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# The effect of difference frequency on electrocommunication: Chirp production and encoding in a species of weakly electric fish, *Apteronotus leptorhynchus*

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ABSTRACT

The brown ghost knifefish, *Apteronotus leptorhynchus*, is a model wave-type gymnotiform used extensively in neuroethological studies. As all weakly electric fish, they produce an electric field (electric organ discharge, EOD) and can detect electric signals in their environments using electroreceptors. During social interactions, *A. leptorhynchus* produce communication signals by modulating the frequency and amplitude of their EOD. The Type 2 chirp, a transient increase in EOD frequency, is the most common modulation type. We will first present a description of *A. leptorhynchus* chirp production from a behavioural perspective, followed by a discussion of the mechanisms by which chirps are encoded by electroreceptor afferents (P-units). Both the production and encoding of chirps are influenced by the difference in EOD frequency between interacting fish, the so-called beat or difference frequency (Df). Chirps are produced most often when the Df is small, whereas attacks are more common when Dfs are large. Correlation analysis has shown that chirp production induces an echo response in interacting conspecifics and that chirps are produced when attack rates are low. Here we show that both of these relationships are strongest when Dfs are large. Electrophysiological recordings from electroreceptor afferents (P-units) have suggested that small, Type 2 chirps are encoded by increases in electroreceptor synchrony at low Dfs only. How Type 2 chirps are encoded at higher Dfs, where the signals seem to exert the greatest behavioural influence, was unknown. Here, we provide evidence that at higher Dfs, chirps could be encoded by a desynchronization of the P-unit population activity.



## 1. Introduction

*Apteronotus leptorhynchus* are a species of weakly electric fish native to South America, and like all species of weakly electric fish they can both produce and detect electric signals. *A. leptorhynchus* emit a continuous wave-type electric organ discharge (EOD) which they use in navigation, prey localization (MacIver et al., 2001; Moller, 1995) and in communication, specifically electrocommunication (Hagedorn and Heiligenberg, 1985). They detect these signals with specialized electroreceptors distributed over the skin. These receptors, in turn, excite sensory afferents that encode the signals and transmit them to the brain.

An animal's perception of the world is limited by the types of information that can be encoded by its sensory receptors and sensory systems. Just as our eyes and ears are responsive to a narrow range of frequencies within the visible and audible range, in weakly electric fish, electroreceptors are tuned to specific stimulus features (Benda et al., 2006; Chacron et al., 2005; Keller et al., 1986; Hopkins, 1976). This limited ability to encode sensory stimuli shapes the detection and perception of conspecific electrocommunication signals and hence is likely to play a central role in shaping behaviour during social interactions.

The EOD frequency is sexually dimorphic in *A. leptorhynchus*; female EOD frequencies range from 600–800 Hz, whereas males emit in the range of 800–1100 Hz (Meyer et al., 1987). For each fish, the frequency of discharge is very regular over time (Moortgat et al., 1998); however, stereotyped frequency and amplitude modulations are common in social situations and are believed to serve as communication signals (Zakon et al., 2002; Zupanc, 2002; Hagedorn and Heiligenberg, 1985). Of these modulations, the most commonly studied is the Type 2 or 'small' chirp (Zupanc et al., 2006; Engler and Zupanc, 2001; Engler et al., 2000), defined as a transient (10–20 ms) frequency excursion associated with a small amplitude decrease (Fig. 1).

When two or more fish are in close proximity, their EODs interact and a beat results – a periodic amplitude modulation (AM) of the EOD with a frequency equal to the difference between the EOD frequencies of the interacting fish, the difference frequency, Df (Fig. 2). When one fish chirps, it results in a transient and rapid AM, and imposes a phase shift of the beat cycle (Fig. 2C). AMs are detected by tuberous electroreceptors and electroreceptor

