The Journal of Experimental Biology 204, 1909–1923 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB3184

ARGININE VASOTOCIN MODULATES A SEXUALLY DIMORPHIC COMMUNICATION BEHAVIOR IN THE WEAKLY ELECTRIC FISH *APTERONOTUS LEPTORHYNCHUS*

JOSEPH BASTIAN*, STEPHANIE SCHNIEDERJAN AND JERRY NGUYENKIM
Department of Zoology, 730 Van Vleet Oval, University of Oklahoma, Norman, OK 73019, USA
*e-mail: jbastian@ou.ed

Accepted 17 March 2001

Summary

South American weakly electric fish produce a variety of electric organ discharge (EOD) amplitude and frequency modulations including chirps or rapid increases in EOD frequency that function as agonistic and courtship and mating displays. In *Apteronotus leptorhynchus*, chirps are readily evoked by the presence of the EOD of a conspecific or a sinusoidal signal designed to mimic another EOD, and we found that the frequency difference between the discharge of a given animal and that of an EOD mimic is important in determining which of two categories of chirp an animal will produce. Type-I chirps (EOD frequency increases averaging 650 Hz and lasting approximately 25 ms) are preferentially produced by males in response to EOD mimics with a frequency of 50–200 Hz higher or lower than that of their own. The EOD frequency of *Apteronotus leptorhynchus* is sexually dimorphic: female EODs range from 600 to 800 Hz and male EODs range from 800 to 1000 Hz. Hence, EOD frequency differences effective in evoking type-I chirps are most likely to occur during male/female interactions. This result supports previous observations that type-I chirps are emitted most often during courtship and mating. Type-II chirps, which consist of shorter-duration frequency increases of approximately 100 Hz, occur preferentially in response to EOD mimics that differ from the EOD of the animal by 10–15 Hz. Hence these are preferentially evoked when animals of the same sex interact and, as previously suggested, probably represent agonistic displays. Females typically produced only type-II chirps. We also investigated the effects of arginine vasotocin on chirping. This peptide is known to modulate communication and other types of behavior in many species, and we found that arginine vasotocin decreased the production of type-II chirps by males and also increased the production of type-I chirps in a subset of males. The chirping of most females was not significantly affected by arginine vasotocin.

Key words: arginine vasotocin, communication, behaviour, weakly electric fish, electrosensory system, *Apteronotus leptorhynchus*.

Introduction

Weakly electric fish generate an electric field around their body that, in conjunction with an array of electroreceptors, comprises an active sensory system enabling the animals to detect and identify objects in their environment and also provides a channel for intraspecific communication (for reviews, see Bullock and Heiligenberg, 1986; Turner et al., 1999). South American weakly electric fish include species that produce a discontinuous or pulse-like electric organ discharge, or EOD, and others that produce a continuous quasi-sinusoidal EOD. Among the latter, the so-called wave-species, several EOD frequency and amplitude modulation patterns have been described that function as communication signals (Hopkins, 1972; Hopkins, 1974a; Hopkins, 1974b; Hopkins, 1974c; Hopkins, 1988; Hagedorn and Heiligenberg, 1985; Hagedorn, 1986).

The well-known jamming avoidance response (JAR) is undoubtedly the most thoroughly studied of these types of behavior (Bullock et al., 1972; Heiligenberg, 1977; Heiligenberg, 1986; Heiligenberg, 1991). The JAR consists of a gradual change in the EOD frequency of a given animal in response to the presence of the EOD of a conspecific with a slightly different frequency. This enlarges the frequency difference between the EODs of the conspecifics, preserving the electrolocation abilities of the animals that would otherwise be jammed by the interfering EODs (Heiligenberg, 1973). In addition, the JAR may facilitate the ability of an animal to discriminate other EOD waveforms (Kramer, 1999). Several additional EOD modulation patterns have been observed in either agonistic or reproductive contexts in both *Eigenmannia virescens* and *Apteronotus leptorhynchus*; these include long rises (moderate EOD frequency increases lasting several seconds), short rises lasting 1–2 s, frequency falls, EOD cessations and chirps (Hopkins, 1974c; Hagedorn and Heiligenberg, 1985; Hagedorn, 1986). This latter behavior,

initially described as 'pings' (Larimer and MacDonald, 1968) and as 'chirps' (Bullock, 1969), consists of very rapid increases in EOD frequency ranging from approximately 50 Hz to well in excess of 500 Hz. Chirps typically last approximately 10–30 ms but, during actual courtship, they can last over 100 ms (Hagedorn and Heiligenberg, 1985). The highest-frequency long-duration chirps also result in a decrease in EOD amplitude which, in the case of *Eigenmannia virescens*, can result in a cessation of the discharge known as an interruption (Hopkins, 1974c).

Chirps are readily evoked under laboratory conditions by stimulating an animal with a sinusoidal signal with an amplitude and frequency that mimic the discharge of a conspecific, and this behavior persists in neurophysiological preparations (Dye, 1987). In addition, both the anatomy and physiology of the sensory and motor circuitry involved in controlling chirping are well understood (for reviews, see Zupanc and Maler, 1997; Metzner, 1999). Studies of chirping by *Apteronotus leptorhynchus* have shown that males are more likely than females to chirp in response to mimics of the discharge of a conspecific, although a subset of females will also chirp (Dye, 1987). Non-chirping females can be induced to chirp following chronic testosterone implants (Dulka and Maler, 1994). Testosterone implants also alter the distribution of substance P, which has been shown to modulate chirping (Weld et al., 1991), in brain areas known to be involved in the control of this behavior (Weld and Maler, 1992; Dulka et al., 1995). In addition to modulating communication behavior, androgens have also been shown to modulate the waveform and fundamental frequency of the EOD, which are sexually dimorphic in this and other species. Both the pacemaker nucleus that drives each cycle of the discharge and the biophysical properties of cells within the electric organ are influenced by steroid hormones (Meyer, 1983; Meyer, 1984, Meyer et al., 1987; Mills and Zakon, 1987; Mills and Zakon, 1991; Zakon et al., 1991; Ferrari et al., 1995; Dunlap et al., 1997; Dunlap et al., 1998; Dunlap and Zakon, 1998; Zakon and Dunlap, 1999).

Observations of *Apteronotus leptorhynchus* during courtship and mating and during stimulation with mimics of the discharge of a conspecific show that chirps can vary, particularly among males, in terms of maximum frequency change, chirp duration and the extent to which EOD amplitude changes (Hagedorn and Heiligenberg, 1985). Zupanc and Maler (Zupanc and Maler, 1993) quantitatively described chirps produced by *Apteronotus leptorhynchus* males and found that the 'typical' male chirp has a duration of approximately 15 ms during which the EOD goes through a frequency excursion of approximately 100 Hz. In addition, they observed chirps of longer duration and of much larger frequency change, but these were seen rarely. Recently, Engler et al. (Engler et al., 2000a; Engler et al., 2000b) observed two clearly identifiable categories of spontaneously occurring and stimulus-evoked chirps. One category, referred to as type-I, was similar to the chirps observed only rarely by Zupanc and Maler (Zupanc and Maler, 1993) while the second category, type-II, had essentially the same characteristics as the more commonly occurring brief chirps described earlier.

The results of this study confirm the existence of two qualitatively different chirps produced by *Apteronotus leptorhynchus* and show that the propensity of animals to produce the two chirp types is sexually dimorphic. With rare exceptions, females only produced the more commonly observed type-II chirps. In addition, in males, the probability of type-I and type-II chirp production varies with the difference between the discharge frequency of an experimental animal and that of a sinusoidal mimic of the discharge of a conspecific. This difference frequency, or DF, determines the beat frequency of the composite signal that the animal receives, and the DF-dependent response differences support the idea that the type-II chirps are important communication signals among individuals of a given gender while the type-I chirps may be more important for male/female interactions (Hagedorn and Heiligenberg, 1985; Hagedorn, 1986). Thus, the roles of type-I and type-II chirps may parallel the roles of long- and short-duration discharge interruptions observed in the related fish *Eigenmannia virescens*. The long-duration interruptions preferentially occur during sexual interactions, while the short-duration behavior is linked to agonistic encounters (Hopkins, 1974c).

Lastly, the effect of arginine-8-vasotocin on male and female chirping behavior was investigated. This neuropeptide and the related peptide arginine vasopressin in mammals have clear-cut modulatory effects on reproductive behavior in many species (for reviews, see Moore, 1992; Moore and Lowry, 1998), and recent studies have demonstrated effects of arginine vasotocin on the vocalization behaviors linked to reproduction of the plainfin midshipman fish *Porichthys notatus* (Goodson and Bass, 2000a; Goodson and Bass, 2000b). We found that intraperitoneal injection of arginine vasotocin caused dose-dependent changes in male behavior, but this treatment was often ineffective in changing female behavior.

Materials and methods

The weakly electric fish *Apteronotus leptorhynchus* (Eigenmann) was used exclusively in this study. Animals were housed singly or in small populations of 2–6 fish at approximately 26 °C. The conductivity of the water in the various holding tanks ranged between 500 and 1000 $\mu S\,cm^{-1}$. Animal care and handling and experimental and surgical protocols were in accord with University of Oklahoma Animal Care and Use Committee guidelines. During experiments, the fish were restrained in an envelope made of nylon mesh and suspended in the center of a plexiglas tank 30.5 cm×30.5 cm×7 cm deep. The tank was filled with water from the animal's home tank, and the water was recirculated between the experimental tank and a reservoir aerated and temperature-controlled to maintain the animal at a temperature between 26 and 27 °C.

Animals used ranged from 145 to 218 mm in total body length. Males were distinguished from females on the basis of

the electric organ discharge frequency, which typically ranges from approximately 600 to 800 Hz in females and from 800 Hz to as high as 1050 Hz in males (Meyer et al., 1987). Head morphology also varied between males and females (as described by Hagedorn, 1986). Males typically had more elongate snouts than females. In addition, the morphology of the cloacal papilla was found to be a reliable indicator of gender in larger animals. In females, this structure is bulbous and lacks pigmentation, resulting in an obvious white appearance. In males, it is more heavily pigmented and is recessed. Sex was verified by dissection of animals with intermediate EOD frequencies.

Recording and stimulation

The electric organ discharge (EOD) was measured between silver chloride electrodes placed near the head and tail, and this head-to-tail EOD was amplified with a World Precision Instruments (WPI, Sarasota, FL, USA) DAM 50 preamplifier (gain 1000× and low- and highpass filters set to 300 Hz and 10 kHz, respectively) and led to a Cambridge Electronic Design (CED, Cambridge, UK) 1401 plus data-acquisition computer running Spike II for Windows. The head-to-tail EOD was analog-to-digital converted at a sampling frequency of 12.5 kHz. The head-to-tail EOD signal was also led to a custom-built Schmidt trigger circuit that produced a single pulse at the negative-going zero-crossing of each EOD cycle. The resulting pulse train, which marked the time of occurrence of each EOD cycle, was also led to the data-acquisition unit and time-stamped with a resolution of 10 μs. Following each experiment, the analog-to-digital recordings of the EOD waveform and the times of each EOD cycle were converted to text files and imported to Matlab (The Mathworks Inc, Natick, MA, USA), and chirp counts and the associated EOD amplitude and frequency changes were measured using the algorithm described below.

