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ARTICLES

Effects of sex, sensitivity and status on cue recognition in the weakly electric fish *Apteronotus leptorhynchus*

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Maintaining a stable social organization necessitates that animals recognize their own dominance status relative to the status of other group members. The weakly electric brown ghost knifefish emits a sexually dimorphic sinusoidal electric organ discharge (EOD) for electrolocation. Dominant males discharge at the highest and females at the lowest EOD frequencies (EODFs). Each individual is most sensitive to its own EODF, which can be modulated for communication. To examine how sensitivity and social status influence an individual's response to different cues, we recorded the electrical signals emitted by 10 males and seven females in response to playbacks of sine waves mimicking a wide range of con- and heterospecific EODFs. While all individuals emit small chirps (LoCs) mostly to stimuli around their own EODF, they are more likely to emit rises (gradual nonchirp signals) to frequencies to which they are less sensitive; males similarly emit larger chirps (HiCs) to frequencies more distant from their own, especially to female mimics. Males with 'dominant' EODFs are less likely to emit rises, stimuli in the female range elicit more rises from both sexes, and females emit rises to male EOD mimics. Although low-ranking male EOD mimics elicit more LoCs from all males, males with lower-ranking EODFs chirp less at high EOD mimics than males with high-ranking EODFs chirp at low EOD mimics. We conclude that (1) although much of the variation in an individual's response is attributable to its sensitivity, individuals recognize sexual and status cues and have some internal representation of their own social status, and (2) whereas LoCs appear to function in intrasexual aggression, HiCs and rises could be used in both courtship and submissive signalling.

To maintain a stable social organization, animals must recognize their own dominance status relative to that of others in the group or at least be able to discriminate between group members of different status (e.g. Colgan 1983; Hurst et al. 1994). How recognition occurs is not often obvious, and the nature of its cognitive template has been the subject of recent debate (Vauclair 1996; Griffin 2001; Johnston & Bullock 2001). Electric fish are a good model system in which to study this question because they constantly emit and monitor a cue, their electric organ discharge (EOD), which conveys their social status. Thus, every animal can instantaneously compare its own EOD with those of conspecifics.

Wave-type gymnotiform electric fish continuously produce a quasisinusoidal EOD at an extremely precise and steady frequency. They detect their own EOD and EODs of conspecifics with sensory receptors called electroreceptors. They use the EOD for two functions: electrolocation and electrocommunication. During electrolocation, a fish monitors the deflections in its electric field produced by nearby objects and thus perceives the electrical properties of its environment (for a review see Bullock & Heiligenberg 1986). The EOD is species, sex and individual specific. Thus, in addition to its role in electrolocation, it can serve as a cue for species, sex and individual recognition. In the brown ghost knifefish, *Apteronotus leptorhynchus*, males have higher EOD frequencies (EODFs) than females, and the dominant, spawning male is the largest and has the highest frequency (Hagedorn & Heiligenberg 1985; Zakon & Dunlap 1999). A dominance hierarchy has not been documented for females, and patterns of female dominance characteristics are unclear. Among nonreproductive females, EODF and size are

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positively correlated only in some social situations (Tallarovic & Zakon, in press), and although females with higher EODFs are more likely to occupy shelters when availability is limited, they are also much more likely than males to share shelters (Dunlap & Oliveri 2002).

Since a fish continuously emits its EOD, the EOD of another individual necessarily interacts with its own. This creates a beat frequency at the difference between the two EOD frequencies. Thus, this unique sensory system must extract external electrical information, such as conspecific signals, from the beat frequencies created by its own behavioural output. It would probably benefit an individual discharging at 750 Hz to distinguish between an approaching dominant male frequency of 900 Hz and one equidistant from its own in the female range (i.e. at 600 Hz), both of which create the same beat frequency. An additional complexity is that, in order to optimize electrolocation, each fish's electroreceptors are most sensitive to its own EODF. Behavioural tuning curves for *A. albifrons* show a symmetrical decrease in sensitivity to higher and lower frequencies, at least near the fish's own EODF (Knudsen 1974). Thus, fish may be differentially sensitive to the EODFs of conspecifics depending on the values of their own EODFs.

In order to know whether the fish respond to these cues differentially, we noted their tendency to produce transient modulations of EOD amplitude and frequency, described as 'chirps' (Bullock 1969), or 'pings' (Larimer & MacDonald 1968) upon presentation of a stimulus. Observations of social groups indicate that fish make short chirps (~15–30 ms) presumably as an aggressive signal and other signals, such as short and long 'rises', to signal submissiveness (Hagedorn & Heiligenberg 1985). Recently, Engler et al. (2000) described four types of spontaneously emitted chirps, SP-1 through 4, distinguishable by their frequency excursions, durations and presence or absence of a baseline frequency undershoot. In this study, we break chirps down into categories of low- and high-frequency excursions (LoC and HiC, respectively).

We asked whether individuals simply respond more to stimuli to which they are more sensitive (Ryan & Keddy-Hector 1992), or if they distinguish between two frequencies to which they are equally sensitive but that carry different information. We presented males and females with an array of EOD mimics spanning a range of con- and heterospecific frequencies, designed to be able to compare responses to stimuli equidistant from an individual's own EODF. If simple tuning accounts for most of the variation in a receiver's response, we expected symmetrical responses to stimulus frequencies in either direction from the individual's own EODF. If the EOD has communicative value, we predicted (1) that the responses to two stimuli to which an individual is equally sensitive should elicit asymmetrical responses, and (2) that individuals should show variation in responses to different absolute stimulus frequencies, regardless of the relative distance from their own EODF. Furthermore, since EODF is correlated with dominance status and hormonal state (e.g. Dulka & Maler 1994), we expected a difference in responses from individuals with EODFs signifying different dominance status, such that fish with dominant EODFs should respond more aggressively (e.g. with more chirps) or less subordinately (e.g. with less rises) to a wider range of stimulus frequencies. To test the latter prediction, we reanalysed the data by examining the responses of high- and low-frequency individuals of each sex to the absolute frequency values of the 'relative' stimuli previously presented.