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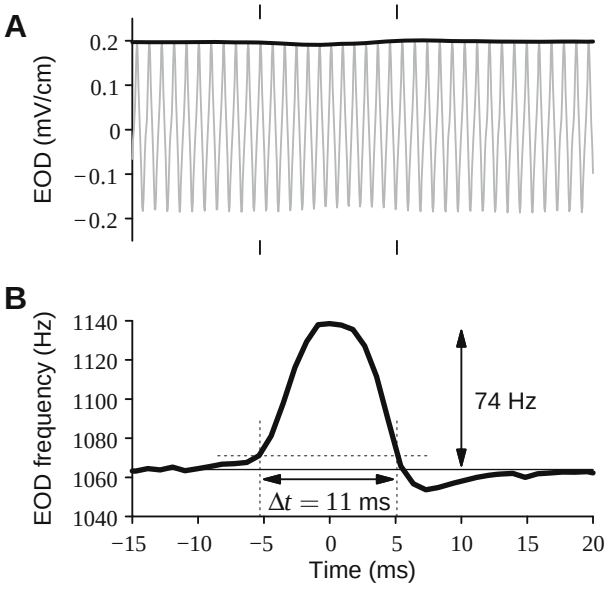


**Fig. 1.** Type 2 chirps: (A) Looking at the EOD waveform of an isolated fish (gray line), a Type 2 chirp (within the tic marks) is only visible as a small decrease in EOD amplitude (black line). (B) Plotting the frequency of the EOD reveals the chirp: a transient ($\Delta t$ = 11 ms) increase in EOD frequency (74 Hz) followed sometimes by a much smaller decrease in EOD frequency.

afferents (P-units) propagate these signals to an electrosensory structure in the hindbrain called the electrosensory lateral line lobe (ELL). From here, signals are sent to a variety of electrosensory and electromotor processing areas, including an indirect pathway to the diencephalic central posterior/prepacemaker nucleus, CP/PPn (Zupanc and Heiligenberg, 1992). The CP/PPn, in turn, influences the medullary pacemaker nucleus (Pn), an endogenous oscillator whose activity determines the frequency of the EOD (Zupanc, 2002). One particular region of the CP/PPn, the PPn-C, is responsible for generating chirps. Activation of the PPn-C induces a frequency shift in the pacemaker which is propagated through relay cell axons to the electrogenic cells of the electric organ, where it results in a brief modulation of the EOD: a chirp (Kawasaki et al., 1988).

In this paper, we will discuss how Type 2 chirp behaviour and chirp sensory encoding are influenced by the beat frequency, Df. It is well established that chirp production rates of *A. leptorhynchus* are influenced by Df (Hupé and Lewis, 2008; Dunlap and Larkins-Ford, 2003; Dunlap, 2002; Bastian et al., 2001; Zupanc and Maler, 1993), and here, for the first time, we show that this is also true for attack counts, and for the strengths of the relationships between chirping in one fish and chirp and attack behaviours of an interacting conspecific. Secondly, we will discuss how Type 2 chirps are encoded in P-units by an enhanced firing rate response that also synchronizes the electroreceptor population at low Dfs (Benda et al., 2005; 2006). We provide new evidence that at even higher Dfs, Type 2 chirps exert a behavioural influence on conspecifics, and suggest that they may be encoded by a desynchronization of the P-unit population response under these conditions.

## 2. The effects of difference frequency on chirp behaviour

In order to understand a particular behaviour from a neuroethological perspective we must understand it on both neurophysiological and behavioural levels. A great deal of work has revealed many of the mechanisms used to encode electric signals in *A. leptorhynchus* (Benda et al., 2005, 2006; Chacron et al., 2005; Wessel et al., 1996). Similarly, much work has been done on characterizing chirp production patterns (Zupanc et al., 2006; Dunlap and Larkins-Ford, 2003; Engler and Zupanc, 2001; Zupanc and Maler, 1993; Bullock, 1969); however, although chirps were first described almost 40 years ago, we are only now beginning to understand their social significance (Hupé and Lewis, 2008; Triefenbach and Zakon, 2008).

