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Hormonal and Body Size Correlates of Electrocommunication Behavior during Dyadic Interactions in a Weakly Electric Fish, *Apteronotus leptorhynchus*

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Brown ghost knife fish, *Apteronotus leptorhynchus,* produce sexually dimorphic, androgen-sensitive electrocommunication signals termed chirps. The androgen regulation of chirping has been studied previously by administering exogenous androgens to females and measuring the chirping response to artificial electrical signals. The present study examined the production of chirps during dyadic interactions of fish and correlated chirp rate with endogenous levels of one particular androgen, 11-ketotestosterone (11KT). Eight males and four females were exposed to short-term (5-min) interactions in both same-sex and opposite-sex dyads. Twenty-four hours after all behavioral tests, fish were bled for determination of plasma 11KT levels. Males and females differed in both their production of chirps and their ability to elicit chirps from other fish: males chirped about 20–30 times more often than females and elicited 2–4 times as many chirps as females. Among males, chirp rate was correlated positively with plasma 11KT, electric organ discharge frequency, and body size. Combined with results from experimental manipulation of androgen levels, these results support the hypothesis that endogenous 11KT levels influence electrocommunication behavior during interactions between two male fish.

Key Words: **electrocommunication; 11-ketotestosterone; steroid; androgen; electric fish; aggression; *Apteronotus leptorhynchus;* social behavior; agonistic behavior.**

The sexually dimorphic electrocommunication signals of weakly electric fish have been quite useful for identifying specific neural mechanisms underlying steroid-dependent behavior (Zakon, 1993, 1996; Dulka and Ebling, 1999; Zakon and Dunlap, 1999). Brown ghost knife fish, *Apteronotus leptorhynchus,* produce a continuous, wave-type electric organ discharge (EOD) that functions in gender identification. Males have a higher EOD frequency than females, and 11-ketotestosterone (11KT) treatment raises and estradiol treatment lowers EOD frequency (Meyer, Leong, and Keller, 1987; Schaefer and Zakon, 1996).

In certain social and experimental contexts, this continuous signal can be modified into brief (~15-ms), discrete signals termed chirps, during which the frequency of the signal transiently increases and the amplitude decreases (Larimer and Macdonald, 1968; Hagedorn and Heiligenberg, 1985; Zupanc and Maler, 1993, 1997). Chirps are occasionally emitted spontaneously, but their rate increases drastically when fish are presented with a conspecific fish or an artificial sinusoidal electric signal near the fish's own frequency (a so-called jamming stimulus) (Engler, Fogarty, Banks, and Zupanc, 2000). Because fish readily attack other fish or the stimulus electrodes in these circumstances, these chirps are often considered aggressive signals. Chirping responses to jamming stimuli are also sexually dimorphic and androgen-sensitive: males chirp more than females (Dulka and Maler, 1994; Dunlap, Thomas, and Zakon, 1998), and androgen (testosterone and dihydrotestosterone) treatment to females increases the rate, probability, and structure of chirping (Dulka and Maler, 1994; Dulka, Maler, and Ellis, 1995).

One advantage of using chirps for examining steroid-dependent behavior is that the brain nucleus that generates these signals, the prepacemaker nucleus-C (PPn-C), is rather small and discrete, and thus it is relatively easy to trace specific neural changes following steroid treatment (Zupanc and Maler, 1997). For example, substance P injections directly into the

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PPn-C stimulates chirping, and treatment with exogenous androgens increases both chirp rate and substance P immunorectivity in the PPn-C (Dulka *et al.*, 1995; Dulka and Ebling, 1999).

