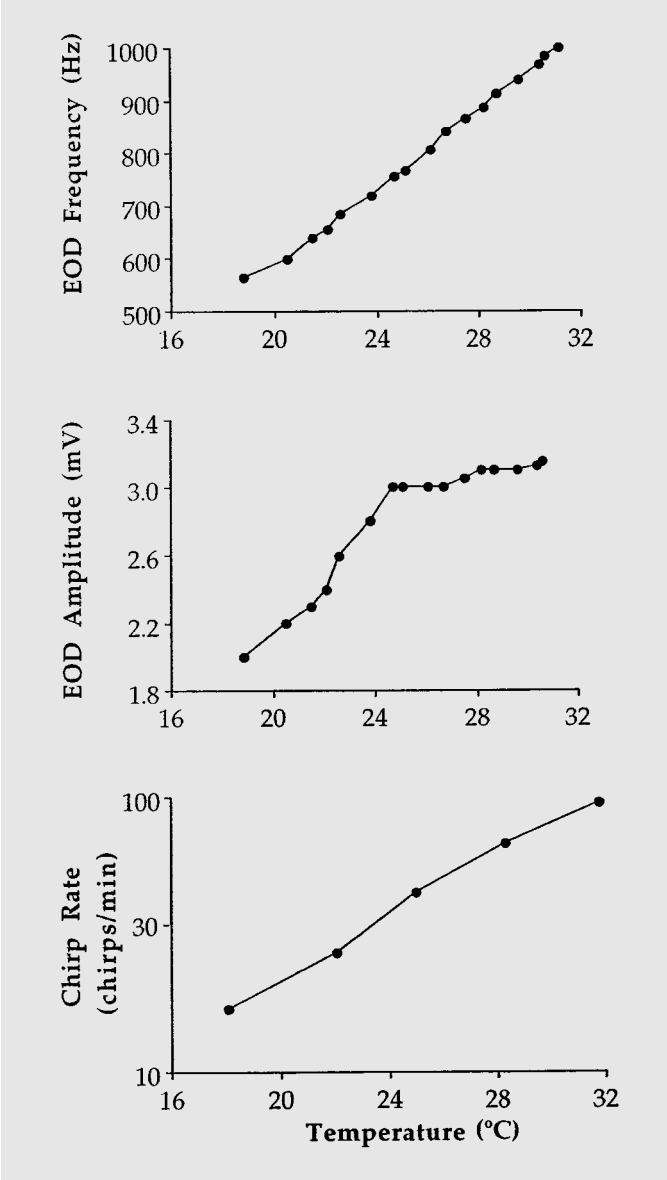

Fig. 1. Effect of temperature on the EOD frequency, EOD amplitude and chirp rate of a representative individual *A. leptorhynchus* during warming. For chirp rate, note the log scale on the ordinate axis.

from which frequency measurements were made coincided with the times of each 0.1 °C change on the digital readout of the thermometer.

Statistics

Q_{10} values were calculated for EOD frequency, amplitude, and chirp rate using the equation:

$$X_1/X_2[10/(T_2–T_1)]$$

where X_1 is the value at the lower temperature (T_1) and X_2 is the value at the higher temperature (T_2) [Schmidt-Nielsen, 1997].

Table 1. Effects of temperature change on electric organ discharge parameters

Parameter	Temp. range (°C)	Warming (W) or Cooling (C)	Q_{10}	95% Conf. Int.
EOD Frequency	18–32	W	1.63*	1.628–1.637
		C	1.60	1.597–1.603
EOD Amplitude	18–25	W	1.96	1.81–2.09
		C	2.08	2.01–2.14
	25–32	W	1.19	1.13–1.23
		C	1.14	1.12–1.16
Chirp Rate	18–32	W	2.97	2.20–3.80
		C	3.37	2.54–4.16
In vitro pacemaker firing frequency	23–27	W	1.81	1.78–1.87
		C	1.81	1.73–1.91

* Significant difference between warming and cooling.

To analyze for temperature-dependent changes in harmonic content of the EOD, we used a repeated measures MANOVA with F2/F1 and F3/F1 as dependent variables and replicate and temperature as within-subjects repeated measures and direction of temperature change (heating vs. cooling) as a between-subjects independent variable. Prior to analysis, F2/F1 and F3/F1 ratios were arcsine transformed. The initial analysis showed that neither replicate ($F = 1.83$, d.f. = 3, $p > 0.05$), direction of change ($F = 0.92$, d.f. = 1, $p > 0.05$) nor any interaction with these variables were statistically significant factors. Thus, in subsequent analyses, we used repeated measures MANOVA with mean F2/F1 and F3/F1 as dependent variables and only temperature as the repeated measure. Fisher's PLSD was used as a post-hoc test to identify significant differences between temperatures. To determine the effect of testing apparatus on waveform, we also used repeated measures MANOVA, with F2/F1 and F3/F1 as dependent variables and replicate and temperature as within-subjects repeated measures and apparatus type (mesh vs. tube) as a between-subjects independent variable. In all tests, p-values < 0.05 were considered significant.