### 2.1. Chirp production in free swimming dyads

In a recent study (Hupé and Lewis, 2008), we examined chirp production by *A. leptorhynchus* during 5 min dyadic interactions in a small test arena. The fish ($n$ = 13) were randomly paired (mean ± sd body length: 12.6 ± 1.7 cm), and further details about

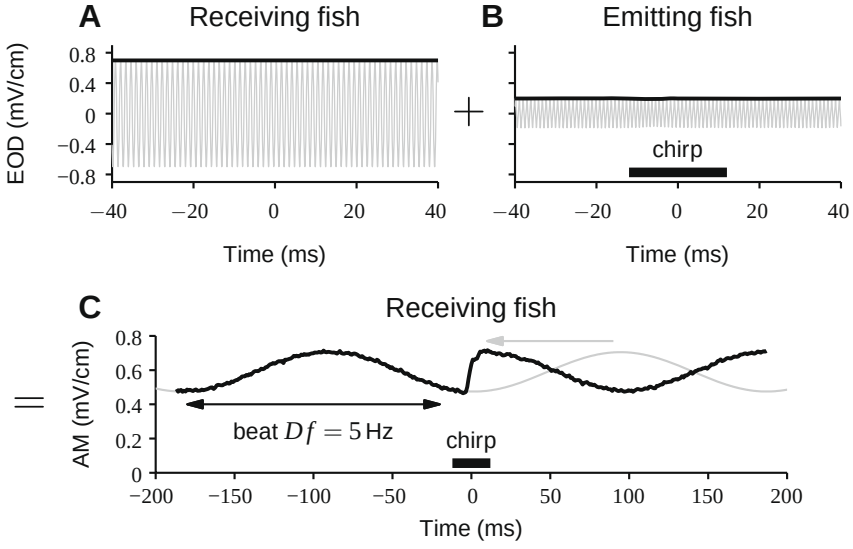


**Fig. 2.** Amplitude modulation evoked by a Type 2 chirp. (A) An individual fish experiences its own EOD (gray line) with constant amplitude (black line). (B) The EOD of a distant fish has a smaller amplitude at the location of the receiving fish. This distant fish emits a Type 2 chirp (thick horizontal bar). (C) The superposition of the two wave forms of the receiving (A) and the emitting fish (B) is the effective electric field that stimulates the electroreceptors of the receiving fish. A slow beat is created (here with frequency Df = 5 Hz) and the chirp (thick horizontal bar) induces an abrupt upstroke of the amplitude modulation (AM). Half a cycle of the beat (gray line) is briefly accelerated (gray arrow) by the chirp.

individual fish and pairings can be found in Hupé and Lewis (2008). Using spectrograms from the electrical recordings for each trial, we noted the times of all chirps produced by each fish. *A. leptorhynchus* produce short and long duration chirps, termed Types 1 and 2 and Types 3–6, respectively (Zupanc et al., 2006; Engler and Zupanc, 2001; Engler et al., 2000). Only short duration (10–20 ms) chirps were observed in this study, and among these, large Type 1 chirps and smaller Type 2 chirps were differentiated based on the size of the frequency excursion associated with each. Type 1 chirps were produced at very low rates. Type 2 chirps were produced at much higher rates and are the predominant chirp type produced by *A. leptorhynchus* under a variety of experimental contexts (Hupé and Lewis, 2008; Smith and Combs, 2008, Zupanc et al., 2006; Engler and Zupanc 2001; Engler et al., 2000). From video recordings of the interactions, we recorded the times of all attacks, defined as any lunge or bite directed at a conspecific. These chirp and attack counts were used to examine how chirp production rates in free swimming fish are influenced by sex, EOD frequency, Df, and the time course of the interaction (Hupé and Lewis, 2008). We also quantified chirp production patterns and the temporal relationship between chirping and attack behaviours using correlation analyses and showed that chirping in one fish is correlated with both chirping and attack behaviours of the interacting conspecific (Hupé and Lewis, 2008). Although both males and females were tested in Hupé and Lewis (2008), here we will only be considering Type 2 chirps produced by males because of the sexual dimorphism in chirp rates; males produce significantly more chirps than females (Hupé and Lewis, 2008; Kolodziejski et al., 2004; Zupanc and Maler, 1993; Maler and Ellis, 1987). In our new analyses, we examine the influence of the difference in the EOD frequencies of the interacting fish (Df) on the temporal relationships between chirping and conspecific behaviours. This is motivated by the strong Df dependence of chirp encoding by electroreceptor afferents (Benda et al., 2005, 2006).