We also addressed the related question of how a sender increases its cue's or signal's active space with respect to a receiver of the opposite sex, given that individuals are tuned best to EODs of their own sex. We would expect elements of either the EOD or its modulations to correlate with properties of the sensory substrate of the opposite sex (Ryan & Keddy-Hector 1992). To test this prediction, we examined male and female EODs and several randomly selected modulations, quantified the distribution of energy in different spectral components and compared these with conspecific sensitivity ranges.

METHODS

Animals

Fish were purchased from a commercial vendor and kept in group and individual tanks (temperature between 25 and 26.5 °C; conductivity between 500 and 1200 μS). *Apteronotus leptorhynchus* EODFs are directly dependent on water temperature. Within a temperature range of 25–27 °C, typical female EODFs range from 500 to 750 Hz, those of males from 800 to 1000 Hz. We haphazardly selected males (N=10) and females (N=7), placed them in a playback arena (see below), and allowed them to acclimatize to the tank for at least 24 h prior to the playback session. Afterwards, we confirmed the sex of the fish by dissection in 10 cases. The experiments complied with all federal, state and local regulations concerning the use of animals.

Set-up of the Playback Arena

A playback arena (100 × 55 cm; water level 9 cm) contained a 50-W thermostat/heater attached to one side of the tank that kept water temperature at 26 ± 0.4 °C (conductivity=600 ± 100 μS). The centre of the arena contained a PVC tube shelter (18 × 5 cm) with its top cut and replaced with plastic mesh to allow visibility of the fish from above. Two plastic mesh partitions at each end of the tank spanned the arena to create two compartments 10 cm wide, behind which two pairs of carbon stimulus electrodes (spaced 15 cm apart) were placed through plastic grating attached to the ends of the tank. Prior to the introduction of the fish, we set and confirmed the amplitude of the stimuli at 1.5 ± 0.1 mV/cm by measuring with a pair of Ag electrodes placed in the centre and parallel to the stimulus electrodes. Because these fish are most active nocturnally, we obstructed light entry to the arena during the trials with a black felt curtain hanging from the ceiling and attached to the sides of the table holding the

tank. We thus ensured that the fish experienced a 12:12 h light:dark regimen and had acclimated to the dark at least 1 h prior to the trials.

Stimulus Presentations

We generated 19 stimuli in Cool Edit Pro (Syntrillium) and presented each separately for 2 min with 60 s of silence between each stimulation. All but one of the stimuli were sine waves and consisted of percentage deviations from the fish's own EODF in both directions: ± 1, 5, 10, 15, 20, 25, 30 and 50%. Because we wanted to compare responses to stimuli to which the fish are equally sensitive, we presented frequencies equidistant from the fish's own EODF in a pairwise fashion, so as to minimize treatment order effect contamination of these most salient comparisons. We randomized the order that each pair was presented, as well as the order of presentation of each stimulus within the pair. We also randomized the side of the tank at which the stimuli of each pair was presented. For example, for a fish discharging at 750 Hz, the +10% stimulus (825 Hz) might have been presented first and from the left side of the tank. The − 10% stimulus (675 Hz) would then immediately have followed on the right side. An additional 'pair' was made of the second harmonic (200% of the EODF) and a control of pink noise (band limit of 5512.5 Hz) at similar amplitude, which was estimated visually. We were interested in responses to the harmonic, as the beat frequencies created when this frequency interacts with slight deviations of the fish's EODF from baseline are similar to those elicited by playbacks of stimuli within a few Hz of the EODF. Since the latter have been extensively shown to elicit a jamming avoidance response (see e.g. Zupanc & Maler 1993), we expected the second harmonic also to jam the fish's sensory system. To compare with responses to the harmonic stimulus, we also presented a stimulus at the fish's own EODF in some cases. To control for habituation, we presented the same random sequence in reverse order 24 h later. For the analysis we averaged the responses to each stimulus from both days. For clarity of presentation, plotted responses to the 95 and 105% stimulus treatments reflect their consolidation with responses to the 99 and 101% treatments, respectively.

EOD Recording and Signal Definitions

EODs were recorded with two perpendicular pairs of carbon electrodes placed across the width and the length of the tank, processed through an A/D converter (Terratech EWS-88MT), digitized at a sampling rate of 11 025 Hz and analysed in Cool Edit Pro. Spectral measurements were made using Fast Fourier Transformation (FFT) at different output sizes, depending on the analysis. Half the sampling rate divided by the output size determines the frequency resolution, the reciprocal of which in turn determines the temporal window over which the FFT is calculated at each cursor position. We generated an FFT (size 2048) of each individual's EOD and calculated the intensity difference between the fundamental and each of the subsequent two harmonic frequencies (Fig. 1a). For one analysis (see below), we then compared the relative intensity of each harmonic in males and females. We did not measure the absolute intensity, because the fish were free swimming, and the recorded amplitude was thus also a function of the fish's position with respect to the recording electrodes.