The discharge of a conspecific was mimicked by a sinusoidal signal produced by a Tektronix FG 501A function generator. The signal was isolated from ground with a WPI A395 analog stimulus-isolation unit and capacity-coupled to large Ag/AgCl electrodes placed approximately 15 cm from either side of the fish. Stimulus field strength was set to 1 mV cm^{-1} prior to placing the fish in the chamber, and the orientations of the stimulation and head-to-tail recording electrodes were carefully adjusted to minimize contamination of the head-to-tail EOD signal by the stimulus field. Throughout each experiment, the stimulus field was also measured using a dipole electrode pair, 1 cm spacing, oriented perpendicular to the long axis of the fish near the operculum. This signal was amplified, analog-to-digital converted at 12.5 kHz, and saved together with the head-to-tail EOD.

The chirp production of an animal varies considerably contingent upon the relative frequencies of the fish and of the stimulation signal (Dye, 1987), and the EOD frequency of an animal also typically changes upon stimulation because of the production of a jamming avoidance response (JAR) or other gradual EOD frequency rises such as the non-selective response (NSR). A frequency-clamp device was therefore used to ensure that a selected difference frequency (DF), defined as the stimulus frequency minus the EOD frequency of the fish, was maintained even when the frequency of the fish changed. A computer system with a Labmaster DMA I/O interface, running LabPac software (Scientific Solutions, Solon, OH, USA), was programmed to measure both the EOD frequency and the stimulus frequency. The difference frequency was determined and compared with a pre-set DF value chosen for a given experiment. A digital-to-analog output was continuously updated according to the difference between the actual and desired DF values. The resulting analog voltage was led to the voltage-controlled frequency input of the function generator, with the result that the stimulus frequency was continuously maintained at the desired value above or below that of the experimental animal. This frequency clamp was used in all experiments in which the desired DF value was between +48 Hz and −48 Hz, but not for experiments using DF values outside this range since little to no change in the EOD of the animal occurred with these larger DF values.

Experimental design and chirp analyses

Each experimental period consisted of an initial 10 s pre-stimulus period during which the baseline EOD of the animal was measured, followed by a 100 s stimulation period during which the sinusoidal stimulus of the desired DF was applied. A final 10 s period with no stimulus followed the end of the stimulation period. Typically, 12 min separated the start of each successive experimental period and, depending on the experiment type, 40–42 experimental periods made up a single experimental session. For studies of the effects of DF sign and magnitude on chirp production, responses to sinusoidal signals of DFs of + and −1, 2, 4, 8, 12, 16, 24, 28, 32, and 48 Hz were recorded. Each stimulus was repeated twice for a total of 40 experimental periods, and different random sequences of DF presentation were used with different fish to minimize any systematic effects of habituation. DF values of + and −4, 12, 48, 100 and 200 Hz were used in a second series of experiments designed to determine chirp responses to a wider range of DFs. Four replicates of each stimulus, randomized for each fish, were used in these sessions, giving a total of 40 experimental periods. For studies of arginine vasotocin on chirp behavior, 42 experimental periods were used; 21 stimulus presentations of −12 Hz were interleaved with 21 DF stimuli at −4 Hz. The first or control phase of these experiments consisted of 12 experimental periods, six at each DF. This was immediately followed by an intraperitoneal injection of 100 or 300 μl of fish saline (Bastian, 1974). A second set of 12 stimulation periods, the saline phase, followed this injection. After this, a second injection of the same volume of saline plus arginine vasotocin (Sigma, St Louis, MO, USA) at concentrations resulting in dosages of 0.005–16 μg g^{-1} was applied and followed by a set of 18 interleaved −12 and −4 Hz DF stimulus periods.

The EOD zero-crossing times and the peak-to-peak amplitude of each EOD cycle were exported to Matlab, where an algorithm counted and categorized chirps and measured the

duration of each chirp, the peak-to-peak (p-p) frequency change and EOD amplitude change. First, the times of EOD zero-crossings were converted to instantaneous frequency, and thresholds were entered for type-II and type-I chirps. Values for these were typically 40 and 150 Hz above the baseline frequency of the animal, which was determined from the initial 10 s of each experimental period. Second, candidate chirps were identified as increases in instantaneous frequency that exceeded the lower threshold only (type-II chirps) or both the low- and high-frequency thresholds (type-I chirps). To be considered a valid chirp, at least seven consecutive instantaneous frequency measurements must remain above the low-frequency threshold. This constraint filtered out occasional spurious frequency increases caused by false EOD triggers. The beginning of each chirp was identified as the time of the first instantaneous frequency value that exceeded the mean plus three standard deviations of 100 instantaneous frequency measurements ending 10 EOD cycles before the first above-threshold value. The end of each chirp was identified as the time of the last instantaneous frequency value either three standard deviations above or below the mean pre-chirp frequency. Chirp duration was measured as the difference between these times. Peak-to-peak frequency change was measured as the maximum minus minimum instantaneous frequency value within each chirp, and the EOD amplitude change was determined as the ratio of the mean peak-to-peak amplitude of the first 10 EOD cycles within the chirp to the mean pre-chirp EOD amplitude. The magnitude of the jamming avoidance response, or non-selective response, was measured as the difference between the mean EOD frequency measured approximately halfway through the stimulation period and the EOD frequency measured during the 10 s pre-stimulus period. The EOD frequency measurement during the stimulation period was made from the longest data segment within which no chirps occurred beginning 35 s after stimulus onset.

Values are presented as means ± 1 S.E.M.

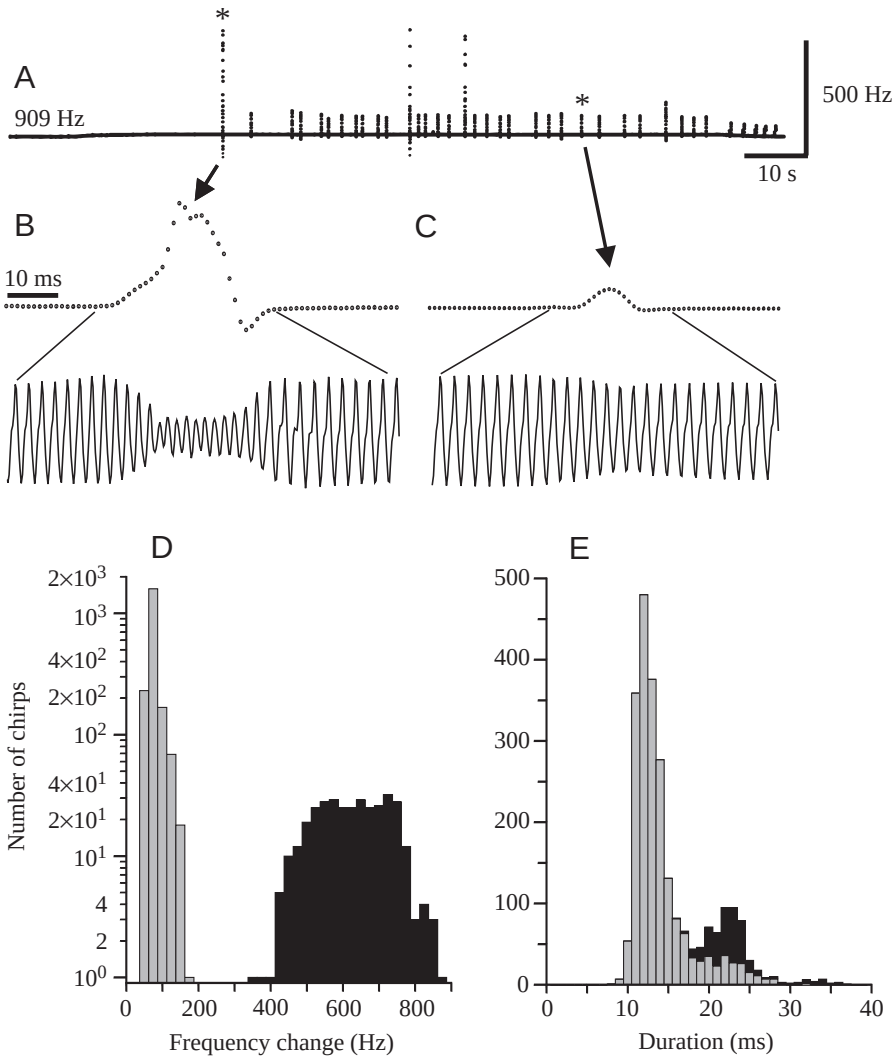

Fig. 1. Examples of chirps produced by a male *Apteronotus leptorhynchus* in response to stimulation with sinusoidal electric fields. (A) Instantaneous electric organ discharge (EOD) frequency plot during stimulation with a −4 Hz difference frequency (DF) stimulus field. Temperature 26.5 °C. (B,C) Instantaneous frequency and EOD waveforms during the type-I (left) and type-II (right) chirps indicated by the asterisks in A. (D,E) Histograms of chirp peak-to-peak frequency change (D) and duration (E) for chirps made by this male during one experimental session. Black columns, type-I; grey columns, type-II chirps.

Results

Chirp characteristics of males and females

The most common chirp type produced by males consists of a brief (10–15 ms) increase in EOD frequency of 75–100 Hz. The EOD amplitude is typically unchanged or slightly reduced during this behavior. These chirps were originally described quantitatively by Zupanc and Maler (Zupanc and Maler, 1993) and more recently termed type-II chirps (Engler et al., 2000a). Depending on the individual fish and on the stimulus conditions, males may also produce a much longer chirp 20–30 ms in duration during which EOD frequency first accelerates to nearly double its normal frequency then briefly decelerates to approximately 100 Hz below the baseline EOD frequency. The EOD amplitude is also usually reduced during these events, which will be referred to as type-I chirps and, under appropriate stimulus conditions, both chirp types appear intermixed, as shown by the instantaneous EOD frequency plot of Fig. 1A. The −4 Hz DF stimulus used for this stimulation

Table 1. *Summary of the characteristics of type-I and type-II chirps produced by a typical male and female* Apteronotus leptorhynchus

	N		Mean duration (ms)		Mean p-p frequency (Hz)		Mean EOD amplitude (%)		Mean JAR
DF	Type-I	Type-II	Type-I	Type-II	Type-I	Type-II	Type-I	Type-II	(Hz)
–12 Hz ♂	345	2080	23.54±0.20	12.38±0.08	642.2±6.50	88.13±0.34	77.80±0.40	95.9±0.03	0.25±0.16
–4 Hz ♂	32	411	28.75±0.97	16.03±0.22	614.1±16.0	97.07±1.45	80.95±0.81	96.6±0.16	9.05±0.81
–12 Hz ♀	0	321	–	15.16±0.21	–	86.38±0.92	–	98.18±0.13	1.50±0.19
–4 Hz ♀	0	716	–	15.95±0.19	–	75.91±0.54	–	99.28±0.16	12.65±0.50

DF, difference frequency; p-p, peak-to-peak; EOD, electric organ discharge; JAR, jamming avoidance response; *N*, number of chirps. Values are means ± S.E.M.

period also evoked a jamming avoidance response of 10.8 Hz. The instantaneous EOD frequencies during the type-I and type-II chirps indicated by the asterisks in Fig. 1A are shown on an expanded time scale together with the EOD waveforms measured between the head and tail of the animal in Fig. 1B,C, respectively. Both chirp types begin with a gradual EOD frequency increase which, in the case of the type-I chirp, is followed by a rapid second phase of EOD acceleration. It is during this second phase of EOD acceleration that the maximum EOD amplitude decrease occurs; as EOD amplitude recovers, its frequency typically falls below baseline levels. The prolonged small EOD amplitude decrease seen after the type-II chirp in Fig. 1C is partly the falling phase of the beat resulting from weak contamination of the head-to-tail EOD by the stimulus field.