### 2.2. Chirp and attack rates are dependent on the difference frequency, Df

In Hupé and Lewis (2008), we reported that freely interacting *A. leptorhynchus* males produce Type 2 chirps at the highest rates when the difference in EOD frequency between the two interacting fish is small, and that the chirp rates tend to decrease as the absolute Df increases (the relationship between chirp rates and Df persists for both positive and negative Dfs). Fig. 3A shows the Type 2 chirp counts of male fish, summed over an entire trial, plotted against Df, and it is evident that chirp rates are highest when the discharge frequencies of the two fish are similar. This trend persists regardless of whether males are paired with a second male or with a female, thus we have pooled male–male and male–female trials here. The inverse relationship between chirping and Df has been observed in *A. leptorhynchus* tested under a variety of experimental conditions (Dunlap and Larkins-Ford, 2003; Dunlap, 2002; Bastian et al., 2001; Engler and Zupanc, 2001; Zupanc and Maler, 1993). In contrast, however, male attack counts tend to increase as Df increases (Fig. 3B), regardless of whether they are paired with another male or with a female.

### 2.3. Chirping influences chirp and attack behaviours in an interacting conspecific

Communication signals, by definition, transmit information from a sender to a receiver (Bradbury and Vehrencamp, 1998) and our detection of this information transfer is limited to changes in subsequent, observable behaviours. Chirps have long been thought to play a role in communication, but direct evidence for this has been conspicuously absent until recently (Hupé and Lewis, 2008; Triefenbach and Zakon, 2008; Zupanc et al., 2006).

To quantify patterning in chirp production between interacting fish, we created chirp-centered cross-correlograms, in which we plot counts of one fish's chirps centered at the time of chirp production by the other fish (Hupé and Lewis, 2008). In the same way, to temporally relate one fish's chirp production with the other fish's attack counts, we created chirp-centered cross-correlograms for one fish's attacks centered at the time of chirp production by the other fish. Only male–male pairings were considered in the correlogram analysis because female chirp and attack counts are much lower than male counts, and too low to represent using correlation analysis. For both correlograms presented, significance was assessed by comparing the calculated cross-correlograms to the null distribution (a flat line) expected if all events occur at random (Hupé and Lewis, 2008).

Fig. 4A depicts the averaged cross-correlogram relating chirp production in one fish with that of the other fish ($n = 14$ fish in 7 trials). From the peaks in the correlogram, occurring at 200–600 ms, we can see that a chirp produced by one fish is often followed by a chirp produced by the other fish, with a preferred latency of 200–600 ms (Hupé and Lewis, 2008). Due to averaging over all fish, this correlogram is symmetrical about $t = 0$ s (because

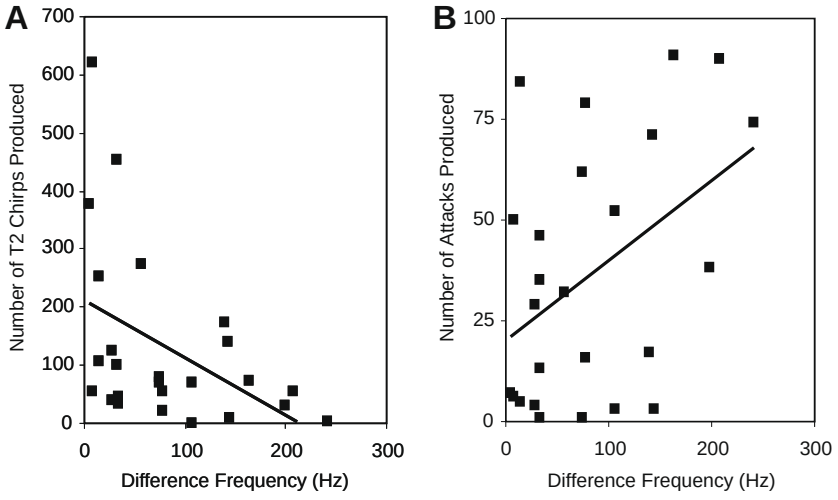


**Fig. 3.** Male Type 2 chirp (A) and attack (B) counts as a function of absolute difference frequency, Df. Chirp rates decrease significantly as Df increases ($n = 25$, F-test, $p = 0.03$); whereas attack counts increase significantly as Df increases ($n = 25$, F-test, $p = 0.03$).