These amplitude modulation artefacts also limited our ability to conventionally define a signal based on amplitude changes alone (e.g. Zupanc & Maler 1993; but see Fig. 1b for amplitude-time plots of chirps from a stationary fish in the absence of a stimulus). To verify the chirp categories determined by Engler et al. (2000), we measured the instantaneous peak frequency every ms on a frequency-time plot (spectrogram) calculated at an FFT size of 2048. Signal onset and offset were determined as deviations of more than 2 Hz from and returns to within 2 Hz of the baseline EODF, respectively. Chirps were defined as frequency modulations greater than 30 Hz and less than or equal to 35 ms. Figure 2 shows a plot of duration and maximum frequency excursion for 30 randomly selected chirps from two males (N=10 each) and two females (N=5 each). From these data, we comprised the more intuitive categories of low (LoC: 30–90 Hz) and high frequency modulations (HiC: >200 Hz), analogous to what Engler et al. (2000) call SP-2 and SP-1 type chirps, respectively. Male and female LoCs differed in their total frequency excursion (males: $\bar{X} \pm \mathrm{SE} = 61.2 \pm 4.5$ Hz; females: 35.8 ± 5.6 Hz) but not in duration (males: 23.1 ± 1.4 ms; females: 23.9 ± 1.7 ms; Fig. 2). Male HiCs were slightly longer than LoCs ($\bar{X} \pm \mathrm{SE} = 27.4 \pm 1.6$ ms) and characterized by an initial increase of 307 ± 13 Hz and a subsequent undershoot of the baseline EODF of 40 ± 18 Hz. These agree with more quantitative examinations of chirp structure by Engler et al. (2000) and Bastian et al. (2001).

For the remainder of the analyses, we categorized each modulation by calculating an FFT of size 4096 after placing the spectrogram cursor in or near the middle of the signal (Fig. 1b). LoCs have a characteristic single bulge of increased energy at higher than baseline frequencies, whereas HiCs have several peaks, including a substantial lower frequency component (Fig. 1a). Four males emitted several chirps that were substantially longer (>200 ms) than those plotted in Fig. 2. Although these correspond to Engler et al.'s (2000) SP-3 and SP-4 distinctions, we include them in our HiC category by virtue of their HiC-typical frequency excursions. All other signals, usually less than 30 Hz in frequency excursion and often of much longer duration than chirps, were collectively termed 'rises' (Hagedorn & Heiligenberg 1985).

Analysis

We counted and compared the numbers of LoCs, HiCs and rises emitted in response to each stimulus by each fish. To normalize variation within individuals in signalling rate, we configured the data as signalling probabilities (i.e. the number of each chirp type and the number of rises as a percentage of the total number of signals). From

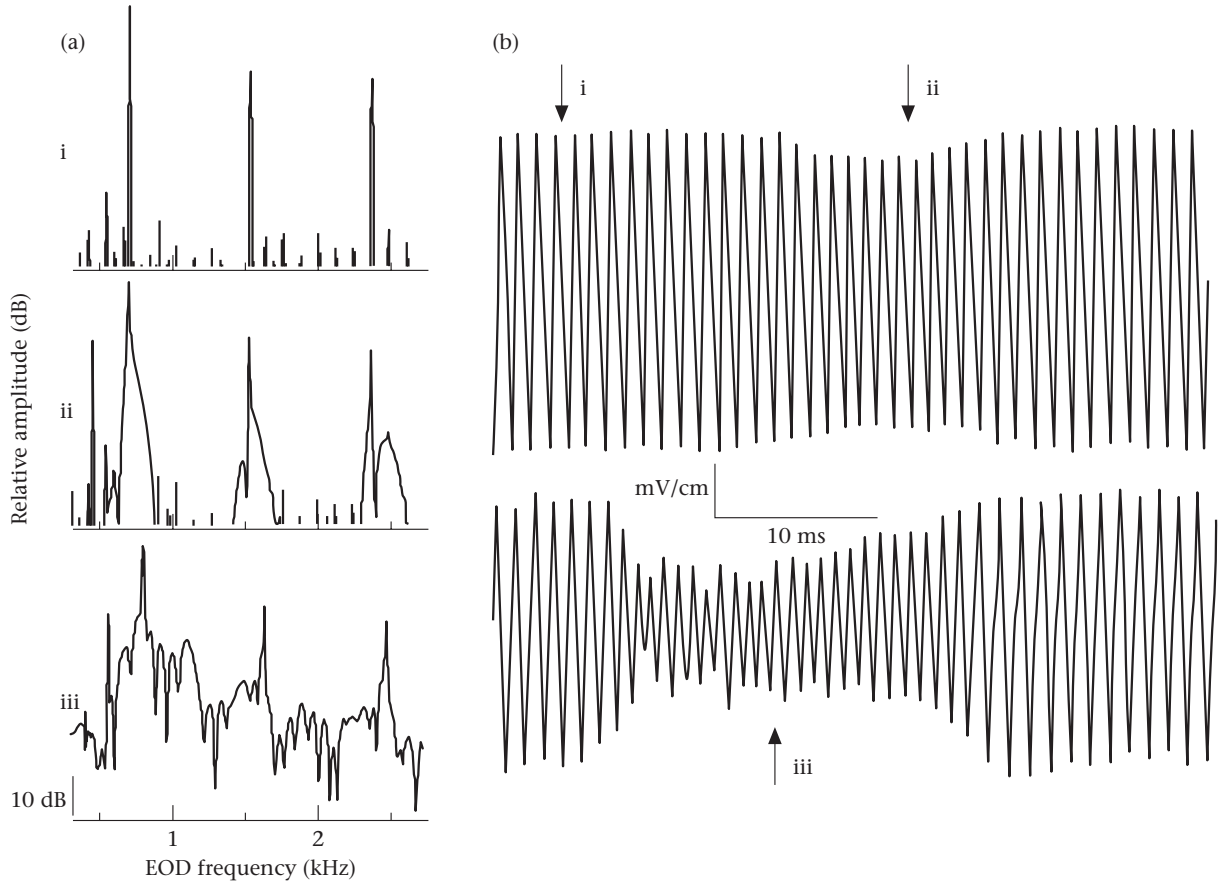

Figure 1. Structure of chirps from a male *A. leptorhynchus*. (a) Spectra (FFT size 4096; frequency resolution 1.3 Hz) of a typical male's unmodulated EOD (i), LoC (ii) and HiC (iii). (b) Oscillograms of the LoC and HiC. Note the greater amplitude deflection of the HiC. Arrows indicate the approximate positions of the cursor in Cool Edit Pro when generating the FFTs shown in (a).

within a small portion of the stimulus array corresponding to the range of species-typical EODFs, we established three 'conspecific stimulus groups' by averaging individual responses to stimuli in each range: 10–15% below, within 5% of, and 10–15% above the fish's own EODF (see Fig. 3).