A histogram of the peak-to-peak EOD frequency changes for 2425 chirps recorded from this animal during 24 stimulus presentations (100 s duration, –12 Hz DF) is shown in Fig. 1D. The distribution is obviously bimodal, and the non-overlapping groups correspond to the peak-to-peak frequency changes associated with type-I (black columns) and type-II (grey columns) chirps. The mean frequency changes of the 2080 type-II and the 345 type-I chirps were significantly different, averaging 88.1±0.3 for type-II chirps and 642.2±6.5 Hz for type-I chirps (P<0.0001, *t*-test). The distribution of chirp durations is shown in Fig. 1E. The mean duration of type-I chirps (23.5±0.2 ms) was also significantly longer than that of type-II chirps (12.4±0.1 ms, P<0.0001, *t*-test). The peak-to-peak amplitude of the EOD was also measured immediately prior to and during each chirp. During type-II chirps, the EOD amplitude averaged 95.9±0.03 % of the normal amplitude, while during type-I chirps EOD amplitude fell to an average of 77.8±0.35 % of normal; these changes are also significantly different (P<0.0001, *t*-test).

Descriptions of the chirps produced by this male to −12 and −4 Hz DF during one experimental session are summarized in Table 1 and, in the case of this fish, several characteristics of type-I and type-II chirps differed significantly contingent on the stimulus DF value. However, these differences were not seen in all cases, and when the mean chirp characteristics of all fish studied are compared, no significant differences emerge contingent on DF magnitude (Table 2). Fewer chirps were produced in response to the lower DF stimulus and, importantly, the ratio of mean numbers of type-II to type-I chirps per animal changed as a function of DF magnitude. This raises the possibility that DF frequency differentially affects the probability of type-I and type-II chirp production.

Eleven males were tested with –12 Hz DF, and nine of these were also tested with –4 Hz DF, as described above; although all animals produced both type-I and type-II chirps, the numbers of each type produced varied widely among

Table 2. *Summary of the characteristics of type-I and type-II chirps produced by 11 male and nine female* Apteronotus leptorhynchus

	N		Mean duration (ms)		Mean p-p frequency (Hz)		Mean EOD amplitude (%)		Mean JAR
DF	Type-I	Type-II	Type-I	Type-II	Type-I	Type-II	Type-I	Type-II	(Hz)
–12 Hz ♂	1731/11	20 889/11	26.63±1.45	15.60±0.52	447.6±41.6	80.28±9.01	83.12±3.00	95.1±0.64	7.41±1.76
–4 Hz ♂	620/5	9135/8	28.00±1.60	16.59±0.31	448.9±63.7	76.91±7.63	85.06±2.03	95.5±7.63	12.9±1.91
–12 Hz ♀	64/9	7107/9	24.48±1.51	16.97±1.21	531.0±129	106.5±29.4	65.29±6.78	93.9±2.81	4.57±1.15
–4 Hz ♀	5/1	3504/7	25.87±0.77*	16.95±1.43	857.0±128*	78.6±20.1	60.43±2.12*	94.2±3.77	13.8±2.05‡

DF, difference frequency; p-p, peak-to-peak; EOD, electric organ discharge; JAR, jamming avoidance response.

Sample sizes (*N*) are given as number of chirps/number of fish.

Entries are grand means unless marked with asterisks, which indicate that means and standard errors are based on the responses of one animal. ‡ indicates the mean jamming avoidance response of seven animals.

individuals. Generally, males produced larger numbers of type-II chirps; the number produced per 100 s −12 Hz DF stimulus epoch ranged from 40 to 262, with a mean of 158.3±23.2 chirps 100 s^{-1}. These individuals consistently produced fewer type-I chirps at this DF. Type-I chirp counts ranged from less than 1 to a maximum of 75, with a mean of 13.11±6.53 chirps 100 s^{-1}. The grand means of chirp characteristics measured for the male fish are summarized in Table 2 together with the mean magnitude of the JAR evoked by the −12 and −4 Hz DF stimuli. Type-I and type-II chirps were significantly different in all measured characteristics and, as expected, JAR magnitude was greater for −4 Hz DF. On average, however, the characteristics of type-I and type-II chirps did not vary with DF.

As has been repeatedly noted (Dye, 1987; Zupanc and Maler, 1993), females are generally far less likely to chirp either spontaneously or in response to mimics of the discharge of a conspecific. However, by screening a large population, individual females were found that would reliably chirp in response to the same stimuli used with males. Fig. 2 illustrates the typical range of chirp types produced by a female. Responses to a 100 s, −4 Hz DF stimulus is shown in Fig. 2A, and the chirps identified *via* the asterisks are shown on an expanded time scale in Fig. 2B,C. This animal produced 321 chirps in response to 21 presentations of a 100 s, −12 Hz DF stimuli. The distributions of peak-to-peak frequency changes and of chirp durations were unimodal (Fig. 2D,E) and averaged 86.38±0.92 Hz for frequency changes and 15.16±0.21 ms for chirp durations. The mean characteristics of chirps produced by this female are summarized in the two lower rows of Table 1. With rare exceptions, the chirps produced by females were similar to the type-II chirps produced by males, and type-I chirps were not recorded.

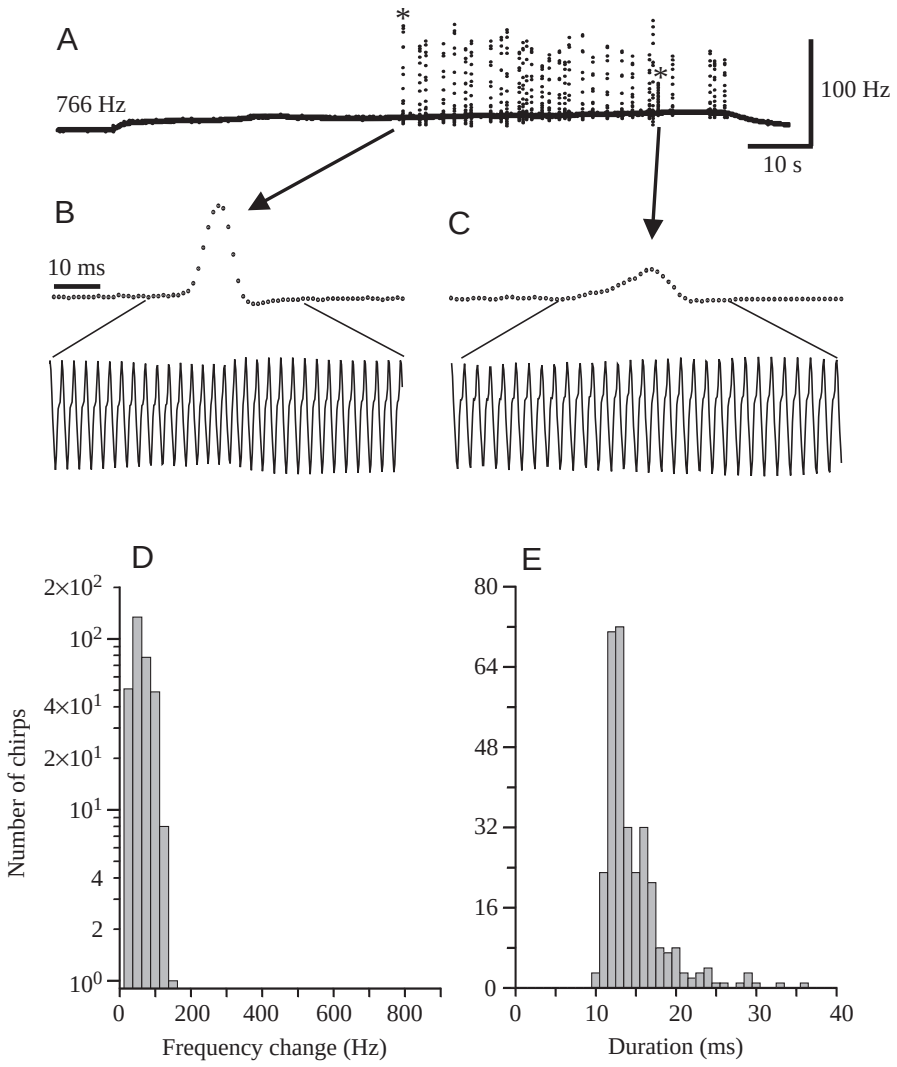

Fig. 2. Examples of chirps produced by a female *Apteronotus leptorhynchus* in response to −4 Hz difference frequency (DF) stimulation. (A) Instantaneous electric organ discharge (EOD) frequency plot. Temperature 26.3 °C. (B,C) Instantaneous frequency and EOD waveforms during the chirps indicated by the asterisks in A. (D,E) Histograms of chirp peak-to-peak frequency change (D) and duration (E) for chirps made by this female during one experimental session.

Nine females were studied as described above and, of these, six produced only type-II chirps while three produced type-II chirps together with small numbers of type-I chirps. On average, the females produced 65.8±20.9 small chirps per 100 s −12 Hz DF stimulus (range 5–170). Of the three females that did produced type-I chirps, one produced a total of 56 in 12 presentations of the −12 Hz DF stimulus while the remaining two fish produced one and six type-I chirps. The grand means of the chirp characteristics measured for this sample of females are also summarized in Table 2. The duration of type-II chirps, their peak-to-peak frequency changes and their EOD amplitude changes did not differ significantly from those of male type-II chirps (*t*-tests). Comparisons of the type-I chirps produced by males and females also showed no significant differences; however, as stated above, the sample size for female type-I chirps was very small.

Differential effects of DF on type-I versus *type-II chirp production*

The numbers of type-I and type-II chirps evoked by 100 s epochs of stimuli having DFs ranging from −48 to +48 Hz were determined for 13 males and six females. Stimuli of different DFs were presented in a different random order in each experiment to reduce any systematic effects of habituation and,

in the case of some females in which habituation was particularly marked, the initial order of DF presentation was reversed in a second experiment on another day. Data from individuals studied in this fashion were averaged. Fig. 3A summarizes the mean production of type-II chirps by males. To normalize for the variation among individuals in total chirp production, the number of chirps produced by a fish at each DF is expressed as a proportion of the total number of chirps produced by that animal during the experimental session for that day (chirp probability). Type-II chirp production showed clear peaks at + and −12 Hz DF and, on average, negative DF stimuli were more effective.

Of the 13 males studied, two did not produce any type-I chirps and two produced a total of 10 or fewer. Data from these four fish were excluded from the analysis of type-I chirp tuning to avoid the effects of high probabilities that result when fish produce few chirps. Type-I chirp production showed a significantly different pattern of DF tuning, as shown in Fig. 3B; the probability that this behavior would occur was lowest at small absolute DF values and increased with larger DF values. A second sample of six males was studied using a broader range of DFs (−200 to +200 Hz), and the tuning of type-I chirping (filled symbols) and type-II chirping (open symbols) in response to these stimuli is shown in Fig. 4A. As indicated above, type-II chirp probability is maximal in response to DFs of approximately ±12 Hz, while type-I chirps are preferentially evoked by much larger DFs. On average, the most effective DF for type-I chirp production is in the neighborhood of −100 Hz.

Six females were also studied with stimulus DFs ranging from −48 to + 48 Hz. These females only produced type-II chirps, and female chirp probability as a function of DF is summarized in Fig. 3C. Although the standard errors of many of the mean probabilities are large, the shape of the tuning curve is similar to that for the production of male type-II chirps. Two females were also studied using large DFs. Females will produce chirps in response to DFs of ±100 and 200 Hz, but these responses rapidly habituated, and tuning curves as shown in Fig. 4A were not completed for females.