Results

EOD Frequency and Amplitude

EOD frequency (discharge rate) was linearly related to temperature (fig. 1, 2), with r-squared values consistently greater than 0.97. The mean Q_{10} during warming (1.63) was slightly and statistically higher ($F = 21.02$, d.f. = 7, $p < 0.0005$) than the Q_{10} during cooling (1.60) (table 1). Q_{10}s showed little variation between individuals (fig. 2).

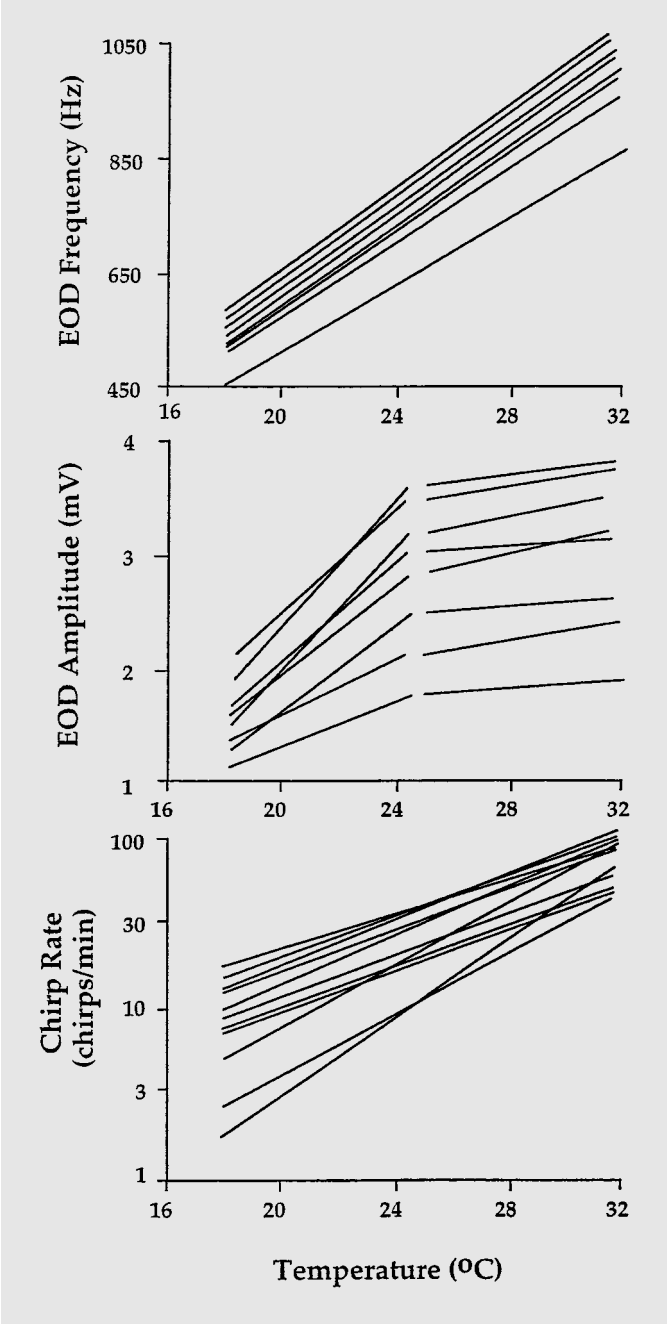

Fig. 2. Effect of temperature on EOD frequency (n = 8), amplitude (n = 8) and chirp rate (n = 11) of all fish tested. Each line represents the least-squares regression for values during warming for an individual. For EOD amplitude, data from temperatures < 25 °C and > 25 °C were analyzed separately (see Methods). For chirp rate, note the ordinate log scale. For mean Q_{10} values of EOD parameters, see table 1.