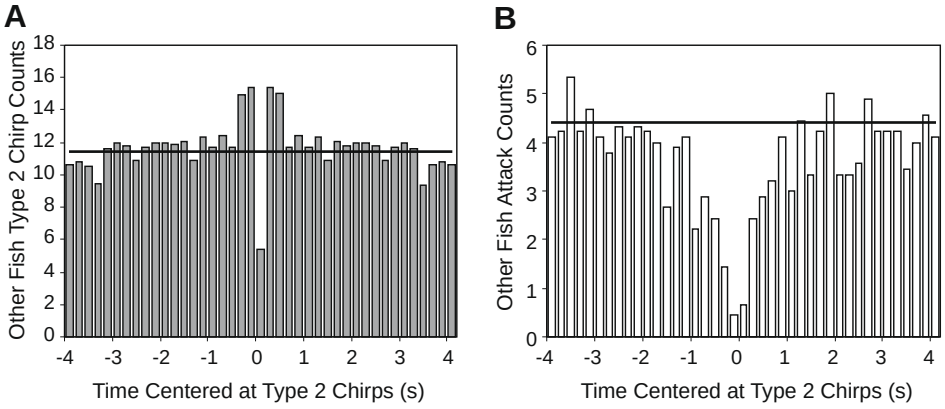


**Fig. 4.** Chirp-centered cross-correlograms temporally relating one fish's chirp production with chirp production (A; averaged over $n = 14$ individuals) and attacks (B; averaged over $n = 9$ individuals) in the other fish. Averages for all male–male trials in which the relevant counts (chirps and attacks) within each bin are greater or equal to 10, are plotted. The thick horizontal line denotes the bin counts expected if chirp production was uncorrelated between the two fish (null hypothesis).

for a given trial, the correlograms for the two interacting fish will be mirror-images of each other); the level of symmetry in individual trials actually varies somewhat. Males tend to echo one another reciprocally, thus chirps often occur in bouts with the two fish alternately producing chirps. Importantly, the magnitude of this 'echo response' does not appear to be based on aggression status (Hupé, unpublished observations). In some trials, the more aggressive fish echoed the less aggressive fish more strongly, whereas in other trials the opposite was true. A similar 'echo response' has been reported in electrically interacting male fish confined to separate PVC tubes, with a similar preferred latency of 500 ms (Zupanc et al., 2006), despite being tested under very different experimental conditions.

Observing chirp production in freely swimming, interacting fish allowed us to relate chirp production with aggressive behaviours directly (Hupé and Lewis, 2008). We reported that the attack rates of both the chirping fish and the fish with which it is interacting (Fig. 4B) are lower than expected at the time of chirp production; a similar trend was recently reported by Triefenbach and Zakon (2008). The attack counts of one fish tend to be less than expected within about 1 s of chirp production in the fish with which it is interacting. The relevance of this negative relationship remains to be determined. Triefenbach and Zakon (2008) suggest that chirps may be used to signal attack motivation and aggression; however, our preliminary results using interactive playbacks suggest that Type 2 chirps may be used to deter aggression (Hupé and Lewis, 2007). The relationships shown in Fig. 4 demonstrate that chirp production in one fish influences signaling and attack behaviours in other fish. Benda and colleagues (2006, 2005) have demonstrated that the encoding of chirps by electroreceptor afferents is dependent on Df, so in the next section we will examine whether the relationships revealed in Hupé and Lewis (2008), namely the magnitude of chirp influence on conspecific chirp and attack behaviour, are also dependent on Df.

### 2.4. Chirp influence on conspecific behaviour is affected by Df

As a novel approach taken in this paper, we test if the magnitude of chirp influence on conspecific behaviour (echo response and attack inhibition, reported in Hupé and Lewis (2008) is affected by Df. In the last section, we saw that chirps produced by one fish are often echoed by the interacting conspecific with a preferred latency of 200–600 ms. In addition to influencing a conspecific's chirp production patterns, we have also seen that chirping is inversely related to conspecific attack counts. We predict that the influence of chirps on conspecific behaviour will vary along with the efficiency with which they are encoded at different Dfs by electroreceptor afferents (Benda et al., 2005, 2006) and hence we ask whether the magnitudes of the 'echo response' and 'attack inhibition' are influenced by Df. To quantify the magnitude of the chirp and attack relationships we calculated the relative difference in the correlogram bin counts within one second of $t = 0$ to their expected counts, for each trial. These values were then plotted against Df (Fig. 5). The 7 male–male trials considered spanned a range of Dfs from 8 to 78 Hz (one male–male trial with a Df of 107 Hz was omitted because one of the fish produced only one chirp during the interaction). For comparison, male–female trials spanned a range of Dfs from 5 to 242 Hz.