The slower long-duration frequency changes (jamming avoidance responses, JARs, and non-selective responses, NSRs) due to stimuli of various DFs were also measured in these experiments and are summarized in Fig. 3D for males (filled symbols) and females (open symbols). As described previously (Dye, 1987), the JAR is maximal for approximately −4 Hz DF and, on average, males produced larger JARs over the range of negative DFs used. *Apteronotus leptorhynchus* only produces a true JAR in response to negative DFs. Since the animal does not lower its firing frequency below its baseline level, responses to positive DF stimuli are not frequency decreases as in other species. Instead, the smaller frequency increase seen in response to positive DF stimuli is termed the 'non-selective response or NSR' (Dye, 1987), and the NSR was also typically larger for males than for females.

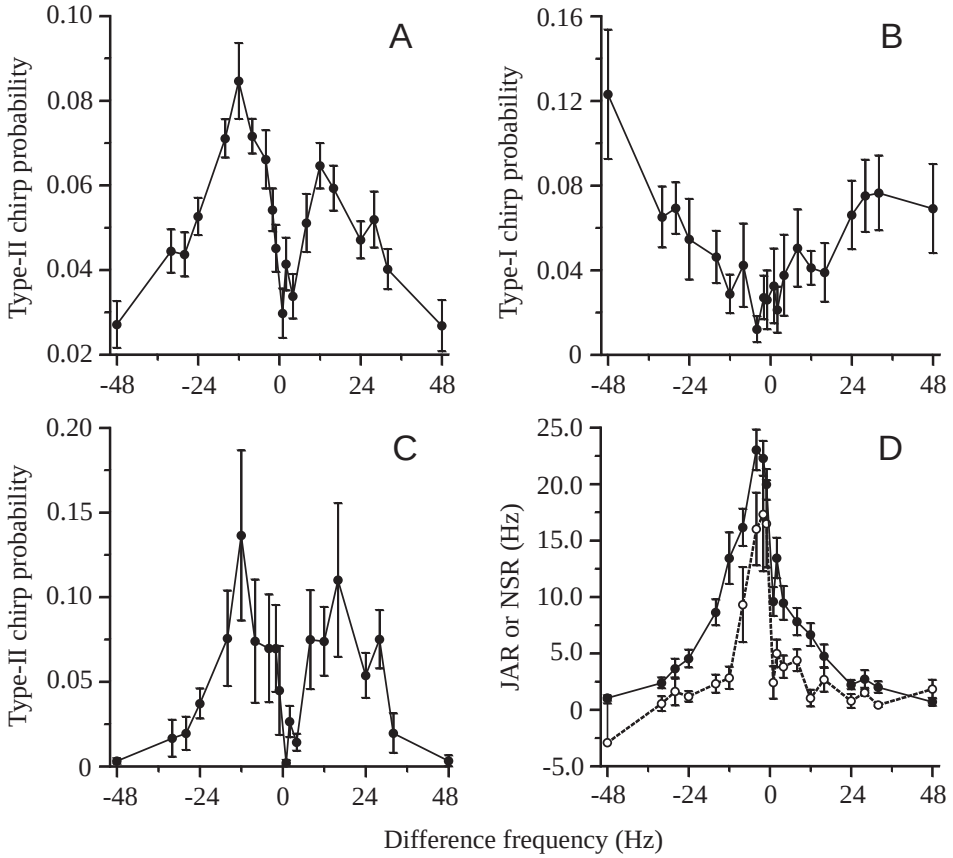

Fig. 3. Tuning curves relating type-I and type-II chirp production to stimulus difference frequency (DF). (A) Probability of male type-II chirps *versus* DF (*N*=13 fish). (B) Probability of male type-I chirps *versus* DF (*N*=9 fish). (C) Probability of female chirps *versus* DF (*N*=6 fish). (D) Magnitude of the jamming avoidance response (JAR) and non-selective response (NSR) *versus* stimulus DF. Filled symbols indicate data from males (*N*=13 fish) and open symbols indicate data from females (*N*=6). Values are means ± S.E.M.

Fig. 4B shows the magnitudes of the JAR and NSR of males evoked by higher-frequency DFs; neither of these types of behavior appears for absolute DFs greater than approximately 100 Hz.

Effects of arginine vasotocin on male chirp probability

In a subset of the males studied, intraperitoneal injections of arginine vasotocin differentially altered type-I and type-II chirp responses in a dose-dependent manner. An example of the effects of arginine vasotocin injection on chirping evoked by −12 Hz DF stimulation is shown in Fig. 5. The instantaneous frequency recording shown in Fig. 5A was taken approximately 120 min after a 300 µl injection of saline and just before the injection of arginine vasotocin. The recording of Fig. 5B was taken 36 min after a 0.08 µg g^{-1} arginine vasotocin injection in the same volume of saline. There was a striking increase in the number of type-I chirps accompanied by a decrease in the number of type-II chirps.

The time courses of experiments for a male in which above- and below-threshold arginine vasotocin injections were used are summarized in Fig. 6 and Fig. 7. Alternating presentations of 100 s epochs of −12 Hz and −4 Hz DF stimuli were presented at 12 min intervals, and type-I and type-II chirp counts are plotted in Fig. 6A,B (type-I) and Fig. 7A,B (type-II). Data from the experiments using above-threshold (0.08 µg g^{-1}) and below-threshold (0.04 µg g^{-1}) arginine vasotocin concentrations are indicated by open and filled circles, respectively. Initially, 12 control stimulus presentations, six at each DF, were applied, and the animal produced 3–5 type-I and 130–180 type-II chirps during each 100 s stimulus presentation. The mean counts for each chirp type during the control phases of these and additional experiments with different arginine vasotocin dosages are shown by the light grey columns of the histograms of Fig. 6C and Fig. 7C.

Following the last control stimulus, the fish was injected with 300 µl of fish saline, and the sequence of six −12 and −4 Hz DF stimuli was repeated. Chirping was often inhibited for a short time following an injection, perhaps as a result of handling the fish. In some experiments, saline alone resulted in significant alterations in the numbers of type-I chirps evoked by subsequent stimuli, as is shown by the filled symbols in Fig. 6A (saline). Mean type-I and type-II chirp counts following saline injections in five separate experiments with this male are shown by the dark grey columns in Fig. 6C and Fig. 7C, respectively.

Various dosages of arginine vasotocin, delivered in 300 µl volumes of Ringer, were injected following the saline phase of the experiments, and chirps were monitored during 18 additional 100 s stimulus periods (nine at −12 Hz DF interleaved with nine at −4 Hz DF). As shown by the instantaneous frequency plots of Fig. 5 and the counts of chirps per 100 s (Fig. 6A,B and Fig. 7A,B; arginine vasotocin), suprathreshold doses (0.08 µg g^{-1}, open symbols) resulted in large increases in the number of type-I chirps and a reduction in the number of type-II chirps evoked by both −12 and −4 Hz DF stimuli. Lower arginine vasotocin doses (e.g. 0.04 µg g^{-1}, filled symbols),

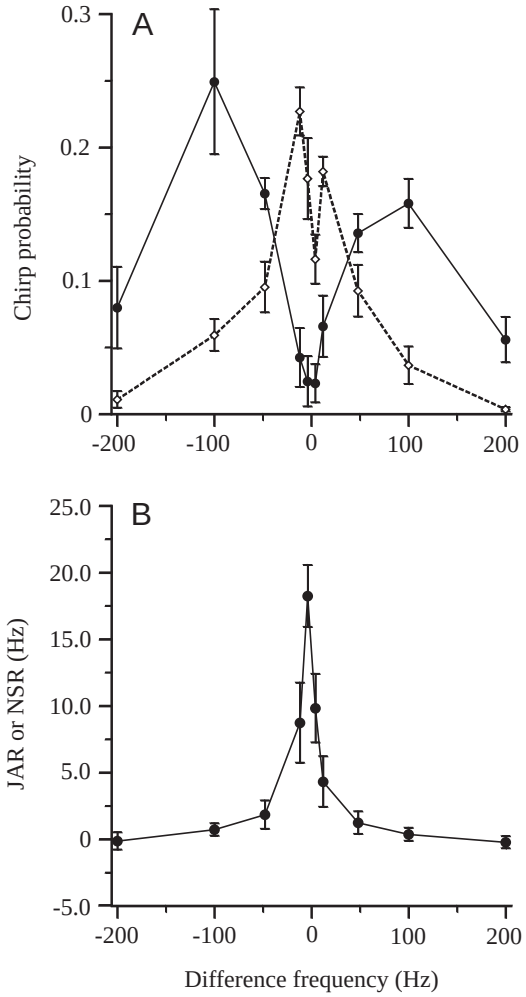

Fig. 4. Tuning curves relating male type-I and type-II chirp probability to a wide range of difference frequency (DF) values. (A) Probability of type-I (filled symbols) and type-II (open symbols) chirp production *versus* stimulus DF (*N*=6 fish). (B) Magnitude of the jamming avoidance response (JAR) and non-selective response (NSR) *versus* stimulus DF (*N*=6 fish). Values are means ± S.E.M.

however, did not alter chirp production above or below that due to saline alone. The asterisks in Fig. 6A and Fig. 7A show the chirp counts from the experimental periods shown in Fig. 5A,B. The effects of administration of five concentrations of arginine vasotocin on type-I and type-II chirp production are summarized in Fig 6C and Fig. 7C, respectively. With arginine vasotocin doses equal to or below 0.04 µg g^{-1}, no significant changes in the mean numbers of chirps of either type were seen compared with chirp responses following saline, but above this threshold consistent increases in type-I and decreases in type-II chirps were seen.

A series of experiments similar to those described with Fig 6 and Fig. 7 was completed for a second male, and similar results were obtained, except that the minimum arginine vasotocin concentration at which significant changes in chirp responses were seen was 0.01 µg g^{-1}. In addition, for this fish, two

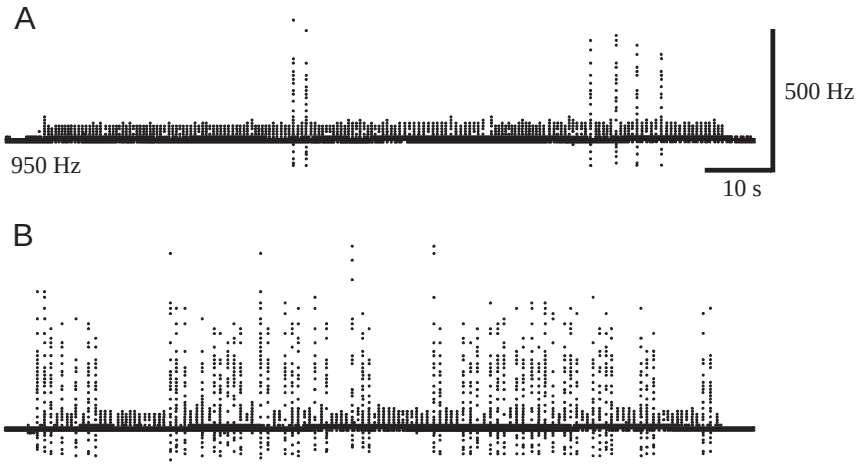

Fig. 5. Chirps produced by a male *Apteronotus leptorhynchus* before and after injection of arginine vasotocin (AVT). (A) Responses to −12 Hz difference frequency (DF) stimulation after a control injection of saline but before AVT injection. (B) Responses of the same animal to −12 Hz DF stimulation following AVT injection. The calibration is the same for A and B. Temperature 27 °C.

additional experiments were performed in which saline alone was injected during the third phase of the experiment (arginine vasotocin concentration 0 μg g^{-1}), and no significant changes in chirp counts were observed.