For the analyses presented in Figs 4 and 5, individual EODFs were calculated from an average of five haphazardly selected EOD measurements in each trial. To assess responses as a function of an individual's status, we divided individuals into two groups per sex of low and high EODF (females: <700 Hz, $\bar{X} \pm \mathrm{SE} = 674 \pm 14$ Hz, $N=4$; >700 Hz, $\bar{X} \pm \mathrm{SE} = 723 \pm 16$ Hz, $N=3$; males: <850 Hz, $\bar{X} \pm \mathrm{SE} = 787 \pm 13$ Hz, $N=5$; >850 Hz, $\bar{X} \pm \mathrm{SE} = 868 \pm 13$ Hz, $N=5$). To assess how responses are affected by the absolute frequency of stimuli, we reanalysed the data after distributing the previous stimulus groups across new treatment groups. These 'treatments' consisted of species-typical frequency ranges spanning 50 Hz each (10 between 575 and 1075 Hz for males, and eight between 525 and 925 Hz for females). We then averaged each individual's responses to stimuli falling within each range group.

We used Student's t tests when comparing any two groups, such as males and females on variables averaged for each fish across all stimulus treatments. We compared larger numbers of treatments with a one-way analysis of variance, ANOVA (Figs 3–5). For overall comparisons across the stimulus treatments depicted in Figs 3 and 5, we used $\alpha=0.1$, because of a substantial decrement in power when comparing this many treatments. When significant differences were found in the overall ANOVAs, we used a Tukey–Kramer test to determine which treatments were different at $\alpha=0.05$. Percentage data in Fig. 3 were normalized using an arcsine transformation prior to the ANOVA.

RESULTS

Sex Differences in Responses to Stimuli as a Function of Relative Frequency

The control stimulus of pink noise only elicited LoC responses in one male and one female and was thus excluded from all analyses. All individuals produced LoCs at some point in the trials, although males produced many more than females (males: $\bar{X} \pm \mathrm{SE} = 59 \pm 11.1$, females: 8 ± 2.4; Student's t test: $t_{15} = -3.7$, $P<0.002$) and at shorter latencies (males: 23.6 ± 6.5 s, females: 91 ± 6.1 s; $t_{15}=51.6$, $P<0.0001$). The patterns of LoC responses to stimuli along the tuning curve were nonrandom and similar for both sexes (ANOVA: males: $F_{17,115}=11.1$,

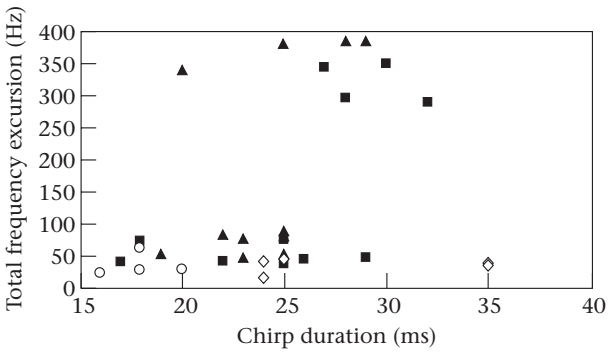

Figure 2. Distribution of LoC (lower) and HiC (upper) chirp types based on correlations between duration and frequency excursion for two females (open symbols) and two males (closed symbols).

$P<0.0001$; females: $F_{16,67}=2.29$, $P<0.01$; Fig. 3a). A comparison between the three conspecific stimulus groups revealed that stimuli within 5% of the fish's own EODFs elicited more LoCs than did more distant stimuli, and this was true for both sexes (males: $F_{2,27}=25.7$, $P<0.0001$; females: $F_{2,18}=4.16$, $P<0.04$; Fig. 3a). Interestingly, stimuli at 200% of the fish's own EODF, which corresponds to the second harmonic, also strongly elicited LoCs in both sexes.

Whereas 80% of the males produced HiCs as well as LoCs, only two of the seven females produced HiCs two orders of magnitude less than males, and were thus excluded from analyses on HiC emissions (males: $\bar{X} \pm \mathrm{SE}=5 \pm 2.9$; females: 0.04 ± 0.03). Figure 3b shows the pattern of male chirps as percentages of total signals across the stimulus trials. An examination of the three conspecific stimulus groups revealed that males were more likely to emit LoCs to frequencies within 5% of their own (ANOVA: $F_{2,27}=3.55$, $P<0.05$; Fig. 3b). Although low incidence of female LoC emissions probably reduced the power of the analysis, this pattern was consistent for female LoC probabilities ($F_{2,18}=2.85$, NS; not shown). In contrast, males were more likely to emit HiCs to lower frequencies, in the female range ($F_{2,27}=5.51$, $P<0.01$; Fig. 3b). The long-duration HiCs (described in Methods: EOD Recording and Signal Definitions) were emitted by four males exclusively in response to stimulus frequencies lower than their own EODFs by at least 15%; these correspond to female EODFs.

The normalized percentages of rise emissions were similar between sexes (males: $\bar{X} \pm \mathrm{SE}=1.1 \pm 0.8$, females: 1 ± 0.3; Student's t test: $t_{15}=-0.3$, NS; Fig. 3c), and visual inspection suggests an inverse trend to LoC emissions. Comparing the three conspecific stimulus groups revealed no significant difference for males (ANOVA: $F_{2,27}=0.97$, NS) but a significantly greater tendency for females to emit rises to frequencies differing more than 5% from their own ($F_{2,18}=5.22$, $P<0.02$; Fig. 3c).