Fig. 5A shows that the magnitude of the echo response increases significantly with Df (Regression, $p = 0.035$). As will be discussed in the following section, Benda et al. (2005, 2006) suggest that Type 2 chirps are encoded in electroreceptor afferents by increases in synchrony only when Dfs are small (<30 Hz). However, the results presented here suggest that the magnitude of the echo response is actually greatest in trials with a large Df. Because chirps must be detected to induce an echo, this implies that even at large Dfs, chirps are being effectively encoded. Fig. 5B shows that the

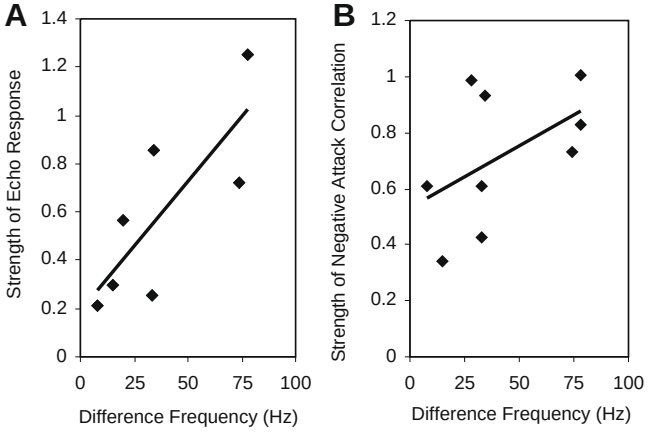


**Fig. 5.** (A) Echo response magnitude ($n = 7$ trials). (B) Attack inhibition ($n = 9$ individuals) as a function of Df. These values correspond to the normalized difference in the observed counts within +/− 1 s of center from the values expected based on the null distribution (=abs(observed − expected)/expected), calculated on a fish by fish basis for all fish considered in the correlograms of Fig. 4, and averaged across the bins considered here. The strength of both relationships tend to increase with increases in Df (Regression, $p < 0.001$ and $p = 0.16$ for Type 2 chirps and attacks, respectively).

strength of the relationship between chirping and low conspecific attack counts also tends to increase as Df increases, although the increase is not significant (Regression, $p = 0.16$). Even at large Dfs, chirps are associated with lower than expected attack rates. Both the chirp and attack results presented here suggest that Type 2 chirps are detectable even when produced at Dfs greater than the threshold for detectability suggested by Benda et al. (2005, 2006). In the next sections, we will review how chirps are encoded by electroreceptor afferents (P-units) at low Dfs and present a novel mechanism by which Type 2 (small) chirps are encoded at higher Dfs.

## 3. P-unit encoding of the EOD and Type 2 chirps

The amplitude and phase modulations of the EOD that result during the interaction of two fish are encoded by three different types of electrosensory receptors in gymnotid fish. Only information that is contained in the activity patterns of these receptor neurons can be further processed by higher brain areas. The ampullary receptors of the passive electrosensory system are sensitive to slow changes of the electric field and thus likely do not play a role in encoding chirps in *A. leptorhynchus*. A closely related species (*Eigenmannia*), however, produces chirps with a DC offset that has been shown to be encoded by ampullary receptors and higher brain areas (Metzner and Heiligenberg, 1991; Heiligenberg et al., 1991). The tuberous receptor afferents of the active electrosensory system can be categorized into two groups: T-units and P-units. T-units fire a spike at each cycle of the EOD and thus can measure the phase of the EOD (Heiligenberg and Partridge, 1981). P-units fire with a lower rate than T-units and encode EOD amplitude (Bastian, 1981; Scheich et al., 1973). In the following discussion we focus on P-units, the predominant type of tuberous electroreceptor in *A. leptorhynchus*.