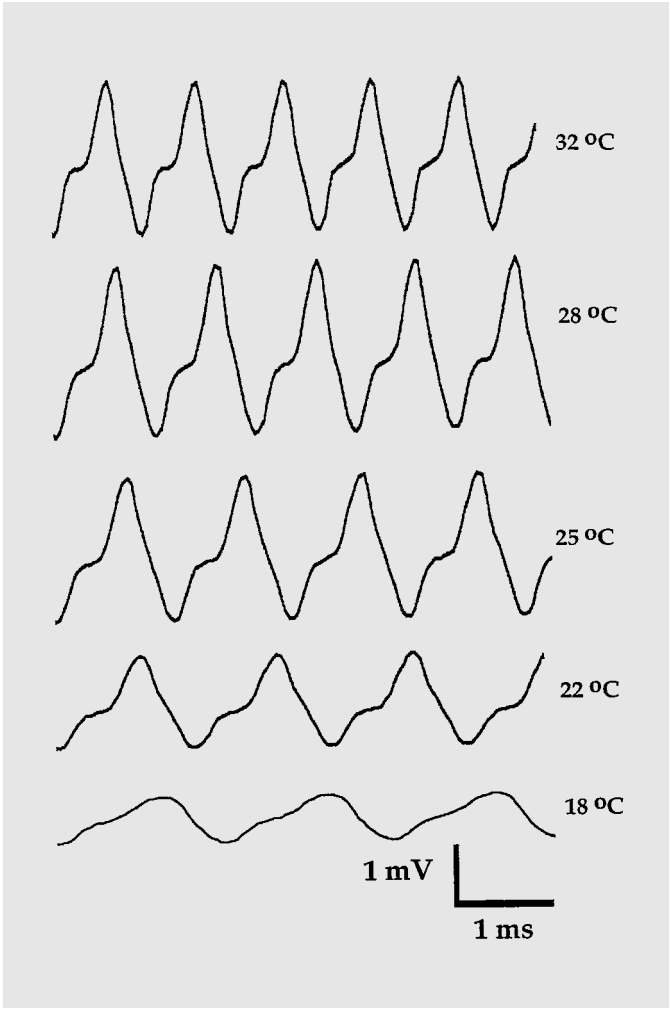

Fig. 3. Temperature-dependent variation in EOD waveform of *A. leptorhynchus* showing traces from a representative individual. For mean harmonic heights of all individuals, see table 2.

By contrast, EOD amplitude was not linearly related to temperature. Instead, amplitude increased steeply with temperature below 25 °C (Q_{10} = 2.02), but increased only gradually above 25 °C (Q_{10} = 1.16; fig. 1, 2; table 1). Our division of the data at 25 °C was based on visual inspection of the data and is thus somewhat arbitrary. We chose not to fit the data with a curvilinear regression because there were no theoretical grounds indicating that a curvilinear model was appropriate. Although the slope was considerably lower at high temperatures, it was significantly greater than zero ($Q_{10} > 1.0$) in seven of eight individuals. The temperature dependence of EOD amplitude was not different during warming and cooling at either low (< 25 °C) or high (> 25 °C) temperature ($F = 2.3$, d.f. = 7, $p > 0.05$).

Table 2. Effects of temperature on relative amplitudes of second (F2/F1) and third (F3/F1) harmonic

	Temperature (°C)					F value	p value
	18	22	25	28	32		
F2/F1	0.835 ± 0.007^{a}	0.813 ± 0.011^{b}	0.798 ± 0.009^{b}	0.812 ± 0.009^{b}	0.844 ± 0.013^{a}	4.61	0.0055
F3/F1	0.704 ± 0.010^{c}	0.798 ± 0.006^{b}	0.820 ± 0.004^{d}	0.825 ± 0.003^{d}	0.844 ± 0.005^{a}	32.26	0.0001

Values with different superscript letters are significantly different from each other ($p < 0.05$).

Table 3. Analysis of variance for the effects of temperature (Temp), test apparatus (tube vs. mesh netting) and their interaction on the relative amplitudes of the second (F2/F1) and third (F3/F1) harmonics

	Variable	F value	df	p value
F2/F1	Temp	8.93	2	0.009
	Apparatus	1.92	1	0.184
	Temp × Apparatus	0.224	2	0.787
F3/F1	Temp	52.9	2	0.0001
	Apparatus	3.19	1	0.092
	Temp × Apparatus	0.019	2	0.98

EOD Waveform and Harmonic Content

The different temperature dependence of EOD frequency and amplitude causes substantial temperature-dependent variation in EOD waveform (fig. 3). Analysis of variance using temperature as the repeated measure demonstrated that both F2/F1 and F3/F1 ratios changed significantly with temperature (table 2). The second harmonic was relatively high at both high and low temperatures and relatively low at intermediate temperatures. Values for F2/F1 at 18 °C (F2/F1 = 0.835) and 32 °C (F2/F1 = 0.844) were significantly higher ($F = 4.61$, d.f. = 7, $p < 0.005$) than at 22 °C (F2/F1 = 0.813), 25 °C (F2/F1 = 0.799) and 28 °C (F2/F1 = 0.812). Values for F2/F1 at intermediate temperatures were not significantly different from each other. The relative harmonic content of F3/F1 showed an increase with temperature (table 2). The relative amplitude of F2 at 32 °C was significantly greater than that at 18 °C ($F = 32.2$, d.f. = 7, $p < 0.0001$); there were no significant differences at other temperatures.

When testing for the effect of test apparatus on waveform, we found an overall effect of apparatus type that approached statistical significance ($F = 1.8$, d.f. = 1, $p < 0.2$; table 3). Fish recorded in mesh netting had F2/F1 and F3/F1 ratios that tended to be lower than those recorded from fish in tubes. However, the interaction between temperature and apparatus type was not close to statistical significance ($F = 0.02$, d.f. = 2, $p > 0.75$; table 3). Thus, although the plexiglass tube may influence the harmonic content of the EOD, we conclude that temperature-dependent changes in harmonic content occur in a similar manner regardless of the fish's immediate physical surroundings.