Sex Differences in Responses to Stimuli as a Function of Absolute Frequency

Figure 4 shows differences between high- and low-frequency males and females in total chirp emission (ANOVA: $F_{3,13}=5.91$, $P<0.01$; Fig. 4a), rise emission ($F_{3,13}=3.56$, $P<0.05$; Fig. 4b) and body length ($F_{3,13}=3.55$, $P<0.05$; Fig. 4c). A within-sex examination of differences between high- and low-EODF individuals revealed no significant differences for chirp emission, but significantly greater rise emissions by high-frequency females and low-frequency males (Fig. 4b). High-frequency males were also larger than low-frequency males.

In the previous section, we examined the effects of stimulus frequencies relative to the individuals' own EODFs. To assess the value of absolute frequency to fish of different sexes and EODFs, we reanalysed the data by grouping responses from high- and low-frequency males and females into new treatment groups consisting of several absolute frequency ranges (see Methods: Analysis). Both males and females emitted LoCs in a nonrandom fashion to the range of absolute frequencies (males: $F_{9,72}=5.4$, $P<0.0001$; females: $F_{7,38}=5.2$, $P<0.0003$; Fig. 5a), and a Tukey–Kramer test revealed a significantly greater response at 800 Hz for males and at 700 Hz for females. This response function was approximated better by the low-frequency males, which are more sensitive near 800 Hz than the high-frequency males (Fig. 5c). The high-frequency males also showed a comparable peak response at 800 Hz. In addition, however, they responded more to the 900 Hz treatment than low-frequency males ($t_6=-2.05$, $P<0.08$; Fig. 5c). There were no apparent interactions between low- and high-frequency females.

The rise emission patterns of both sexes shifted from the LoC emission patterns and were also nonrandom (ANOVA: males: $F_{9,72}=1.7$, $P<0.1$; females: $F_{7,38}=2.6$, $P<0.03$; Fig. 5b). The Tukey–Kramer test revealed a significantly greater male response to female-typical stimulus frequencies near 700 Hz than to low-male frequencies. Females had two peak responses, at 650 Hz and at 850 Hz (Fig. 5b), although the data in the 850-Hz group were significantly different only from the low-frequency male and female stimuli.

Sex Differences in EOD Harmonic Content

A comparison of the intensity differences between the fundamental frequency and the second harmonic yielded no significant sex difference (males: -16.8 ± 2.4 dB; females -10.9 ± 2.4 dB; Student's t test: $t_{10}=-1.7$, NS). However, females had significantly more relative energy in their third harmonics than males (males: -18.8 ± 1.3 dB; females -12.3 ± 1.3 dB; $t_{10}=-3.6$, $P<0.005$).

DISCUSSION

Responses to Cue Mimics as a Function of Sensitivity and Status

Past studies have shown that weakly electric fish chirp when presented with a stimulus close to their own EODF (e.g. Larimer & MacDonald 1968; Dye 1987; Zupanc & Maler 1993; Dunlap et al. 1998). In this study, we extended the stimulus array to cover the entire range of