Although such studies have begun to identify the neural mechanisms underlying steroid-dependent electrocommunication behavior, little is known about how endogenous levels of steroids correlate with the production of socially elicited chirps. Thus far, our understanding of the hormonal regulation and social context of chirping comes from two sorts of studies. First, researchers have treated fish with exogenous androgens, confined them within a behavioral testing apparatus (a "chirp chamber") and presented brief (0.5–1 min), sinusoidal jamming stimuli that, in some ways, resemble the signal of a conspecific fish (Maler and Ellis, 1987; Zupanc and Maler, 1993; Dulka and Maler, 1994; Dulka *et al.*, 1995; Dunlap *et al.*, 1998; Engler *et al.*, 2000). Dulka *et al.* (1995) showed that females treated with testosterone and dihydrotestosterone chirp at higher rates and have a modified chirp structure compared control-treated females. Under these circumstances, the hormone profile and the stimuli are manipulated experimentally, but they are artificial compared to what occurs in fish during normal social interactions. Moreover, no study has yet documented the androgen dependence of chirping in males. Second, one study (Hagedorn and Heiligenberg, 1985) examined chirping in the context of an established social group. In this case, the fish received relatively realistic social stimuli, but the description of chirping behavior was limited by the difficulty of following an individual's stimulus environment and its electrical responses within the "chorus" of a large social group. In addition, the hormonal state of the interacting fish was not examined.

The present study bridged these two approaches. The interactions of pairs of fish were described in testing situations in which electrical behavior of both interacting fish could be measured over an intermediate time scale (5 min). The social contexts of fish were experimentally modified, chirp rates during dyadic interactions were quantified, and the chirp rate was correlated with endogenous hormonal state and body size. Thus, in contrast to previous studies, the stimuli came from real fish and were modified experimentally, the chirp rates of all interacting fish were quantified, and endogenous levels of androgens were determined. Finally, the relationship between androgens and chirping behavior, which had been examined previously only in females, was examined in males. Although both chirp rate and chirp structure are likely important for social communication (Hagedorn and Heiligenberg, 1985; Zupanc and Maler, 1993; Dulka *et al.*, 1995; Engler *et al.*, 2000), only chirp rate was examined. Chirp rates depended heavily on the sex of both fish in an interacting pair. Among males, chirp rate varied positively with a cluster of correlated variables that included endogenous androgen (11-KT) concentrations, EOD frequency, and body size.

MATERIALS AND METHODS

Apteronotus leptorhynchus were obtained from commercial dealers and housed in individual tanks that were part of a 1520-liter circulating system. Water conditions were held at a constant temperature (28.2°C), light cycle (12L:12D), and water conductivity (800 μS). Fish were fed frozen brine shrimp every 2 days. All procedures used in this study adhered to ethical standards of animal use specified by the National Institutes of Health (DHEW Publication 80-23).

To verify the stability in the fish's EOD frequency, the EOD of each fish was recorded weekly through two bare wires mounted on a Plexiglas rod that was held within 1 cm of the fish's tail. The signal was amplified by a Grass P15 amplifier, and the frequency was measured with a FLUKE voltmeter in the frequency counter mode.

Testing Apparatus

The test apparatus was designed to record the chirp responses of fish to the presence of free-swimming conspecific fish. To distinguish the electrical signals of each fish and to minimize the interference of the signals in the recordings, fish were separated by a physical barrier during the interaction. Behavioral tests were conducted in an aquarium ($25 \times 25 \times 10$ cm) that was divided in half by a plastic grid (1×1 cm) covered with nylon mesh (1×1 mm). Fish had full electric, olfactory, acoustical contact and partial visual and vibrational contact with each other. Each compartment of the aquarium was fitted with a pair of silver electrodes through which the electric activity of the fish in that compartment was recorded. The signals collected by each set of electrodes were amplified with a Grass P-15 amplifier and then recorded on separate channels of a four-channel Vetter video recorder. The signal from one of the electrodes was also sent to an audio speaker so that one of the fish could be monitored continuously during the behavioral test. Although signals from both fish could be detected

from each electrode, it was easy to distinguish between fish because the signal from the fish in the compartment with the electrode was always higher in amplitude than the signal from the fish in the adjacent compartment.

The water in the test aquarium was replaced every day with fresh water from the fishes' home aquarium system, aerated with an airstone, and maintained at 28.2°C with a thermostatically controlled heater. Because gymnotiforms are most active nocturnally, all behavioral tests were conducted under dim red-light conditions during the dark phase of the light cycle.

Behavioral Tests

Eight males and four females were used in a round-robin experimental design. The gender of each fish was identified prior to behavioral testing by the sexual dimorphism in EOD frequency (Dunlap *et al.*, 1998) and confirmed after completing all tests by examining the gonads (see below).