Chirping

Chirp rate increased with temperature at a relatively high rate ($Q_{10} = 3.17$) (fig. 1, 2; table 1), and there was no statistical difference in the slope during warming and cooling ($F = 5.9$, d.f. = 11, $p > 0.05$). This Q_{10} was significantly higher than that of EOD frequency ($F = 31.11$, d.f. = 11, $p > 0.0005$). Q_{10} of chirp rate also showed considerably more individual variation than that of EOD frequency or amplitude (fig. 2).

In vitro Firing Frequency

In vitro pacemaker firing frequency increased with temperature with a Q_{10} of 1.82 and did not differ during warming vs. cooling (table 1; fig. 4). The mean Q_{10} of neurons within an individual did not differ significantly among individuals and was similar to the mean Q_{10} of all neurons pooled together. The Q_{10} of in vitro firing frequency (1.82) was statistically higher than that of EOD frequency (1.615) ($F = 70.51$, d.f. = 4, $p < 0.0001$).

Discussion

Our results show that frequency (discharge rate) and amplitude components of an electrocommunication signal respond differently to temperature change (fig. 1, 2; table 1). As a consequence, the harmonic content of the signal also changes with temperature (table 2), and thus temperature has qualitative as well as quantitative effects on the production of this communication signal. We also found that an

EOD modulation (chirp) has a different temperature dependence than the continuous EOD, which likely reflects its reliance on chemical rather than electrotonic synapses.

Apteronotus leptorhynchus is native to the neo-tropics. The environment through much of its equatorial range is approximately 23–28 °C with relatively little temperature variation [Westby, 1988; Hagedorn and Keller, 2000]. However, the full distribution of this species is not known, and it is possible that it inhabits waters at the periphery of its range with more drastic temperature changes. For example, another gymnotiform electric fish, *Brachyhypopomus pinnecaudatus*, lives in streams in the southern part of its range that vary diurnally between 18 and 33 °C, and seasonally between 9 and 33 °C. These temperature variations also cause changes in waveform and harmonic content as well [Caputi et al., 1998; Silva et al., 1998]. Thus it is plausible that the temperature changes we conducted in the laboratory (18–32 °C) are relevant to the fish's natural environment. However, the ecological significance of our results can only be clarified by more detailed mapping of the species distribution and the associated thermal environment.

EOD Frequency

Many neurophysiologists studying electric fish have temperature adjusted their data using a Q_{10} of 1.5 based on the results of Enger and Szabo [1968]. Enger and Szabo pointed out several shortcomings in their methods which we sought to improve. They used only a single individual of each genus, they did not state which species they used within each genus, their measurement of 'discharge frequency was determined rather crudely … on an oscilloscope', the rate of temperature change was inconsistent, and they made only a rough estimation of the Q_{10} for each species. When exposing many individuals to an even rate of temperature change, we calculated the mean Q_{10} of *Apteronotus leptorhynchus* EOD frequency at 1.62. For many studies, this distinction may be trivial, but for studies carefully examining social, hormonal or hydrological influences on EOD frequency, this difference could be important. For example, take the case in which a researcher measures an EOD frequency of 800 Hz at room temperature (22 °C) and adjusts this value to 28 °C. Using a Q_{10} of 1.5 rather than 1.62 would yield values of 1020 and 1068 Hz, respectively (fig. 5). This difference could be significant considering the range of EOD frequency changes caused by maturation, social stimuli and hormonal manipulation range from ten to several hundred hertz [Hagedorn and Heiligenberg, 1985; Meyer et al., 1987; Dulka and Maler, 1994; Schaefer and Zakon, 1996].

The only other study of thermal influences on EOD frequency in an electric fish with a continuous, wave-type

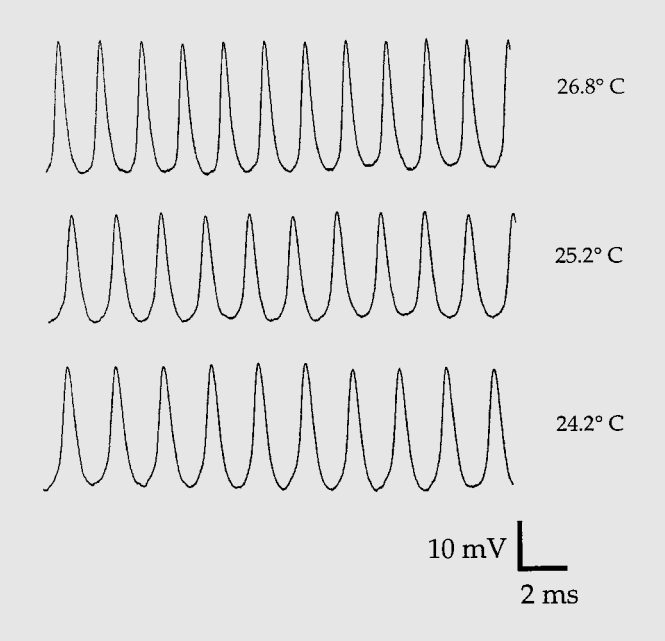

Fig. 4. Extracellular recording of a single neuron in an in vitro pacemaker nucleus, illustrating change in firing frequency with temperature.