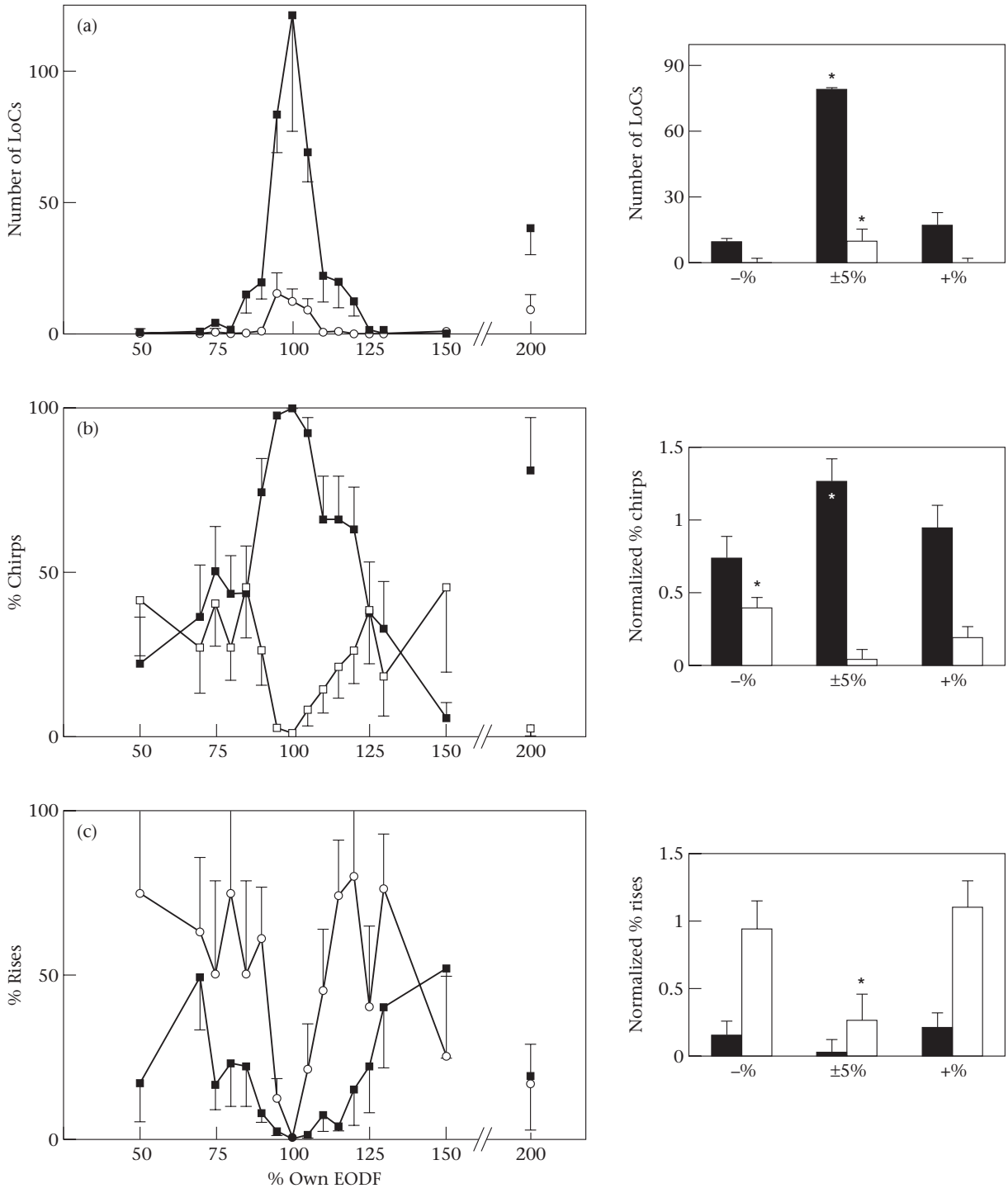

Figure 3. Signal emissions as a function of the percentage difference in stimulus frequency from the fish's own EODF. (a) Numbers of LoCs for females (○; *N*=7) and males (■; *N*=10). (b) Male LoC and HiC chirps (■ and □, respectively), and (c) female (○) and male (■) rises as a percentage of total signal emissions. Panels on the right show responses averaged over three ranges of conspecific stimulus presentations (−%: 10–15% below; ±5%: within 5% of; +%: 10–15% above the fish's own EODF). **P*<0.05 for comparisons between the range groups for each sex. Note that the panels for (b) and (c) depict arcsine-transformed percentages used in the ANOVAs on proportionate data. See Methods for clarification.

conspecific EODFs to ask whether individuals would discriminate between and respond differently to frequencies to which they were equally sensitive, as one would expect given that these stimuli carry different information about sex and social status. We were further interested in how an individual's own status might influence its responses.

An individual's sensitivity influences its signal emissions. In our playback trials, males and females responded

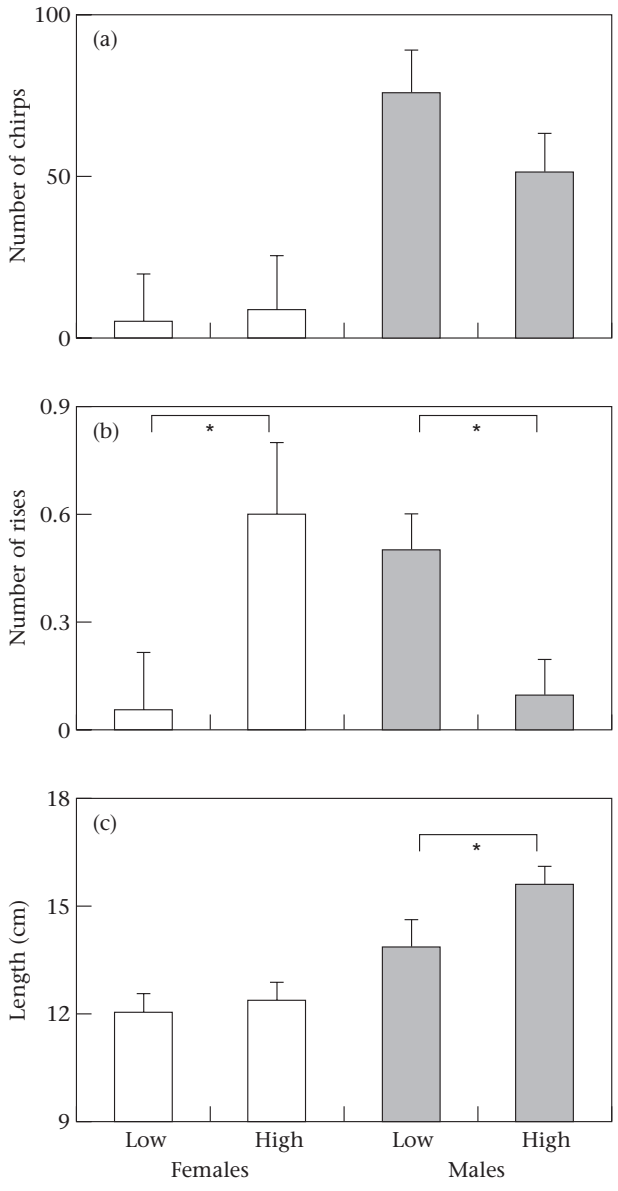

Figure 4. Characteristics of low- and high-EODF females (□, *N*=4 and 3, respectively) and males (■, *N*=5 each): (a) total chirp emission, (b) total rise emission and (c) body length. *P<0.05 for within-sex comparisons.

similarly to frequencies to which they were presumably most sensitive (i.e. within 5% of their own EODF; Fig. 3). Individuals of both sexes emitted more LoCs to similar frequencies than to more distant frequencies, consistent with a concurrent study by Bastian et al. (2001). These smaller chirps are probably those other researchers have elicited with playbacks of frequencies that jam the sensory system (e.g. Larimer & MacDonald 1968; Dye 1987; Zupanc & Maler 1993; Dunlap et al. 1998). Interestingly, there was a marked increase in LoC emissions at the second harmonic similar to that seen during the jamming avoidance response. Coupled with the fact that the fish presumably show a sensitivity peak here, this suggests that signals close to the second harmonic also jam the fish's sensory system. In contrast, the tendency for males to emit HiCs increased with stimulus deviation from the fish's own EODF, but significantly so only for lower, female-typical, frequencies. Similarly, the patterns of male and female rise emissions resembled those of male HiCs. Thus, signalling responses in general symmetrically increased or decreased away from an individual's own EODF, depending on the emitted signal type, and were thus significantly a function of an individual's sensitivity.