The effects of arginine vasotocin injections were studied in a total of 12 males using a protocol similar to that described in conjunction with Fig. 6 and Fig. 7. Large arginine vasotocin doses (200 μg per animal; approximately 15 μg g^{-1}) were used to ensure above-threshold concentrations. For each fish, the mean numbers of type-I and type-II chirps evoked during the six −12 and −4 Hz DF stimuli following the saline injection were compared with the mean chirp counts from the stimuli of the same DF following the saline+arginine vasotocin injection. Fish were then divided into sub-populations depending on whether or not significant changes (P<0.05, t-tests) occurred following arginine vasotocin injection. In all cases, following arginine vasotocin injection, the mean numbers of type-II chirps per stimulation period were significantly decreased relative to saline treatment. Fig. 8A summarizes the changes in type-II chirp production in response to −12 Hz DF stimulation. The response of each animal during each stimulus presentation was converted to the percentage of the mean number of chirps produced during the first six stimulus presentations (control phase) to normalize for individual differences in the absolute numbers of chirps produced. The grand mean type-II chirp production for all fish during these control stimulus presentations was 167.9±23.3 chirps 100 s^{-1}. Type-II chirp production showed a steady, roughly exponential, decay during the control period and through the saline phase of the experiments, and saline injection had no effect on chirp production beyond that attributable to habituation (Fig. 8A).

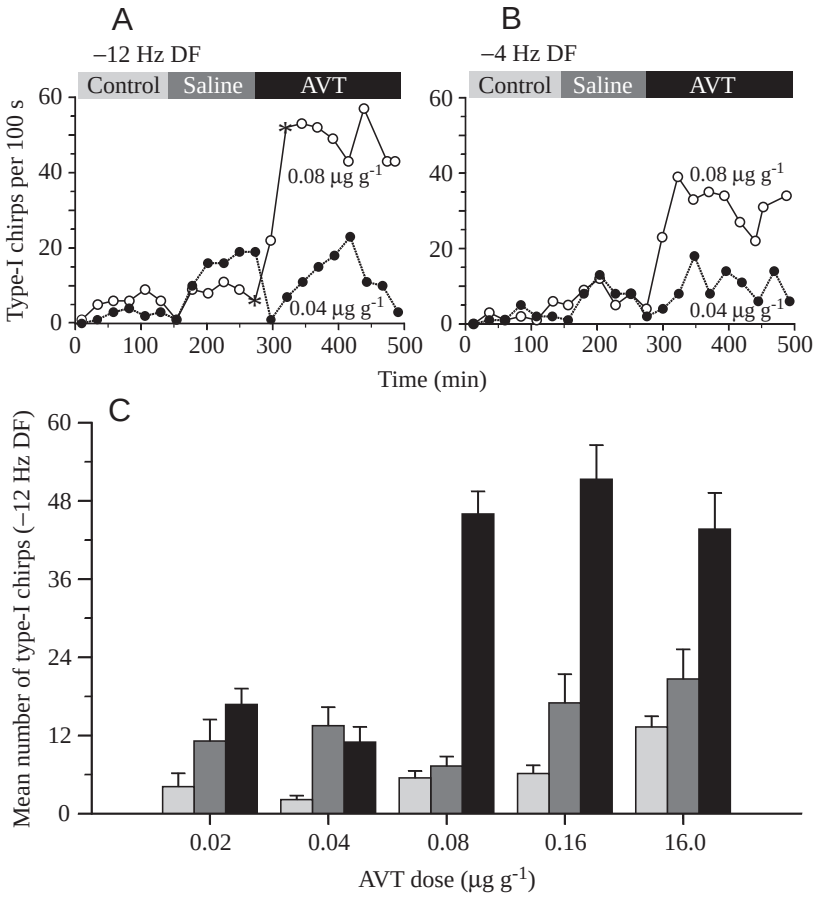

Fig. 6. Effects of arginine vasotocin (AVT) on the production of type-I chirps by a single male. (A) Counts of type-I chirps per stimulus presentation during control conditions, following saline injection and following 0.04 (filled symbols) and 0.08 μg g^{-1} (open symbols) doses of AVT. Asterisks indicate chirp counts from the recordings of Fig. 5A,B. (B) Type-I chirp counts from the −4 Hz difference frequency (DF) stimulus presentations interleaved with the −12 Hz DF stimuli. (C) Histograms summarizing the mean numbers of type-I chirps evoked by −12 Hz DF stimulation during the six control stimuli (light gray columns), during the six stimuli following saline injection (dark gray columns) and during nine stimuli following the indicated AVT doses (black columns). Values are means ± S.E.M.

However, following arginine vasotocin injection, the mean responses show a rapid decay to approximately 55% of the control chirp rate. A similar pattern of changes in type-II chirp production was seen for responses to −4 Hz DF stimulation.

Following arginine vasotocin injection, five of the 12 males also showed significant increases in type-I chirp production, six showed no significant change and one showed a significant decrease. Of the five that increased chirp production, one produced no type-I chirps during the control phase or following saline injection, but produced an average of 4.3 type-I chirps per post-arginine-vasotocin stimulus period. The remaining four fish that showed significant increases following arginine vasotocin injection produced an average of 8.46±2.08 type-I chirps per control stimulus presentation. Data from these fish were normalized to the mean control responses, as described above, and are plotted in Fig. 8B (open symbols). Of the seven fish that did not show significant increases following arginine vasotocin injection, five produced at least one type-I chirp per stimulus presentation, and the number of type-I chirps per control stimulus averaged 23.4±12.3. The mean chirp counts from this sample, normalized as described above, are plotted in Fig. 8B as filled symbols. Although arginine vasotocin affected the rates at which type-II and in some males the rates at which type-I chirps were produced, this treatment had no effect on the characteristics of either chirp type. The peak-to-peak frequency change, the duration and the degree to which EOD amplitude was modulated were not altered by arginine vasotocin injection.

The effects of saline and arginine vasotocin injection on the magnitude of the jamming avoidance response are shown in Fig. 8C. The JARs of males showing significant increases in type-I chirps (open symbols) and those that did not (filled symbols) changed in a similar fashion following arginine vasotocin injection. In both cases, the magnitude of the JAR was increased, but the increase restored this behavior towards values seen at the start of the experiments. That is, this treatment seemed to reduce the effects of habituation.

The differences among males in terms of the numbers of type-II chirps produced during control runs, following saline injection and following arginine vasotocin injection were not correlated with fish size, which ranged from 145 to 218 mm, or resting EOD frequency, which ranged from 789 to 1032 Hz (mean 933.5±20.2 Hz). EOD frequencies were corrected to a standard temperature of 27 °C using a Q_{10} of 1.56 (Engler et al., 2000a). The discharge frequencies of all but one were above 880 Hz and within the range typical for males (Meyer et al., 1987). The lowest-frequency animal was killed and verified to be male. Although no definitive link between individual characteristics and type-I chirp production was established, we did observe that the highest-frequency animal within a population tank was most likely to produce type-I chirps and to show increases following arginine vasotocin injection. This suggests that position in a dominance hierarchy may influence this behavior.

Effects of arginine vasotocin on female chirp probability

Nine females ranging in length from 176 to 190 mm in length with EOD frequencies ranging from 670 to 836 Hz (mean 739.9±16.8 Hz) were studied using the same protocol as in the studies of males. As described above, most females only produced type-II chirps; hence, the following analyses were restricted to this chirp type. The same arginine vasotocin dose (200 μg per animal) was used with females. The mean chirp production of each animal during the six −12 Hz DF stimulation periods following saline injection was compared with the mean chirp production during the nine stimulus periods following

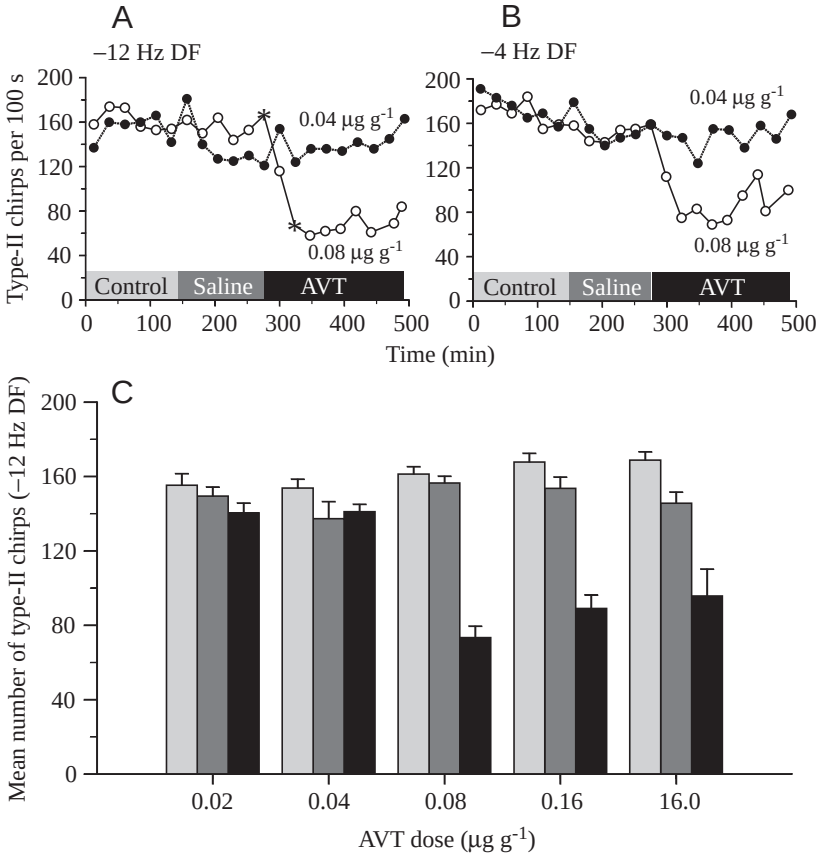

Fig. 7. Effects of arginine vasotocin (AVT) on the production of type-II chirps by a single male. (A) Counts of type-I chirps per stimulus presentation during control conditions, following saline injection and following 0.04 (filled symbols) and 0.08 μg g^{-1} (open symbols) doses of AVT. Asterisks indicate chirp counts from the recordings of Fig. 5A,B. (B) Type-I chirp counts from the −4 Hz difference frequency (DF) stimulus presentations interleaved with the −12 Hz DF stimuli. (C) Histograms summarizing the mean numbers of type-I chirps evoked by −12 Hz DF stimulation during the six control stimuli (light gray columns), during the six stimuli following saline injection (dark gray columns) and during nine stimuli following the indicated AVT doses (black columns). Values are means ± S.E.M.