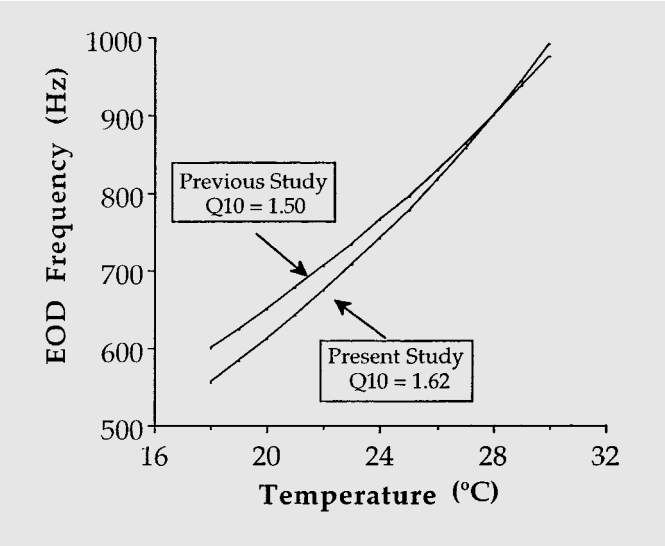

Fig. 5. Effect of temperature on the EOD frequency, contrasting the Q_{10} values found in the present ($Q_{10} = 1.62$) and a previous study [$Q_{10} = 1.5$; Enger and Szabo, 1968]. See Discussion for details.

EOD was done on *Eigenmannia virescens*. Feng [1976] found that between ~18 and 25 °C, the Q_{10} was roughly similar (~1.5) to that which we report for *Apteronotus*. However, at higher temperatures the Q_{10} decreased such that, above 26 °C, EOD frequency was essentially temperature independent (Q_{10} = ~1.0). By contrast, the Q_{10} of EOD frequency in *Apteronotus* was constant across a similar range of temperatures. This species difference could be due to differences in the composition of the electric organ and its synapse type. As mentioned above, the electric organ of *Apteronotus* is neurogenic, comprised only of neural tissue that is electrically coupled in the central nervous system. In *Eigenmannia*, the electric organ is myogenic. It derives embryologically from muscle, and, like muscle, its excitation is mediated through cholinergic synapses. Given intrinsic limitations to the firing rate of muscle-like cells, we speculate that, above 26 °C, the EOD frequency simply cannot increase any further. In the example shown by Feng [1976], EOD frequency plateaued at ~550 Hz at 26 °C. If it were to have the same Q_{10} at low and high temperatures, the EOD frequency would be more than 700 Hz at 30 °C. Perhaps biophysical constraints (e.g. limitations on the rate of synaptic transmission) simply do not permit firing at such a high frequency in a myogenic system.

We found a small but statistically significant hysteresis in EOD frequency: the Q_{10} was slightly higher during warming (1.63) than during cooling (1.60) (table 1). Such differences have been found inconsistently in other studies of electric fish [Enger and Szabo, 1968; Feng, 1976]. In our case, the small hysteresis might have been caused by slight differences in the lag time between temperature changes in the water and body temperature responses during warming and cooling. However, given the relatively small size of the effect, we consider the average (1.615 or 1.62) as an adequate temperature coefficient for future studies of *Apteronotus* EOD frequency. Hysterisis was not found in any other parameter.

EOD Amplitude

In contrast to the constant Q_{10} of EOD frequency in *Apteronotus*, the Q_{10} of EOD amplitude varied significantly with temperature change (fig. 1, 2; table 1). This is the first demonstration of temperature-dependence of EOD amplitude in any wave-type electric fish. However, Caputi et al. [1998] found in the pulse-type fish *B. pinnecaudatus* that EOD amplitude also does not change linearly with temperature. This is due in part to the fact that the positive and negative phases of the pulse respond differently to temperature change. In other nerve preparations, there is considerable variation in the relation between amplitude and temperature. Despite this variation, one general property emerges: Q_{10} is higher at low temperatures than at high temperatures [reviewed in Janssen, 1992]. The temperature dependence of EOD amplitude in *Apteronotus* bears out this generality.