For each test, a fish was placed in one half of the aquarium. This fish, termed the "focal" fish, was allowed to acclimate for 10 min, at which time a second fish, termed the "stimulus" fish, was placed into the other half of the aquarium. The fish were physically separated to minimize interference between the fishes' signals. The electric signals of each fish were recorded simultaneously and continuously for each fish for 5 min. Each fish's EOD frequency was recorded and the number of chirps each fish produced per 15 s was counted by monitoring the fish live during the test or by subsequently replaying the videotape. In the rare cases when the identity of the chirping fish was ambiguous (about 15 cases of a total of 5883 total chirps), the chirp was attributed to neither fish and excluded from the data set. The fish were returned to their home aquarium immediately after the behavioral trial. The differnce frequency for each trial was calculated as the difference in EOD frequency between the two fish.

All fish were paired with each other both as focal and stimulus fish, for a total of 132 behavioral tests conducted over a 19-day period. The order of treatment (focal vs stimulus) and the order of gender pairing (male/male vs male/female vs female/female) were assigned randomly. No fish was tested more than once per day.

Blood Collection and Radioimmunoassay

At 1400–1600 h, 1 day (24–26 h) after the conclusion of all behavioral tests, fish were anesthetized in 0.075% 2-phenoxyethanol (Sigma, P-1126) and bled from the caudal vein. A heparinized 25-gauge needle was inserted into the ventral tail, and 50–150 μl of blood was drawn into a heparinized syringe. All blood samples were collected within 3 min of capture to minimize the effect of handling stress on hormone levels. Then the body length and mass of each fish was measured, their peritoneal cavites were opened, and the gonads were removed and weighed. The Gonadosomatic index (GSI) was calculated as (gonad mass)(100)/body mass. The blood was stored on ice for 1–3 h until centrifugation. Plasma was stored at −20°C until hormone analysis.

Plasma 11KT levels were determined by radioimmunoassay in the laboratory of P. Thomas, University of Texas Marine Science Institute. This assay was used previously for *Apteronotus* (Dunlap *et al.*, 1998) and validated for another gymnotiform fish, *Sternopygus* (Zakon, Thomas, and Yan, 1991), in the Thomas laboratory. Steroids were extracted from 50–100 μl of plasma with a 70:30 mixture of hexane and ethyl acetate. The aqueous portion was removed, the solvent layer was evaporated with nitrogen, and the hormone residue was reconstituted in phosphate buffer. Each sample was incubated 2 to 3 h at room temperature with an antibody to 11KT and tritiated 11KT. The unbound fraction was removed by charcoal and centrifugation (4°C), and the bound fraction was counted in a scintillation counter. The intra-assay variation was 8.2%; all samples were run in a single assay.

Based on previous studies, the 11KT antibody (Helix Biotech Ltd.) cross-reacts 17% with dihydrotestosterone and 9% with testosterone (Zakon *et al.*, 1991). Because the antiserum was not completely specific and steroids were not separated chromatographically, the values presented for circulating 11KT concentrations should only be considered approximations; the true values are probably slightly lower than those reported.

Statistics

The data were analyzed using SOREMO statistical software designed specifically to assess dyadic interactions in a round-robin experimental design (Warner, Kenny, and Stoto, 1979; Kenny, 1994). The model assumes that each animal in a dyad both produces behavioral responses and acts as stimuli to which other animals respond. The program-generated covariance matrices of all variables for both focal and stimulus fish and determined how much of the variance in the focal fishes' chirp rate was attributable to (1) its own

TABLE 1
Sexual Differences in Fish Used in the Experiment

	Males (n = 8)	Females (n = 4)	F test	P value
Body length (cm)	16.9 ± 0.9	14.6 ± 0.2	3.32	<0.05
Body mass (g)	13.2 ± 2.5	7.4 ± 0.9	4.48	<0.05
EOD frequency (Hz)	985 ± 12	788 ± 20	81.61	<0.001
Plasma 11KT (ng/ml)	8.78 ± 2.0	1.3 ± 0.05	6.58	<0.01
Gonadosomatic Index	0.23 ± 0.03	1.24 ± 0.17	61.15	<0.0001

independent characteristics ("actor effect"), (2) the characteristics of stimulus fish with which it was paired ("partner effect"), and (3) particular dyadic combinations ("relationship effect"). The program also calculated whether there was significant reciprocity between the two fish (i.e., the degree to which the chirping of one fish influences the chirping of another).