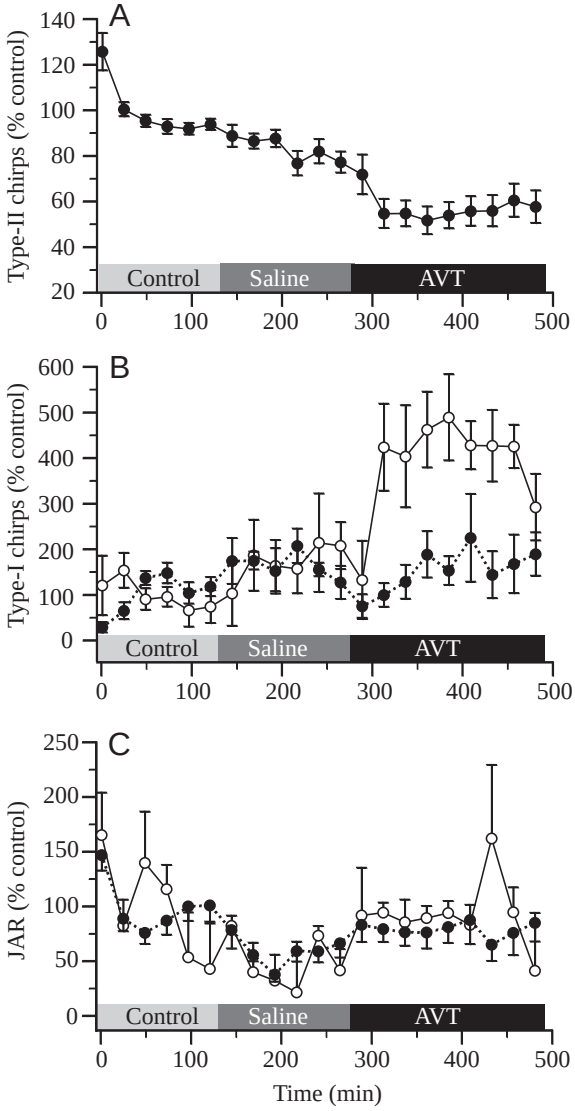

Fig. 8. Summary of the effects of arginine vasotocin (AVT) on male type-I and type-II chirp production in response to −12 Hz difference frequency (DF) stimuli. (A) Mean type-II chirp production during control conditions, following saline injection and following AVT injection. (B) Mean type-I chirp production during control conditions, following saline injection and following AVT injection. Open symbols, mean responses of animals showing significant responses to AVT (mean chirp rate following AVT > mean rate following saline, $P \leq 0.05$, *t*-tests). Filled symbols, mean responses of animals showing non-significant responses to AVT. (C) Mean magnitudes of the jamming avoidance response (JAR) during control conditions, following saline injection and following AVT injection. Open symbols indicate JAR responses of animals showing significant effects of AVT and filled symbols indicate JAR responses of animals showing non-significant effects of AVT injection. Values are means ± S.E.M. (N=12).

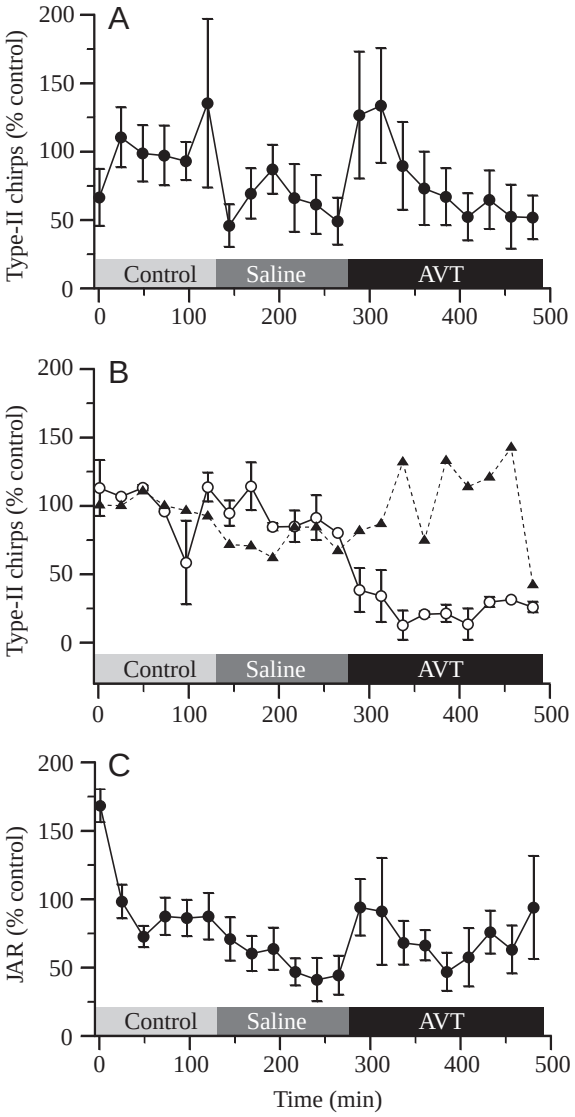

Fig. 9. Summary of the effects of arginine vasotocin (AVT) on female chirp production in response to −12 Hz difference frequency (DF) stimuli. (A) Mean type-II chirp production by six females showing non-significant (mean chirp rate following AVT not different from mean rate following saline, P>0.05, *t*-tests) effects of AVT injection. Light grey bar indicates control phase, dark grey bar indicates saline phase and black bar indicates AVT phase of the experiments. (B) Open circles, mean type-II chirp production of two females showing significant decreases following AVT injection. Filled triangles, responses of a single female showing a significant increase in type-II chirp production following AVT injection. (C) Mean magnitude of the jamming avoidance response (JAR) produced during control conditions, following saline injection and following AVT injection for nine females. Values are means ± S.E.M.

arginine vasotocin injection. Arginine vasotocin caused no significant change in chirp production in six of the nine females, and the mean numbers of chirps produced during each of the stimulus periods, expressed as a percentage of the mean control response, are plotted in Fig. 9A. Although more variable than in males, female type-II chirp production also decreased during the control and saline phases of the experiment, and this probably reflects habituation to the stimulus. A peak in chirp counts immediately followed the arginine vasotocin injection, but this increase was not sustained and analysis of the pooled data from

these six animals also showed no significant difference between chirp production following arginine vasotocin *versus* saline injection.

Two of the remaining three females showed significant decreases in chirp production following arginine vasotocin injection (Fig. 9B, open circles) and one female showed a significant increase (Fig. 9B, filled triangles). The decrease in female chirp production is similar to that seen for male type-II chirps (Fig. 8A). The increase in chirping shown by a single female following arginine vasotocin injection is essentially a return to the initial or control level of responsiveness.

Fig. 9C summarizes the effects of saline and arginine vasotocin injection on the magnitude of the JAR evoked by the −12 Hz DF stimulus. As in the case of males, arginine vasotocin, but not saline, resulted in small increases in the JAR approximately reversing the decrease due to habituation.

Neither EOD frequency nor fish length within this sample was correlated with chirp production or the presence of a significant arginine vasotocin response. However, there may be a threshold size below which females do not chirp, since a large population of fish was screened and animals smaller than those studied here typically did not chirp at all.

Discussion

Studies of chirps produced by *Apteronotus leptorhynchus* during agonistic encounters, courtship and spawning (Hagedorn and Heiligenberg, 1985; Hagedorn, 1986), of spontaneously occurring chirps (Engler et al., 2000a) and of those evoked by electrosensory stimuli designed to mimic the discharges of conspecifics indicate that these animals produce at least two types of chirp (Zupanc and Maler, 1993; Engler et al., 2000b). The results presented here confirm the recent observations of Engler and Zupanc (Engler and Zupanc, 2000) and show that, particularly in males, two qualitatively different chirps are produced. The more commonly produced type-II chirps are thought to be agonistic signals, while the type-I chirps may function as intersexual signals such as male advertisement. As has also been noted previously, females chirp less readily than males (Dye, 1987; Zupanc and Maler, 1993; Dulka and Maler, 1994), and the typical female chirp is similar to the male type-II chirp.

The EOD frequency of *Apteronotus leptorhynchus* is sexually dimorphic; female discharges typically have fundamental frequencies ranging from 600 to 800 Hz, while discharges of males usually fall within the range 800 to 1000 Hz (Meyer et al., 1987). Dye (Dye, 1987) found that the number of chirps evoked under experimental conditions was a function of the frequency difference (DF) between the EOD mimic used as a stimulus and the animal's own discharge frequency and that the best absolute DF frequency for evoking chirps was between 6 and 14 Hz (12 Hz DF in this study). As suggested by Dye (Dye, 1987), given the sexual dimorphism of the discharges in this species, difference frequencies as low as 12 Hz are unlikely to occur when animals of opposite sex come into close proximity, making it unlikely that the type-II chirps function as intersexual signals. Type-II chirps are more likely to be related to intrasexual behavior, such as territoriality and agonistic behavior, since appropriate DFs for evoking these chirps are more likely to occur when animals with similar EOD frequencies encounter one another. The tuning characteristics of type-I chirps, however, are compatible with their role in courtship and mating since DFs ranging from 100 to 200 Hz are most likely to occur as a result of the interactions between male and female EODs.

Sensory-motor integration and chirp production

The summation of an individual's EOD with that of a conspecific, or with a sinusoidal mimic as used in these studies, results in a beat waveform consisting of a continuous pattern of amplitude and phase modulations repeated at the difference frequency or DF. Two categories of tuberous electroreceptor afferent, the P-receptor (probability coder) and T-receptor (tonic receptor) afferents, encode the amplitude and timing, respectively, of the EOD (Scheich et al., 1973). A third category of electroreceptor, ampullary receptors, is specialized to respond to low-frequency electric signals (direct current to approximately 50 Hz), and these receptors are known to respond to the chirps or EOD interruptions produced by the related fish *Eigenmannia virescens* (Metzner and Heiligenberg, 1991; Metzner and Heiligenberg, 1993). Higher electrosensory centers evaluate the information provided by the tuberous afferents, enabling the animals to determine both the frequency and the sign of the DF; this information is used to initiate and control the well-studied jamming avoidance response (for a review, see Heiligenberg, 1991). Chirps occur spontaneously (Engler et al., 2000a), so a second EOD or its mimic is not strictly necessary for the production of this behavior, but the presence of beats greatly increases chirping. Although the beat frequencies or DFs that maximally evoke the JAR and chirps are different, the same populations of electroreceptor afferents encode these stimuli. However, separate regions within the electrosensory lateral line lobe (ELL), the recipient of the receptor afferent projection, contribute to the control of these types of behavior (Metzner and Juranek, 1997; Metzner, 1999).

Tuberous receptor afferents branch within the ELL, resulting in three somatotopic projections terminating within the lateral, centrolateral and centromedial ELL subdivisions (Heiligenberg and Dye, 1982), and the frequency–response characteristics of ELL pyramidal cells, the principal efferent neurons of these subdivisions, differ. Centromedial pyramidal cells are most responsive to low-frequency amplitude modulations that are effective in evoking the JAR, while those within the lateral map are most sensitive to higher amplitude-modulation rates such as those that evoke chirps (Shumway, 1989). The separate roles of these ELL subdivisions in providing the information necessary for the JAR and chirp production were clearly demonstrated by Metzner and Juranek (Metzner and Juranek, 1997), who showed that bilateral lesions of the lateral ELL subdivision exclusively abolished chirping and that bilateral lesions of the centromedial ELL subdivision exclusively abolished the JAR. Type-I and type-II chirps seem

to be distinct types of behavior; they are characterized by non-overlapping peak-to-peak frequency changes, durations and changes in EOD amplitude. In addition, these behavior patterns are tuned to quite different DF values and, at least in males, arginine vasotocin injection alters the animals' propensity to produce these behaviors in opposite directions. These results suggest the presence of further specializations within the electrosensory processing regions that enable the animals to distinguish the high beat rates of 50–200 Hz, which primarily evoke type-I chirps, from the lower rates that evoke type-II chirps. Subsets of ELL pyramidal cells, particularly those of the lateral segment (Shumway, 1989), are responsive to higher amplitude-modulated frequencies, but it is not known whether these ELL cells can be further subdivided into groups specialized to process the different amplitude modulations that preferentially evoke these chirp types. Additional studies of the initial stages of electrosensory processing focusing on much higher beat rates are needed.