However, individuals discriminated between EODFs indicating different sexes and social and/or reproductive status. Colgan (1983) reviews an abundant body of literature on recognition of individuals and features of their status (see also Swaisgood et al. 2000). In this study, recognition of sexual and status cues was particularly evident (1) by peak rise responses of both sexes to female stimulus frequencies and of females to male stimulus frequencies, and (2) by a peak male chirp response to low male stimulus frequencies (Fig. 5). Males have higher EODFs than females, and their EODFs are correlated with body size (Zakon & Dunlap 1999; Fig. 4c), levels of 11-ketotestosterone (Dunlap 2002) and with dominance and spawning access (Hagedorn & Heiligenberg 1985). In contrast to males, obvious dominance patterns for females have not been shown. Variation in status and clear, honest indicators thereof may be under weaker selection in females of this polygynous species, although some evidence indicates a similar relationship between EODF and status in females as in males (Dunlap & Oliveri 2002; Tallarovic & Zakon, in press). None the less, EODF is both a good indicator of sex and a 'badge of status' in males (Rohwer & Rohwer 1978). Furthermore, EODF is sufficient as an indicator in that recognition does not necessitate other cues or interactive sequential assessment of fighting ability (Simpson 1968; Clutton-Brock & Albon 1979; Caldwell 1987; Waas & Colgan 1994). It would be intriguing to determine to what extent individuals can cheat by controlling these 'badges of status' or whether androgens constrain cue honesty (Rohwer & Rohwer 1978).

The results of the current study reveal not only recognition of male dominance cues but also differential discrimination dependent on the receiver's status. The androgen 11-ketotestosterone also increases chirp rate in males (Dulka & Maler 1994; Dunlap et al. 1998; Dunlap 2002). High-EODF males did not chirp more than low-EODF males in this study (Fig. 4a), but whereas low-EODF males chirped much less at high than at low male mimics, the high-EODF males showed a similar response to both (Fig. 5c). Recognition of EODFs thus does not follow a simple, all-encompassing paradigm like, 'if higher in frequency than myself, the stimulus probably represents a larger individual, therefore chirp less', as only the males with lower-ranking EODFs adhere to this pattern.

Individuals must therefore have some internal representation of their own social status, a conclusion not meant necessarily to evoke or exclude a conscious process (Griffin 2001). One possibility for such a representation is a recognition template for absolute spectral characteristics (Evans 1993; Elepfandt et al. 2001), a form of 'perfect pitch', that differs between high- and low-frequency males, such that individuals 'know' which end of the

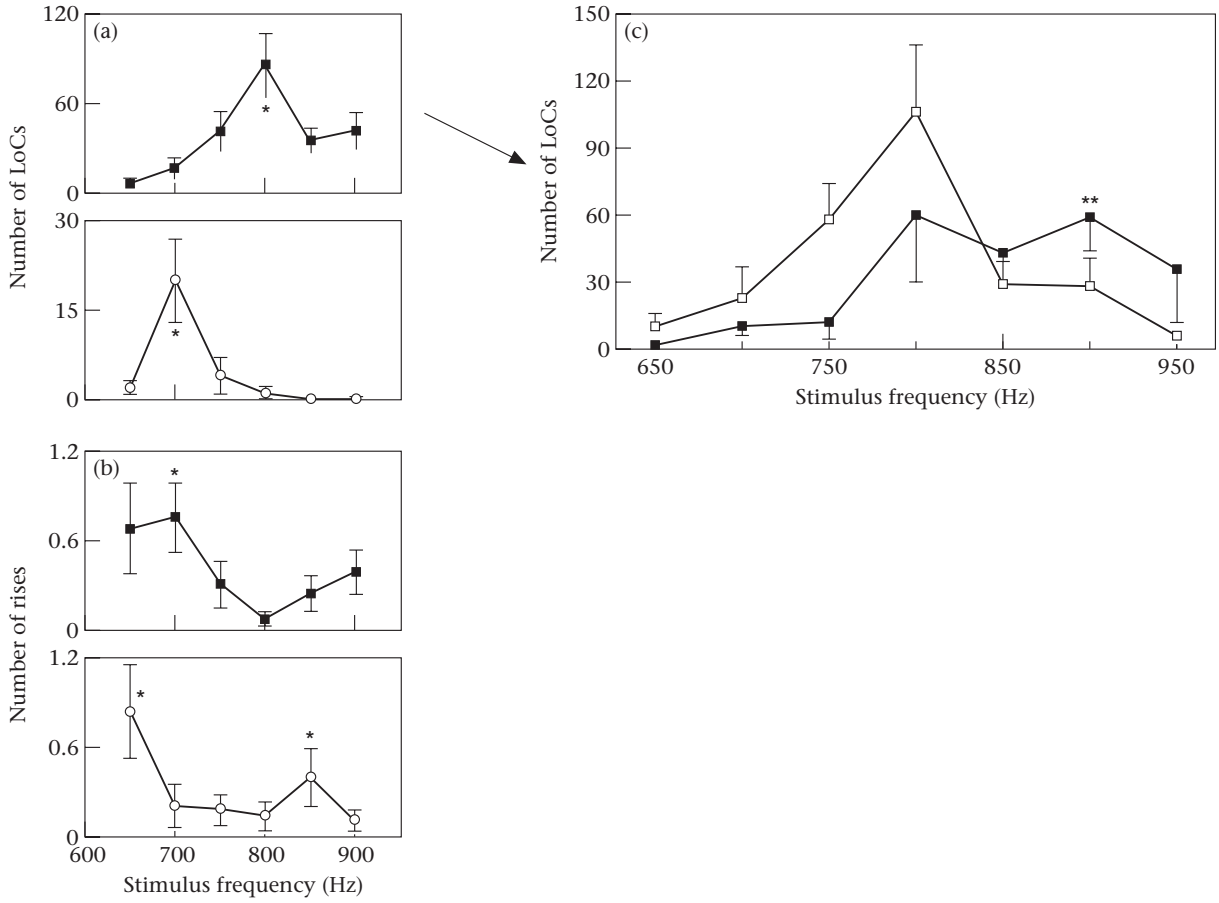

Figure 5. Numbers of (a) LoCs and (b) rises as a function of absolute stimulus frequency for females (○; $N=7$) and males (■; $N=10$). *$P<0.05$. (c) Male LoC responses in (a) broken down for low- and high-EODF males (□ and ■, respectively, $N=5$ each). **$P<0.08$.