The biophysical basis for the relationship between temperature and spike amplitude has been debated since the 1930's [Gasser, 1931; Hodgkin and Katz, 1949; Hodgkin and Huxley, 1952; Hlavova et al., 1970], and to our knowledge, no thorough formulation has emerged. Hodgkin and Katz [1949] argued that the decay phase (repolarization) was slowed more than the rise phase (depolarization) as temperature decreased, resulting in greater amplitudes at low temperatures. However, this only explains a negative relation between amplitude and temperature and does not account for the positive relationship or temperature-independence found commonly in certain preparations. In *Apteronotus*, the discontinuity in Q_{10} at ~25 °C might be explained by a non-linear temperature sensitivity of the sodium channels. EOD amplitude is largely controlled by sodium currents. The Q_{10}s of peak sodium currents in rat myelinated nerve fibers are 1.9 at low temperatures and 1.1 at high temperatures [Schwartz, 1986], quantitatively very similar to those of EOD amplitude in *Apteronotus*. A qualitatively similar inverse relation between temperature and the Q_{10} of sodium conductance has been reported for rabbit [Chui et al., 1979] and squid [Kimura and Meves, 1979] nerve preparations. In all these species, the relatively low Q_{10}s at high temperatures may indicate that spike amplitude approaches a biophysical 'ceiling' in which all relevant channels are open and thereby transmembrane currents are maximal.

EOD Waveform and Harmonic Content

Given the above temperature-dependence of EOD frequency and amplitude, it is clear that the shape of the wave changes drastically with temperature: the wave has a low amplitude and long period at low temperature (18 °C), a high amplitude and intermediate period at intermediate temperature (25 °C), and high amplitude and short period at high temperature (32 °C) (fig. 3). As in the pulse-type fish *B. pinnecaudatus* [Caputi et al., 1998], the temperature-dependence of EOD waveform in *A. leptorhynchus* results from temperature affecting temporal (i.e. duration or frequency) and amplitude components of the wave differently. The temperature-dependent modifications of waveform are manifest in changes in the harmonic content. Interestingly, the relative amplitude of the second and third harmonics differ in their response to temperature change. F2 was relatively weak at temperatures that fish typically experience

(22–28 °C) and strong at temperature extremes (18 and 32 °C). By contrast, F3 was weak at low temperature and increased directly with temperature.

Temperature-dependent changes in harmonic content may influence the function of the EOD in electrocommunication. In another gymnotiform species, *Eigenmannia*, fish exhibit different responses to artificial stimuli depending on the harmonic content of the stimuli [Kramer, 1985]. By inference, *Apteronotus* might respond differently to high temperature signals (relatively high second and third harmonics), intermediate temperature signals (relatively low second and third harmonics) and low temperature signals (high second harmonic, low third harmonic). However, there could also be temperature-dependent changes in the sensory processing by electroreceptive pathways that compensate or otherwise modify the responses to these qualitatively different signals. In *Eigenmannia* and *Sternopygus*, the best frequency of tuberous electroreceptors and the EOD frequency shift in the same direction during temperature change, but it appears that electroreceptive input and electromotor output may respond with different Q_{10}s (~2.8 for best frequency vs. ~1.5 for EOD frequency) [Hopkins, 1976]. Similar studies on acoustic communication in crickets [Pires and Hoy, 1992] and frogs [Gerhardt, 1978] have shown that temperature affects signal reception and production in parallel ways, and consequently information transfer is conserved during temperature changes.

Chirp Rate

Chirping and its control by the PPn-C has become an important system for examining the neuronal control of behavioral plasticity [Zupanc and Maler, 1997], yet, until now, nothing was known about the thermal dependence of this behavior. We found that chirp rate was more sensitive to temperature change than the frequency or amplitude of the continuous EOD. The relatively high Q_{10} of chirp rate likely results from the involvement of chemical synapses in modulating the activity of the electrically coupled circuit underlying the continuous EOD. As stated earlier, several inputs from the electroreceptive and other sensory systems converge on the prepacemaker nucleus, and it is likely that the slowing of chirp production at low temperatures is caused by temperature changes affecting chemical transmission in regions before the prepacemaker-relay cell synapse. The relatively large individual variation in Q_{10} of chirp rate, ranging from ~2 to 6 (fig. 2), may also indicate that temperature differentially affects individuals' motivational state.

In *Eigenmannia*, Feng [1976] also found that EOD modulations are more temperature sensitive than the continuous EOD. He examined the temperature dependence of the jamming avoidance response (JAR) in which a fish slowly shifts its EOD frequency away from an applied frequency. Similar to chirps, the JAR is produced through monosynaptic input from the prepacemaker to the pacemaker. However, it originates from a separate part of the prepacemaker (sPPn) and acts through NMDA-type receptors [Heiligenberg et al., 1996; Zupanc and Maler, 1997]. The Q_{10} for both the magnitude of the JAR and EOD frequency increased as the fish cooled, and the Q_{10} of the JAR (2–8) was consistently higher than that of the EOD frequency (~1–2).