The ELL efferents project to the n. praeminentialis dorsalis (nPd) and the torus semicircularis, and the frequency–response characteristics of cells within both of these structures have also been studied. Neurons within the torus are clearly specialized to respond preferentially to beats of different frequencies. Toral neurons of the related fish *Eigenmannia virescens* can be separated into three categories, low-pass, band-pass or high-pass, on the basis of their responsiveness to beats of different frequencies, and the morphological and biophysical properties of neurons within these categories are well understood (Rose and Call, 1992; Rose and Call, 1993; Rose et al., 1994; Fortune and Rose, 1997; for a review, see Rose and Fortune, 1999). The low-pass cells, which respond preferentially to beat rates of approximately 10 Hz, are not only well suited for processing information important for the JAR but are also candidates for selectively contributing to the production of type-II chirps. High-pass neurons, which often show no response attenuation to beat rates in excess of 30 Hz, are candidates for contributing to type-I chirp production. However, the responses of these neurons to beat rates in excess of approximately 50 Hz have yet to be studied.

The toral efferents project to the diencephalic n. electrosensorius (nE) and to the optic tectum (Carr and Maler, 1986; Keller et al., 1990), the former structure providing the link between electrosensory processing and the motor output circuitry that controls the electric organ (Bastian and Yuthas, 1984; Heiligenberg et al., 1991). Extensive studies of the anatomy and physiology of the nE in *Apteronotus leptorhynchus* and in the closely related fish *Eigenmannia virescens* have identified subdivisions responsible for increasing (the nE$\uparrow$) and decreasing (the nE$\downarrow$) the EOD frequency. Important differences exist in the control of the EOD frequency of *Apteronotus leptorhynchus* and *Eigenmannia virescens* (Heiligenberg et al., 1996), but in both species, cells of the nE$\uparrow$ are linked to the production of chirps. Cells within this region and another nE subdivision, the nE_{beat}, are driven by beat patterns that evoke chirping, but it is not yet known whether categories of nE cell exist that have frequency–response characteristics correlated with the tuning of type-I and type-II chirps to different DF values.

Subdivisions of the nE project to the prepacemaker (PPn) and the sublemniscal prepacemaker (SPPn) nuclei, and efferents from these regions modulate the firing frequency of cells in the pacemaker nucleus, thereby controlling the electric organ discharge frequency. The production of two types of chirps, similar to the type-I and type-II chirps described herein and by Engler et al. (Engler et al., 2000a), can be evoked by stimulation of a subdivision of the PPn, the PPn-chirp or PPnC. Single spikes in individual PPnC neurons, evoked by intracellular current injection, resulted in small EOD accelerations 5–10 ms in duration that resembled type-II chirps, while ionophoresis of glutamate, which activated larger populations of neurons, evoked longer-duration chirps with larger EOD accelerations that were followed by a reduction in frequency reminiscent of the type-I chirps (Kawasaki et al., 1988). These effects of single *versus* multicellular stimulation of PPnC neurons led to the hypothesis that smaller chirps (type-II) are evoked when individual or small populations of PPnC cells are active, but if larger populations of these cells are activated then type-I chirps are produced. The categorical differences between type-I and type-II chirps, together with the absence of intermediate forms, suggests that the transition from small to large numbers of active PPnC neurons is highly nonlinear and, as proposed by Engler et al. (Engler et al., 2000a), dendritic specializations of PPnC neurons, including electrotonic coupling among dendrites (Zupanc, 1991), may be correlates of the switch that selects type-I *versus* type-II chirp production.

The observations that chirps can be evoked in neurophysiological preparations and that type-I *versus* type-II chirps can be selectively evoked by stimulation with appropriate DF values should facilitate further studies aimed at defining the sensory mechanisms enabling the selection of different chirp types as well as testing hypotheses regarding the control of these patterns of behavior by PPnC.

Neuroendocrine modulation of chirping

Sex steroids (Dulka and Maler, 1994; Dunlap et al., 1997; Dunlap et al., 1998; Dunlap and Zakon, 1998; Zakon and Dunlap, 1999), the monoamines norepinephrine, dopamine and serotonin (Maler and Ellis, 1987) and at least one peptide, substance P (Weld et al., 1991), can modulate chirping behavior in *Apteronotus leptorhynchus.* It is also known that the distribution of substance-P-like immunoreactive neurons is sexually dimorphic and modifiable by androgens (Weld and Maler, 1992; Dulka et al., 1995; Dulka and Ebling, 1999). The distributions of several other substances potentially capable of modulating this behavior, including somatostatin (Sas and Maler, 1991; Zupanc et al., 1994), galanin (Yamamota et al., 1992) and Met-enkephalin (Richards and Maler, 1996), have also been described. A detailed description of the distribution of arginine vasotocin within the brain of *Apteronotus leptorhynchus* is not yet available, but its presence in the anterior hypothalamus and in preoptic areas has been documented (Johnston and Maler, 1992). In addition, recent

studies of *Eigenmannia virescens* have shown that electrical stimulation of the preoptic area evokes chirp-like behavior in this fish (Wong, 2000). Unlike stimulation of either the nE or the PPnC, preoptic stimulation evokes chirp-like responses after a very long latency, perhaps indicating indirect influences of preoptic activity on the PPnC neurons. Preliminary experiments with *Apteronotus leptorhynchus* show that electrical stimulation of the preoptic areas also evokes chirping behavior and, interestingly, only type-I chirps have been evoked by this technique. Anatomical results confirm synaptic connections reciprocally linking the preoptic to the prepacemaker regions. The prepacemaker region projects directly to the preoptic area, but the reciprocal connection is probably indirect *via* the preglomerular nucleus (Wong, 1997; Zupanc and Horschke, 1997). Additional studies aimed at determining the distribution of arginine-vasotocin-containing neurons within the brain of *Apteronotus leptorhynchus* are needed. It will be particularly interesting to determine whether differences in the distributions of arginine-vasotocin-containing neurons can be correlated with the sex and reproductive status of individuals, as has been demonstrated in other species (Boyd et al., 1992; Foran and Bass, 1998; Marler et al., 1999; Godwin et al., 2000; Moore et al., 2000).

Arginine vasotocin and its mammalian counterpart arginine vasopressin have been shown to modulate a variety of behaviors, including those linked to communication and reproduction, in many species (for reviews, see Moore, 1992; Moore and Lowry, 1998). In the present study, arginine vasotocin was administered intraperitoneally rather than directly into brain ventricles or into specific nuclei. This method avoids potentially confounding effects of anesthesia and stress resulting from surgery and also enables individual animals to be tested multiple times. However, intraperitoneal injection of arginine vasotocin is expected to have wide-ranging physiological consequences including changes in the blood-vascular system and the renal system as well as possible multiple central nervous system effects. Hence, the changes in chirp behavior observed could be due to indirect effects of arginine vasotocin. To assess whether this substance is directly involved in the control of chirping requires additional studies in which chirping is initiated while arginine vasotocin is applied locally to specific regions of the central nervous system such as the n. electrosensorius, prepacemaker nuclei and preoptic regions.

The effects of arginine vasotocin on chirping behavior show general similarities to the effects of intraperitoneal arginine vasotocin injections on vocal behavior in several anuran species. For example, in gray treefrogs *Hyla versicolor* and other species, arginine vasotocin has been shown to increase the production of advertisement calls, to reduce the rate of release calling in males and also to reduce release call production in some females (Tito et al., 1999; Moore, 1992). These changes are interpreted as indicating increased sexual receptivity following arginine vasotocin injection. Similarly, the arginine-vasotocin-induced decreases in the production of type-II chirps and increases in the production of type-I chirps may indicate decreased agonistic and increased reproductive tendencies in males. The absence of clear effects of arginine vasotocin in females may reflect differences in the reproductive status of individuals tested or that a related peptide, such as isotocin, is involved in controlling chirping in females. Studies of peptide modulation of fictive vocalizations in the midshipman fish *Porichthys notatus* have shown that arginine vasotocin applied directly to the preoptic areas modulates the characteristics of vocalizations associated with courtship activity in parental, mate-calling males but not in females or sneak-spawning males. Isotocin, however, modulated vocalizations normally not associated with reproductive behavior in females and sneak-spawning males (Goodson and Bass, 2000b).

This research was supported by NIH grant NS12337.

References

Bastian, J. (1974). Electrosensory input to the corpus cerebelli of the high frequency electric fish *Eigenmannia virescens*. *J. Comp. Physiol.* **90**, 1–24.

Bastian, J. and Yuthas, J. (1984). The jamming avoidance response of *Eigenmannia*: properties of a diencephalic link between sensory processing and motor output. *J. Comp. Physiol.* A **154**, 895–908.

Boyd, S. K., Tyler, C. J. and De Vries, G. J. (1992). Sexual dimorphism in the vasotocin system of the bullfrog (*Rana catesbeiana*). *J. Comp. Neurol.* **325**, 313–325.

Bullock, T. H. (1969). Species differences in effect of electroreceptor input on electric organ pacemakers and other aspects of behavior in electric fish. *Brain Behav. Evol.* **2**, 85–118.

Bullock, T. H., Hamstra, R. A. and Scheich, H. (1972). The jamming avoidance response of high-frequency electric fish. *J. Comp. Physiol.* **77**, 1–48.

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

Carr, C. E. and Maler, L. (1986). Electroreception in gymnotiform fish: Central anatomy and physiology. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 319–373. New York: Wiley.

Dulka, J. G. and Ebling, S. L. (1999). Testosterone increases the number of substance P-like immunoreactive neurons in a specific sub-division of the lateral hypothalamus of the weakly electric brown ghost knifefish, *Apteronotus leptorhynchus*. *Brain Res.* **826**, 1–9.

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

Dulka, J. G., Maler, L. and Ellis, W. (1995). Androgen-induced changes in electrocommunicatory behavior are correlated with changes in substance P-like immunoreactivity in the brain of the electric fish, *Apteronotus leptorhynchus*. *J. Neurosci.* **15**, 1879–1890.

Dunlap, K. D., McAnelly, M. L. and Zakon, H. H. (1997). Estrogen modifies an electrocommunication signal by altering the electrocyte sodium current in an electric fish, *Sternopygus*. *J. Neurosci.* **17**, 2869–2875.

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

Dye, J. (1987). Dynamics and stimulus-dependence of pacemaker control during behavioral modulations in the weakly electric fish, *Apteronotus*. *J. Comp. Physiol.* A **163**, 175–185.

Engler, G., Fogarty, C. M., Banks, J. R. and Zupanc, G. K. H. (2000a). Spontaneous modulations of the electric organ discharge in the weakly electric fish, *Apteronotus leptorhynchus*: a biophysical and behavioral analysis. *J. Comp. Physiol.* A **186**, 645–660.

Engler, G., Fogarty, C. M., Banks, J. R. and Zupanc, G. K. H. (2000b). Spontaneous modulations of the electric organ discharge in the weakly electric fish, *Apteronotus leptorhynchus*: a biophysical and behavioral analysis. *Soc. Neurosci. Abstr.* **26**, 1520.

Ferrari, M. B., McAnelly, M. L. and Zakon, H. H. (1995). Individual

variation and androgen modulation of the sodium current in electric organ. *J. Neurosci.* **15**, 4023–4032.