Based on in vivo single unit recordings from P-type afferents (P-units), Benda et al. (2005) concluded that Type 2 chirps evoke transiently enhanced firing rate responses, if the chirps occur on Dfs less than about 23 Hz. At Df = 60 Hz the firing rate response to a Type 2 chirp was on average indistinguishable from the response to the beat. Higher Dfs were not used in this study. The study by Benda et al. (2006) focuses on Type 1 chirps, but also shows the summed population activity that has been recorded with silver wire hook electrodes from the posterior branch of the anterior lateral line nerve in response to Type 2 chirps, using Dfs up to 100 Hz. The results confirmed that Type 2 chirps evoke an enhanced response only at Dfs below about 23 Hz. These findings are in strong discrepancy to the behavioural data introduced above where we demonstrated that Type 2 chirps have a significant behavioural effect at Dfs much greater than this. In the following sections we first repeat our findings for low Dfs and then present a novel analysis of the population activity data at high Dfs revealing a possible code for Type 2 chirps in this context.

### 3.1. Type 2 chirps synchronize P-unit population at low beat frequencies

The response of P-units to Type 2 chirps strongly depends on the beat frequency (Df). An example is shown in Fig. 6. At low beat frequencies (about Df < 30 Hz, Fig. 6A left column) the firing

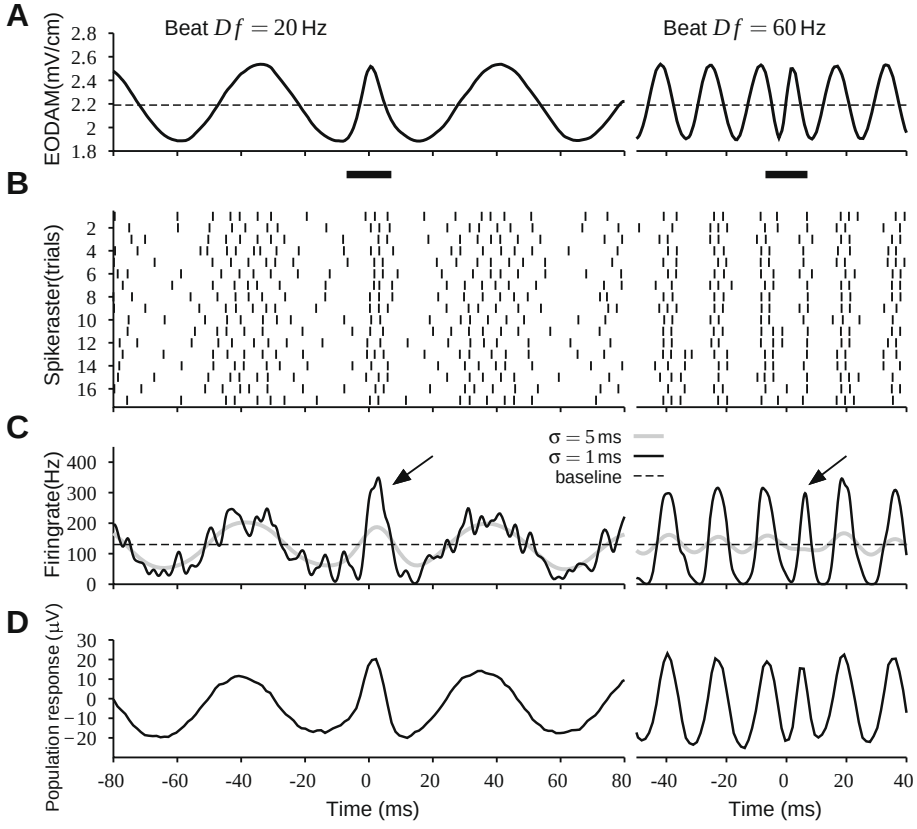


**Fig. 6.** Response of P-units to small, Type 2 chirps. (A) The stimulus is a beat AM with frequency Df = 20 Hz (left column) and Df = 60 Hz (right column) and Type 2 chirps of size 60 Hz at $t = 0$ (thick horizontal bars). The dashed line marks the baseline EOD amplitude. (B) Spike raster obtained from a single unit recording. (C) Firing rate computed from the spike trains in B using Gaussian kernels with standard deviation 1 ms (black line) and 5 ms (gray line). The transiently increased firing rate during the chirp on the 20 Hz beat (left column) increases synchrony (arrow). At higher beat frequencies (60 Hz, right column) the response to the chirp is slightly weaker than to the beat (arrow). The dashed line is the baseline firing rate. (D) The population response resembles the firing rates obtained from single unit recordings using the 1 ms kernels. Shown are recordings from the trunk electroreceptor nerve of another fish in response to similar stimuli.