I also determined whether variance in male focal chirp rate and EOD frequency were significantly correlated with independent variables (body size, plasma 11KT concentration, GSI). Correlations between the chirp rate of stimulus fish with their body size and hormonal variables were not examined. Females were few in number (n = 4) and displayed low rates of chirping, so individual variation among females was not analyzed. Because of the intercorrelations among independent variables, principle components analysis was used to determine whether variables could be reduced to orthogonal factors that contribute separately to variance in chirp rate and EOD frequency.

Sex differences were determined using ANOVA. In all tests, $P < 0.05$ was considered significant.

RESULTS

Variation between Sexes

Mean body length, body mass, EOD frequency, and plasma 11-KT (ng/ml) were significantly greater in males than females (Table 1).

In conjunction with these physiological and morphological differences, males and females differed in both their production of chirps and their ability to elicit chirping in other fish: males chirped about 20–30 times more often than females and elicited two to four times as many chirps as females (Fig. 1). Chirp rates were significantly different (F = 8.08, df = 3, $P < 0.001$) in all four dyadic combinations. Males chirped at least once in all (56/56) pairings with other males and in 88% (28/32) of pairings with females; females chirped at least once in 56% (18/32) of pairings with males and 25% (3/12) of pairings with other females. This apparent effect of gender on both the production and stimulation of chirps is supported by the statistical analysis, which indicates that a significant fraction of the variance in chirping is attributable to the sex of both the actor (43.8%; T = 7.03, df = 10, $P < 0.001$) and partner (10.3%; T = 3.10, df = 10, $P < 0.01$).

The average difference frequency was 38.1 ± 3.7 Hz between males, 44.1 ± 9.7 Hz between females, and 223.5 ± 14.1 Hz in opposite-sex dyads.

Individual Variation among Males

There was substantial variation among focal males in chirp rate. The mean chirp rate of an individual focal male averaged over seven interactions with stimulus males ranged from 12.5 ± 7.4 to 129 ± 24.3 chirps/5 min (Fig. 2). Approximately one third (34.3%) of the variation in focal male chirping was due to consistent differences among individuals (actor effect), none of the variation was due to consistent differences among males in eliciting chirps (partner ef-

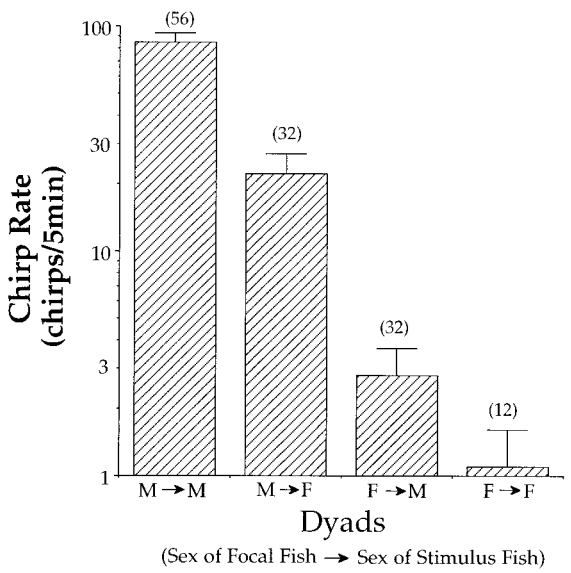

FIG. 1. Mean chirp rate (± SE) of focal fish during same-sex and opposite-sex dyadic interactions. M, male; F, female. Numbers in parentheses indicate the number of behavioral tests for each pairing. Note the log scale on the ordinate. All values are statistically different from each other ($P < 0.005$).