In vitro Pacemaker Firing Frequency

The Q_{10} of the in vitro pacemaker firing frequency was similar to, but consistently higher than that of the EOD frequency in intact animals (table 1, fig. 4). This difference suggests that the Q_{10} of the EOD frequency is influenced by temperature dependent processes in other cells of the electromotor circuit (i.e. the relay cells and the electromotor neurons), and not just the pacemaker neurons. For example, electromotor neurons oscillate endogenously at frequencies that, at least under stable temperatures, are similar to the EOD frequency [Schaefer and Zakon, 1996]. If their firing rate or axonal conduction also follows a Q_{10} close to 1.6, they could act as a limiting step in determining the Q_{10} of EOD frequency. Alternatively, temperature dependent processes in higher brain regions that tonically influence pacemaker activity might also limit Q_{10} of EOD frequency.

Conclusion

Behavioral performance of ectothermic animals often changes quite predictably with temperature. Yet the simplicity of this relationship often masks the fact that component processes generating behavior respond differently to temperature change [Rome, 1990; Montgomery and MacDonald, 1990]. Clarifying how behavioral coordination (or limitation) emerges from the variety of temperature-dependent sub-processes is a major challenge of integrative biology.

Even within the relative simplicity of the electromotor system of gymnotiform fish, it appears that several features of the communication system vary in their response to temperature change. First, temporal and amplitude components of the EOD respond differently to temperature change, yielding qualitative as well as quantitative changes in the communication signal during temperature fluctuations [table 2; Caputi et al., 1998]. Secondly, modulation of the EOD (i.e. chirping and the JAR) is more temperature-

dependent than the continuous EOD [table 1; Feng, 1976]. Thirdly, the temperature dependence of the EOD frequency differs from that of the neuronal pacemaker that generates the EOD (table 1). Finally, it appears that electromotor and electroreceptive processes may differ in their thermal-dependence [Hopkins, 1976]. More research is required to elucidate both the mechanistic causes of this variation and its significance for electrocommunication and electrolocation.

Acknowledgments

We thank D. Cruz-Uribe for help with harmonic analysis, D. Reuman for statistical advise, Y. Lu for fish care, and J. Larkins-Ford for help with data collection. This research was supported in part by NIH grants to K. Dunlap (F32 NS09633) and G.T. Smith (F32 NS10150).

References

Bennet, M., C. Sandri, and K. Akert (1978) Neuronal gap junctions and morphologically mixed synapses in the spinal cord of a teleost, *Sternarchus albifrons* (Gymnotoidei). Brain Res., *143:* 43–60.

Bullock, T.H., and W. Heiligenberg (eds.) (1986) Electroreception. Wiley, New York.

Caputi, A.A., A.C. Silva, and O. Macadar (1998) The electric organ discharge of *Brachyhypopomus pinnicaudiatus*: effects of environmental variables on waveform generation. Brain Behav. Evol., *52:* 148–158.

Chui, S.Y., H.E. Mrose, and J.M. Ritchie (1979) Anomalous temperature dependence of sodium conductance in rabbit nerve compared with frog nerve. Nature, *279:* 327–328.

Dulka, J.G., and L. Maler (1994) Testosterone modulates female chirping behavior in the weakly electric fish, *Apteronotus leptorhynchus*. J. Comp. Physiol. A, *174:* 331–343.

Dunlap, K.D., P. Thomas, and H.H. Zakon (1998) Diversity of sexual dimorphism in electrocommunication signals and its androgen regulation in a genus of electric fish, *Apteronotus*. J. Comp. Physiol. A, *183:* 77–86.

Dunlap, K.D., and H.H. Zakon (1998) Behavioral actions of androgens and androgen receptor expression in the electrocommunication system of an electric fish, *Eigenmannia virescens*. Horm. Behav., *34:* 30–38.

Elekes, K., and T. Szabo (1985) Synaptology of the medullary command (pacemaker) nucleus of the weakly electric fish *(Apteronotus leptorhynchus)* with particular reference to comparative aspects. Exp. Brain. Res., *60:* 509–520.

Ellis, D., and T. Szabo (1980) Identification of different cells types in the command (pacemaker) nucleus of several gymnotiform species by retrograde transport of horseradish peroxidase. Neuroscience, *5:* 1917–1929.

Enger, P.S., and T. Szabo (1968) Effects of temperature on discharge rate of the electric organ of some gymnotids. Comp. Biochem. Physiol. A, *27:* 625–627.

Feng, A.S. (1976) The effect of temperature on a social behavior of weakly electric fish *Eigenmannia virescens*. Comp. Biochem. Physiol. A, *55:* 99–102.

Gasser, H.S. (1931) Nerve activity as modified by temperature changes. Am. J. Physiol., *97:* 254–270.

Gerhardt, H.C. (1978) Temperature coupling in the vocal communication system of the gray tree frog, *Hyla versicolor*. Science, *199:* 992–994.

Hagedorn, M., and W. Heiligenberg (1985) Court and spark: electric signals in the courtship and mating of gymnotid fish. Anim. Behav., *33:* 254–265.