frequency spectrum they are on. A simpler, related explanation for the differential chirp responses is that individuals differ in their sensitivities, such that males with lower-ranking EODFs may be less sensitive to high EODFs than higher-ranking males are to low EODFs. Alternatively, the differential responses could be explained by a difference in 'confidence'. This anthropomorphism is potentially corroborated, for example, by the phenomenon of prior residency effect whereby residents tend to fight harder and win against larger intruders simply because of ownership (Krebs 1982). Such differential motivational predisposition is probably influenced by gonadal state (Neat et al. 1998) and androgen levels (Rohwer & Rohwer 1978; Wingfield & Marler 1988). Social experience, such as during hierarchy formation, is in turn likely to be an important factor affecting motivational predisposition (e.g. McMann 1993) and androgen levels (Carlson et al. 2000).

The results of previous studies suggest that only a small percentage of females chirp during stimulus playback (Dye 1987; Zupanc & Maler 1993; Dunlap et al. 1998; Dunlap 2002). Although females in the present study chirped less than males, all females chirped at least once. The discrepancy between this latter result and the current literature testifies to the importance of several differences in experimental paradigm. Our stimulus array covered the entire range of conspecific EODFs and we extended the length of the playback trials to 120 s per stimulus. As females had an average LoC latency of 91 s, this could explain why past presentations of shorter duration than a minute have failed to elicit chirps from females (Dye 1987; Zupanc & Maler 1993). Also, in past studies stimuli have been presented across the width of a plastic tube that constrained the fish, a paradigm which boasts clarity of EOD recordings at a cost of presenting an unnatural electric field geometry (e.g. Dye 1987; Zupanc & Maler 1993). In our study the relative position of the stimulus electrodes more closely mimicked a distant fish, and we allowed our fish to move around freely (see also Dunlap 2002). It is possible that confinement decreases chirp propensity in females due to stress effects, and/or that females in this study chirped because they had more space than in other studies about which to feel territorial.

Communicative Value of the EOD and its Modulations

It is clear from the above discussion that EODF cues are sufficient to represent sex and male status and elicit different signalling responses, as expected. We had also hypothesized that in order to communicate effectively with the opposite sex, there should be elements of the

EOD and/or modulations thereof that somehow tap into the contrasexual sensory channel (Ryan & Keddy-Hector 1992).

We found that females have more relative energy in their harmonics than do males. This difference was more pronounced at the third harmonic, which for females is a frequency that approximates the male second harmonic. For example, the third harmonic of a female EOD at 600 Hz would have appreciable energy at 1800 Hz, which is incidentally also the frequency of the second harmonic of a male discharging at 900 Hz. Since males show increased sensitivity at their second harmonic, this implies a potentially interesting coevolved match of female signalling and male tuning. Perhaps females increase their cues' active space by allotting more energy to frequencies in the male range.

Since the frequency excursions of LoCs are restricted to the range of maximal consexual sensitivity, our results are consistent with the implication that the short LoCs are used in intrasexual aggressive interactions (Hagedorn 1986). Compared with LoCs, HiCs have an appreciable portion of their energy at frequencies approaching the female EOD range (Fig. 1a), during the terminal undershoot of the HiC. It remains to be tested whether this undershoot is merely a biophysical constraint due to the inactivation of sodium channels in the pacemaker nucleus, or whether it has any functional significance, such as communication with females. However, most of the energy of a HiC is at frequencies above the baseline EODF, corresponding to the range of stimulus frequencies that are also likely to elicit HiCs. If various chirp parameters have evolved to increase the signals' active space, the increased HiC emission probability at both female frequencies and those typical of dominant males suggests that this type of chirp could serve the function of simultaneously signalling reproductive intent and submission. Alternatively, this signal category's greater frequency excursions could merely serve the function of increasing the active space for aggressive communication with individuals of more distant EODFs.

The functions of rises are also unclear. The suggestion that rises are submissive signals (Hopkins 1974) is substantiated by our finding that low-EODF males emit more rises than high-EODF males (Fig. 4). Thus, smaller males are more likely to emit rises than males with more reproductive potential and presumably higher associated dominance. When examining the responses to absolute frequency, males also showed a peak in rise emissions to female frequencies. Although this pattern was stronger for males with low-ranking EODFs (not shown), suggesting they could be submissive to larger females, male rises could thus also function in signalling courtship. Peak rise emissions by females to male stimuli are consistent with this hypothesis. It has long been suggested that spawning necessitates mitigation of aggression (Bastock 1967). Perhaps signals employed to communicate courtship and submission are at least to some extent borne out of a similar motivational condition. However, if rises do signal submission to some extent and female EODF is indeed positively correlated with status (Dunlap & Oliveri 2002), it is unclear why high-EODF females emit more rises (Fig. 4) and why low female stimulus frequencies elicit more rises from females (Fig. 5). A concurrent study has revealed that female rises can be broken down into several categories, some or all of which may in turn have different communicative value (Tallarovic & Zakon, in press). Thus, it is probable that by consolidating rises into a single category we are missing meaningful differences between rise subtypes, which may be used differentially in intra- and intersexual communication.

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