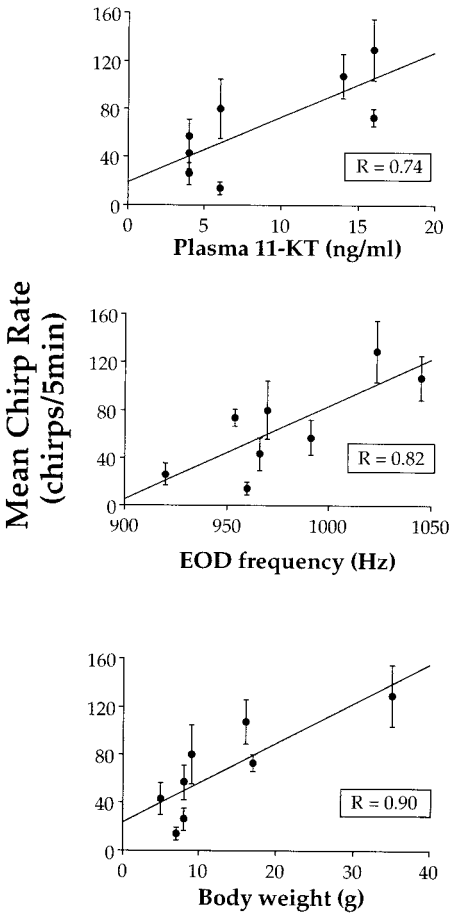

FIG. 2. Correlations between mean chirp rate and plasma 11-ketotestosterone, EOD frequency, and body weight in male *Apteronotus leptorhynchus* ($n = 8$). Each point represents the mean ± SE chirp rate of an individual focal male averaged over interactions with seven other males. R is the least-squares regression coefficient; all correlations are statistically significant (see Results). Correlations among variables are presented in Table 2.

fect), and the remaining 65.7% was due to random variation among dyads.

Among males, the mean chirp rate of the focal fish was significantly and positively correlated with the body length ($R = 0.876$, $T = 2.77$, $df = 6$, $P < 0.05$), mass ($R = 0.900$, $T = 3.16$, $df = 6$, $P < 0.02$), EOD frequency ($R = 0.824$, $T = 2.57$, $df = 6$, $P = 0.05$), and plasma 11KT ($R = 0.742$, $T = 2.12$, $df = 6$, $P = 0.05$) of the focal fish (the actor) (Fig. 2; Table 2). However, the chirp rate of the focal fish did not correlate significantly with body size, EOD frequency, or plasma 11KT of the stimulus fish (the partner) ($P > 0.05$). Among males, the mean chirp rate did not correlate significantly with the mean difference frequency ($P > 0.05$). Simlarly, difference frequency was not a significant predictor of chirp rate among behavioral trials ($P > 0.05$).

Body length, body mass, EOD frequency, and plasma 11KT were significantly and positively correlated with each other; GSI correlated significantly with plasma 11KT, but not with other variables (Table 2). In the priniciple components analysis, body size varibles and plasma 11KT loaded heavily on the first factor, which correlated strongly with both chirp rate ($R = 0.847$) and EOD frequency ($R = 0.813$). GSI loaded heavily on the second factor, which did not correlate significantly with either chirp rate or EOD frequency ($P > 0.05$). Because of the high degree of correlation among body size variables and 11KT ($R > 0.9$, Table 2) and the low sample size ($n = 8$), principle components analysis was unable to further identify combinations of variables (factors) that meaningfully partitioned variance in chirp rate or EOD frequency.

Time Course of Chirping and Reciprocity

The overall pattern of chirping was generally a low level of chirping (~1–2 chirps/15 s) interrupted by erratic bursts of rapid chirping (~10–30 chirps/15 s). There was substantial variation in when during the tests these bursts occurred, but, on average, focal males chirped slightly and significantly ($P < 0.05$)

TABLE 2

Regression Coefficients (R) of Correlated Variables in Male Focal Fish

	Chirp rate	Body length	Body mass	EOD frequency	Plasma 11KT
Body length	0.876*				
Body mass	0.900*	0.967*			
EOD frequency	0.824*	0.762*	0.684*		
Plasma 11KT	0.742*	0.935*	0.846*	0.733*	
GSI[a]	0.282	0.542	0.427	0.629	0.720*

[a] Gonadosomatic Index (see Materials and Methods). * Statistically significant ($P < 0.05$).