Hagedorn, M., and C. Keller (2000) Species diversity of gymnotiform fishes in Manu Bioreserve, Pakita, Peru. *In* Biodiversity of Manu Park (ed. by D. Wilson and A. Sandoval).

Heiligenberg, W., W. Metzner, C. Wong, and C. Keller (1996) Motor control of the jamming avoidance response of *Apteronotus leptorhynchus*: evolutionary changes of a behavior and its neuronal substrates. J. Comp. Physiol. A, *179:* 653–674.

Hlavova, A., D. Abramson, B. Rickert, and J. Taslo (1970) Temperature effects on duration and amplitude of distal median nerve action potential. J. Appl. Physiol., *28:* 808–812.

Hodgkin, A.L., and A.F. Huxley (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol., *117:* 500–544.

Hodgkin, A.L., and B. Katz (1949) The effect of temperature on the electrical activity of the giant axon of the squid. J. Physiol., *109:* 240–249.

Hopkins, C.D. (1976) Stimulus filtering and electroreception: Tuberous electroreceptors in three species of gymnotid fish. J. Comp. Physiol. A, *111:* 171–207.

Janssen, R. (1992) Thermal influences on nervous system function. Neurosci. Biobehav. Rev., *16:* 399–413.

Joyner, R.W. (1981) Temperature effects on neuronal elements. Fed. Proc., *40:* 2814–2818.

Kimura, J.E., and H. Meves (1979) The effect of temperature on the asymmetrical charge movement in squid giant axons. J. Physiol., *289:* 479–500.

Kramer, B. (1985) Jamming avoidance in the electric fish *Eigenmannia*: harmonic analysis of sexually dimorphism waves. J. Exp. Biol., *119:* 41–69.

Meyer, J.H., M. Leong, and C.H. Keller (1987) Hormone-induced and maturational changes in electric organ discharges and electroreceptor tuning in the weakly electric fish *Apteronotus*. J. Comp. Physiol. A, *160:* 385–394.

Montgomery, J.C., and J.A. MacDonald (1990) Effects of temperature on nervous system: implications for behavioral performance. Am. J. Physiol., *259:* R191–R196.

Moortgat, K.T., C.H. Keller, T.H. Bullock, and T.J. Sejnowski (1998) Submicrosecond pacemaker precision is behaviorally modulated: the gymnotiform electromotor pathway. Proc. Natl. Acad. Sci. USA, *95:* 4684–4689.

Nobile, M., E. Carobe, H.D. Lux, and H. Zucker (1990) Temperature sensitivity of Ca currents in chick sensory neurons. Pflüg. Arch., *415:* 658–663.

Pires, A., and R.R. Hoy (1992) Temperature coupling in cricket acoustic communication. I. Field and laboratory studies of temperature effects on calling song production and recognition in *Gryllus firmus*. J. Comp. Physiol. A, *171:* 69–78.

Rasnow, B., C. Assad, and J.M. Bower (1993) Phase and amplitude maps of the electric organ discharge of the weakly electric fish, *Apteronotus leptorhynchus*. J. Comp. Physiol., *172:* 481–491.

Rome, L.C. (1990) Influence of temperature on muscle recruitment and muscle function in vivo. Am. J. Physiol., *259:* R210–R222.

Schaefer, J.E., and H.H. Zakkon (1996) Opposing actions of androgen and estrogen on in vitro firing frequency of neuronal oscillators in the electromotor system. J. Neurosci., *16:* 2860–2868.

Schmidt-Nielsen, K. (1997) Animal Physiology: Adaptation and Environment. Cambridge University Press, Cambridge, UK.

Schwartz, J.R. (1986) The effect of temperature on Na currents in rat myelinated nerve fibers. Pflüg. Arch., *406:* 397–404.

Silva, A.C., L. Quintana, P. Errandonea, and O. Macadar (1998) Temperature variation within its natural range evokes changes in electric organ discharge waveform in *Brachyhypopomus pinnicaudatus*. Internat. Cong. Neuroethol., Abst. #SP 314.

Tokunaga, A., K. Akert, C. Sandri, and M. Bennett (1980) Cell types and synaptic organization of the medullary electromotor nucleus in a constant frequency weakly electric fish, *Sternarchus albifrons*. J. Comp. Neurol., *192:* 407–426.

Waxman, S., G. Pappas, and M. Bennett (1972) Morphological correlates of functional differentiation of nodes of Ranvier along single fibers in the neurogenic electric organ of the knife fish *Sternarchus*. J. Cell Biol., *53:* 210–224.

Westby, G.W.M. (1988) The ecology, discharge diversity and predatory behaviour of gymnotiform electric fish in the coastal streams of French Guiana. Behav. Ecol. Sociobiol., *22:* 341–354.

Zupanc, G.K.H., and L. Maler (1997) Neuronal control of behavioral plasticity: the prepacemaker nucleus of weakly electric gymnotiform fish. J. Comp. Physiol. A, *180:* 99–111.