Foran, C. M. and Bass, A. H. (1998). Preoptic AVT immunoreactive neurons of a teleost fish with alternative reproductive tactics. *Gen. Comp. Endocr.* **111**, 271–282.

Fortune, E. S. and Rose, G. J. (1997). Passive and active membrane properties contribute to the temporal filtering properties of midbrain neurons *in vivo*. *J. Neurosci.* **17**, 3815–3825.

Godwin, J., Sawby, R., Warner, R. R., Crews, D. and Grober, M. S. (2000). Hypothalamic arginine vasotocin mRNA abundance variation across sexes and with sex change in a coral reef fish. *Brain Behav. Evol.* **55**, 77–84.

Goodson, J. L. and Bass, A. H. (2000a). Vasotocin innervation and modulation of vocal–acoustic circuitry in the teleost *Porichthys notatus*. *J. Comp. Neurol.* **422**, 363–379.

Goodson, J. L. and Bass, A. H. (2000b). Forebrain peptides modulate sexually polymorphic vocal circuitry. *Nature* **403**, 769–772.

Hagedorn, M. (1986). The ecology, courtship and mating of gymnotiform electric fish. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 497–525. New York: Wiley.

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

Heiligenberg, W. (1973). Electrolocation of objects in the electric fish *Eigenmannia* (Rhamphichthyidae, Gymnotioidei). *J. Comp. Physiol.* **87**, 137–164.

Heiligenberg, W. (1977). Principles of electrolocation and jamming avoidance in electric fish. In *Studies of Brain Function*, vol. 1 (ed. V. Braitenberg), pp. 1–85. New York: Springer Verlag.

Heiligenberg, W. (1986). Jamming avoidance responses. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 613–649. New York: Wiley.

Heiligenberg, W. (1991). *Neural Nets in Electric Fish*. Cambridge, MA: MIT Press.

Heiligenberg, W. and Dye, J. (1982). Labeling of electroreceptive afferents in a gymnotoid fish by intracellular injection of horseradish peroxidase: the mystery of multiple maps. *J. Comp. Physiol.* **148**, 287–296.

Heiligenberg, W., Keller, C. H., Metzner, W. and Kawasaki, M. (1991). Structure and function of neurons in the complex of the nucleus electrosensorius of the gymnotiform fish *Eigenmannia*: Detection and processing of electric signals in social communication. *J. Comp. Physiol.* A **169**, 151–164.

Heiligenberg, W., Metzner, W., Wong, C. H. and Keller, C. H. (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.

Hopkins, C. D. (1972). Sex differences in electric signaling in an electric fish. *Science* **176**, 1035–1037.

Hopkins, C. D. (1974a). Electric communication in fish. *Am. Sci.* **62**, 426–437.

Hopkins, C. D. (1974b). Electric communication in the reproductive behavior of *Sternopygus marcus* (Gymnotoidei). *Z. Tierpsychol.* **35**, 518–535.

Hopkins, C. D. (1974c). Electric communication: Functions in the social behavior of *Eigenmannia virescens*. *Behaviour* **50**, 270–305.

Hopkins, C. D. (1988). Neuroethology of electric communication. *Annu. Rev. Neurosci.* **11**, 497–535.

Johnston, S. A. and Maler, L. (1992). Anatomical organization of the hypophysiotrophic systems in the electric fish, *Apteronotus leptorhynchus*. *J. Comp. Neurol.* **317**, 421–437.

Kawasaki, M., Maler, L., Rose, G. J. and Heiligenberg, W. (1988). Anatomical and functional organization of the prepacemaker nucleus in gymnotiform electric fish: the accommodation of two behaviors in one nucleus. *J. Comp. Neurol.* **276**, 113–131.

Keller, C. H., Maler, L. and Heiligenberg, W. (1990). Structural and functional organization of a diencephalic sensory–motor interface in the gymnotiform fish *Eigenmannia*. *J. Comp. Neurol.* **293**, 347–376.

Kramer, B. (1999). Waveform discrimination, phase sensitivity and jamming avoidance in a wave-type electric fish. *J. Exp. Biol.* **202**, 1387–1398.

Larimer, J. L. and MacDonald, J. A. (1968). Sensory feedback from electroreceptors to electromotor pacemaker centers in gymnotids. *Am. J. Physiol.* **214**, 1253–1261.

Maler, L. and Ellis, W. G. (1987). Inter-male aggressive signals in weakly electric fish are modulated by monoamines. *Behav. Brain Res.* **25**, 75–81.

Marler, C. A., Boyd, S. K. and Wilczynski, W. (1999). Forebrain arginine vasotocin correlates of alternative mating strategies in cricket frogs. *Horm. Behav.* **36**, 53–61.

Metzner, W. (1999). Neural circuitry for communication and jamming avoidance in gymnotiform electric fish. *J. Exp. Biol.* **202**, 1365–1375.

Metzner, W. and Heiligenberg, W. (1991). The coding of signals in the electric communication of the gymnotiform fish, *Eigenmannia*: From electroreceptors to neurons in the torus semicircularis dorsalis of the midbrain. *J. Comp. Physiol.* A **169**, 135–150.

Metzner, W. and Heiligenberg, W. (1993). The neuronal processing of communicatory signals in *Eigenmannia*. *J. Comp. Physiol.* A **173**, 722–726.

Metzner, W. and Juranek, J. (1997). A sensory brain map for each behavior? *Proc. Natl. Acad. Sci. USA* **94**, 14798–14803.

Meyer, J. H. (1983). Steroid influences upon the discharge frequencies of a weakly electric fish. *J. Comp. Physiol.* A **153**, 29–37.

Meyer, J. H. (1984). Steroid influences upon discharge frequencies of intact and isolated pacemakers of weakly electric fish. *J. Comp. Physiol.* A **154**, 659–668.

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

Mills, A. and Zakon, H. H. (1987). Coordination of the EOD frequency and pulse duration in a weakly electric wave fish: The influence of androgens. *J. Comp. Physiol.* A **161**, 417–430.

Mills, A. and Zakon, H. H. (1991). Chronic androgen treatment increases action potential duration in the electric organ of *Sternopygus. J. Neurosci.* **11**, 2349–2361.

Moore, F. L. (1992). Evolutionary precedents for behavioral actions of oxytocin and vasopressin. *Ann. N.Y. Acad. Sci.* **652**, 156–165.

Moore, F. L. and Lowry, C. A. (1998). Comparative neuroanatomy of vasotocin and vasopressin in amphibians and other vertebrates. *Comp. Biochem. Physiol.* **19**C, 251–260.

Moore, F. L., Richardson, C. and Lowry, C. A. (2000). Sexual dimorphism in numbers of vasotocin-immunoreactive neurons in brain areas associated with reproductive behaviors in the roughskin newt. *Gen. Comp. Endocr.* **117**, 281–298.

Richards, S. and Maler, L. (1996). The distribution of Met-enkephalin like immunoreactivity in the brain of *Apteronotus leptorhynchus*, with emphasis on the electrosensory system. *J. Chem. Neuroanat.* **11**, 173–190.

Rose, G. J. and Call, S. J. (1992). Evidence for the role of dendritic spines in the temporal filtering properties of neurons: The decoding problem. *Proc. Natl. Acad. Sci. USA* **89**, 9662–9665.

Rose, G. J. and Call, S. J. (1993). Temporal filtering properties of midbrain neurons in an electric fish: Implications for the function of dendritic spines. *J. Neurosci.* **13**, 1178–1189.

Rose, G. J., Etter, N. and Adler, T. B. (1994). Responses of electrosensory neurons in the torus semicircularis of *Eigenmannia* to complex beat stimuli: testing hypotheses of temporal filtering. *J. Comp. Physiol.* **175**, 467–474.

Rose, G. J. and Fortune, E. S. (1999). Mechanisms for generating temporal filters in the electrosensory system. *J. Exp. Biol.* **202**, 1281–1289.

Sas, E. and Maler, L. (1991). Somatostatin-like immunoreactivity in the brain of an electric fish (*Apteronotus leptorhynchus*) identified with monoclonal antibodies. *J. Chem. Neuroanat.* **4**, 155–186.

Scheich, H., Bullock, T. H. and Hamstra, R. H. J. (1973). Coding properties of two classes of afferent nerve fibers: high frequency electroreceptors in the electric fish *Eigenmannia*. *J. Neurophysiol.* **36**, 39–60.

Shumway, C. A. (1989). Multiple electrosensory maps in the medulla of weakly electric gymnotiform fish. I. Physiological differences. *J. Neurosci.* **9**, 4388–4399.

Tito, M. B., Hoover, M. A., Mingo, A. M. and Boyd, S. K. (1999). Vasotocin maintains multiple call types in the gray treefrog, *Hyla versicolor*. *Horm. Behav.* **36**, 166–175.

Turner, R. W., Maler, L. and Burrows, M. (1999). (eds) *Electroreception and Electrocommunication. J. Exp. Biol.* **202**, 1167–1458.

Weld, M. M. and Maler, L. (1992). Substance P-like immunoreactivity in the brain of the gymnotiform fish, *Apteronotus leptorhynchus*: presence of sex differences. *J. Chem. Neuroanat.* **5**, 107–129.

Weld, M. M., Maler, L., Quirion, R. and Kas, S. (1991). Sexually dimorphic distribution of substance P and its role in the regulation of communication in an electric fish. *Soc. Neurosci. Abstr.* **17**, 1407.

Wong, C. J. H. (1997). Afferent and efferent connections of the diencephalic prepacemaker nucleus in the weakly electric fish, *Eigenmannia virescens*: Interactions between the electromotor system and the neuroendocrine axis. *J. Comp. Neurol.* **383**, 18–41.

Wong, C. J. H. (2000). Electrical stimulation of the preoptic area in *Eigenmannia*: evoked interruptions in the electric organ discharge. *J. Comp. Physiol.* A **186**, 81–93.

Yamamoto, T., Maler, L. and Nagy, J. I. (1992). Organization of galanin-like immunoreactive neuronal systems in weakly electric fish (*Apteronotus leptorhynchus*). *J. Chem. Neuroanat.* **5**, 19–38.

Zakon, H. H. and Dunlap, K. D. (1999). Sex steroids and communication signals in electric fish: A tail of two species. *Brain Behav. Evol.* **54**, 61–69.

Zakon, H. H., Thomas, P. and Yan, H. (1991). Electric organ discharge frequency and plasma sex steroids levels during gonadal recrudescence in a natural population of the weakly electric fish *Sternopygus macrurus. J. Comp. Physiol.* A **169**, 493–499.

Zupanc, G. K. H. (1991). Clustering of cell bodies, bundling of dendrites and gap junctions: Morphological substrates for electrical coupling in the prepacemaker nucleus. *Neurosci. Lett.* **129**, 29–34.

Zupanc, G. K. H., Ce'cyre, D., Maler, L., Zupanc, M. and Quirion, R. (1994). The distribution of somatostatin binding sites in the brain of gymnotiform fish, *Apteronotus leptorhynchus*. *J. Chem. Neuroanat*. **7**, 49–63.

Zupanc, G. K. H. and Horschke, I. (1997). A distinct population of neurons in the central posterior prepacemaker nucleus project to the nucleus preopticus periventricularis in the weakly electric gymnotiform fish, *Apteronotus leptorhynchus. Brain Res*. **776**, 117–125.

Zupanc, G. K. H. and Maler, L. (1993). Evoked chirping in the weakly electric fish *Apteronotus leptorhynchus*: a quantitative biophysical analysis. *Can. J. Zool*. **71**, 2301–2310.

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