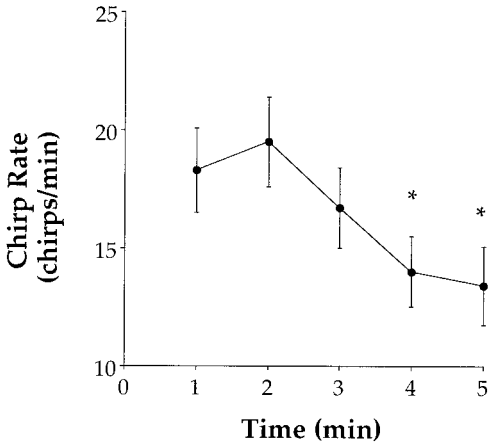

FIG. 3. Chirp rate in focal males ($n = 8$) during the time course of 5-min dyadic interactions with other males. Chirp rates are significantly lower ($P < 0.05$) in the last 2 min than in the first 3 min.

more during the first 3 min of the test than during the final 2 min (Fig. 3).

When analyzing overall chirp production during the 5-min tests, there was a nonsignificant reciprocity correlation ($R = 0.373$, $P > 0.05$) between actor and partner, indicating that the chirp rate of one male does not influence the chirp rate of another. The lack of reciprocity was born out when examining chirp rates at a finer temporal scale. In qualitative inspection of the data collected at 15-s intervals, the individuals' profiles of chirping showed no obvious or consistent relationship to each other. That is, brief bouts of chirping by one fish appeared to neither stimulate nor inhibit subsequent chirping by the other fish.

DISCUSSION

Correlation of Chirping with Endogenous 11-Ketotestosterone

Chirp rates of focal males during dyadic interactions correlated positively with endogenous levels of 11KT. On its own, this result does not necessarily indicate that elevated chirp rates are caused by high levels of endogenous 11KT, since chirp rates were also correlated with body size and EOD frequency (Fig. 2, Table 2). However, combined with experimental studies showing that exogenous androgens stimulate the production of chirps toward artificial stimuli (Dulka and Maler, 1994; Dulka *et al.*, 1995), the results of the present study support the idea that natural variations of androgens are causally important in regulating the chirp rate during dyadic male interactions. Hagedorn *et al.* (1985) showed that the dominant fish in a mixed-sex social group was the largest male with the highest EOD frequency. The correlations between chirp rate, body size, EOD frequency, and 11KT levels in dyadic interactions suggest that dominant males within social groups also have the highest 11KT levels.

In other vertebrates (Wingfield, Hegner, Dufty, and Ball, 1990), including some teleost fish (Olivera, Almada, and Canario, 1996), social behavior influences steroid secretion as well as vice versa. Thus, one possible interpretation of the correlation between endogenous 11KT levels and chirp rate is that the behavior of some fish (e.g., the large fish with high EOD frequency) stimulated certain social interactions that subsequently caused an increase in 11KT secretion. In theory, this possibility could be tested by determining whether chirp rate correlated more closely with pretest or posttest levels of 11KT fish (Olivera *et al.*, 1996). However, the small body sizes of these fish (about 10 g) makes serial bleeding unfeasible. Alternatively, one could examine social influences on steroid production by comparing androgen levels in fish that had repeatedly interacted with other males with those housed in isolation.

In this study I have addressed how endogenous hormonal state and social context influence chirp rate only. It is important to note that chirp structure can also vary by sex and androgen treatment (Hagedorn and Heiligenberg, 1985; Dulka *et al.*, 1995). Males and females produce chirps that differ primarily in duration and frequency change. It has been assumed that short (~15-ms) chirps with small frequency changes (~80 Hz) function in aggression, while long chirps (25 ms) with large frequency changes (~450 Hz) function in courtship (Hagedorn and Heiligenberg, 1985; Zupanc and Maler, 1997; Bastian, Schneiderjan, and Nguyenkim, 2001). It would thus be useful to test whether chirp structure differs in opposite-sex vs same-sex dyadic interactions.

Comparing Chirping Responses to Natural and Artificial Stimuli

Chirp responses to conspecifics in this experiment are only partially similar to responses in the standard testing regime using artificial stimuli in a chirp chamber. The present study confirmed the previously reported sexual dimorphism in the chirp production in *A. leptorhynchus* (Zupanc and Maler, 1993; Dulka and Maler, 1994; Dunlap *et al.*, 1998) and, in addition, demonstrated that sexes differ in the rate of chirping

they elicit (Fig. 1). In studies using a chirp chamber, a fish is typically presented with a sine wave stimulus near its own frequency (a jamming stimulus) and thus it receives a same-sex frequency. Under these conditions, males chirp much more than females, and the present study using real social stimuli shows this sexual difference in chirping is most pronounced during same-sex interactions.

However, sexual differences in chirp rate are far less pronounced during opposite-sex interactions (Fig. 1). Perhaps most surprising is that females chirp about three times as much toward males as toward females. This was unexpected considering that difference frequencies were greater in male–female pairs than in female–female pairs and chirp responses in a chirp chamber vary inversely to difference frequencies (Dye, 1987; Bastian *et al*., 2001). The fact that both sexes chirp more toward males than females suggests that fish respond to the "maleness" of the stimulus and not just the jamming quality of the stimulus. Although males clearly elicit more chirping than females, we know little about what specific features of males are the effective stimuli that provoke chirping. Olfactory, visual, mechanosensory, and electrosensory regions of the brain all send inputs to the PPn-C (Metzner, 1999) and sexually dimorphic signaling in any of these modalities may explain why males are more potent elicitors of chirping than females.

Maximal chirp rates of males during dyadic interactions (i.e., those during episodic bursts of chirping, approximately 70 chirps/min) were comparable to those exhibited in response to brief (30-s) jamming sine wave stimuli. However, average chirp rates were far lower in dyadic interactions [17 chirps/min to real fish vs ~65/min to electrodes (Dunlap, 1998)]. Previous chirp chamber studies have shown that chirp rate depends on both the difference frequency between the stimulus and the fish's own EOD frequency (Dye, 1987; Bastian *et al.,* 2001) and the amplitude of the stimulus (Dunlap, 1998). In the present study, males were exposed to other males whose frequencies were not typically as close to the fish's own frequency (average difference frequency, 38.1 ± 3.7) as a standard jamming stimulus (difference frequency, 0–10 Hz), and this could possibly contribute to lower overall chirp rates in dyadic encounters. However, among dyads, difference frequency did not correlate with chirp rate, indicating that difference frequency is not an important factor in chirping between fish. Fish in the present study were free to move away from the stimulus fish where the amplitude was lower, while fish in chirp chambers must stay within a close range of the stimulus. Thus, differences in the average stimulus amplitude may have contributed to the overall lower chirp rates in this study compared to chirp chamber studies.

Unlike the standard evoked chirping assay, dyadic interactions have the possibility of reciprocal trading of chirps. Behavioral and neurobiological studies suggest that chirp stimuli might affect chirp production. Previously, I found that males chirped less toward recordings of an EOD containing chirps than toward an identical EOD that did not contain chirps (Dunlap and Zakon, 1998). In addition, chirp-sensitive neurons in electrosensory regions of the brain in a closely related species, *Eigenmannia*, make indirect connections with the prepacemaker nucleus (Heiligenberg, Keller, Metzner, and Kawasaki, 1991; Wong, 1997). However, in the present study, there was no evidence that a male's overall chirp production is influenced by the chirp rate of another male during 5-min dyadic interactions. A qualitative inspection of the data on short time scales (15 s) seemed to support this finding: brief bouts of chirping by one male did not appear to modify chirp production by another male. Nevertheless, the possibility of short-term reciprocity in chirp interactions needs to be tested more quantitatively.

The quantitative difference in chirping in dyadic interactions and chirp responses in chirp chamber stimuli raises the question of whether behavior in a chirp chamber is a good predictor of chirping behavior during real social interactions. If so, we can continue to use the chirping chamber as an adequate assay of social behavior in future hormonal and comparative studies. If not, our behavioral methods will have to be modified for us to draw more relevant conclusions about the regulation and diversification of this electrocommunication behavior. In addition, the methodology in the present study allowed fish to communicate in several modalities, but they were unable to interact physically. Thus, it will be important to compare these interactions across the barrier to the behavior of free-swimming dyads and, ultimately, the behavior in social groups.

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