rate of a single neuron (Fig. 6B and C) as well as the summed activity of the whole population of P-units (Fig. 6D) is modulated by the beat AM. Individual spikes between trials (Fig. 6B) but also between different units (not shown, see Benda et al., 2006) are not well synchronized. A Type 2 chirp, however, induces a strong, transient increase in firing rate, since the resulting AM during a chirp is faster than the adaptation processes known in P-units (Benda et al., 2005). This increase in firing rate also transiently synchronizes the P-units, as can be seen in the spike raster (Fig. 6B) as well as in the population response (Fig. 6D, Benda et al., 2006). The population activity is the voltage resulting from the summed activity of all P-unit fibers in the lateral line nerve that are picked up by the hook electrode. This population response will increase when more spikes in the population of P-units are synchronized.

### 3.2. At high beat frequencies larger Type 2 chirps desynchronize the population

At higher beat frequencies (Df > 30 Hz, Fig. 6 right column), on the other hand, the P-units phase lock to the beat and the population becomes synchronized. The AM induced by the chirp is still faster than the beat, but it fails to induce a clearly visible response. At a first glance this seems to be surprising, since at such a Df the fish clearly showed a behavioural response to Type 2 chirps (Fig. 5). However, careful inspection of the spike raster (Fig. 6B) suggests that the firing response might actually be reduced by the chirp at these higher Dfs.

In Fig. 7 we compare the population response to a moderately fast beat (Df = 60 Hz as in Fig. 6 right column) and four chirps that differ in their size (i.e. in the maximum elevation of the EOD frequency) and thus in the resulting phase shift of the AM. This example indicates that larger Type 2 chirps reduce the P-unit population response more drastically than smaller chirps (compare Fig. 7C and D with Fig. 7A and B) and thus desynchronize the population. This suggests that the P-unit population can indeed encode Type 2 chirps in trials involving relatively large Dfs.

### 3.3. Dependence of synchrony on stimulus frequency explains chirp response patterns

How can it be that one and the same chirp in the context of a low beat frequency enhances the P-unit population response, whereas at a high beat frequency reduces it? A possible explanation for these contradictory results is based on the analysis shown in Fig. 8A. There, the degree of synchrony of the P-unit population is quantified as the standard deviation of the population activity for many different beat frequencies ranging from Df = 5 to 300 Hz (averaged over $n = 4$ fish). The data show that the level of synchrony increases for Dfs up to about 60 Hz. For higher Dfs the degree of synchrony is then gradually reduced again, resulting in a unimodal relationship (Benda et al., 2006). A chirp briefly increases the EOD frequency and therefore also increases the beat frequency by exactly this frequency for the short duration of the chirp (if the chirp emitting fish has the higher EOD frequency, as was the case in the electrophysiological recordings). Consequently, a rough estimate of the population response during the chirp would be that it equals the response to a beat with a frequency Df plus the size of the frequency excursion associated with the chirp. At low beat frequencies a chirp therefore synchronizes the P-unit population, since the population response increases with small increases in beat frequency. If, on the other hand, the beat frequency is at 60 Hz or higher, then the population is already synchronized and the chirp causes a desynchronization, since the population response now falls off for small increases in frequency. We have, therefore, two ranges of beat frequencies that are roughly separated by the maximum in the population response shown in Fig. 8A near Df = 60 Hz.

Unfortunately, no electrophysiological data exist for the complementary scenario where the chirp emitting fish has the lower EOD frequency. When the higher frequency of two fish chirps, it causes a transient increase in the Df, or beat frequency; whereas, if the lower frequency of two fish chirps, it causes a transient decrease in the beat frequency. We propose that when the lower frequency fish chirps at Dfs below about 60 Hz (where P-units are

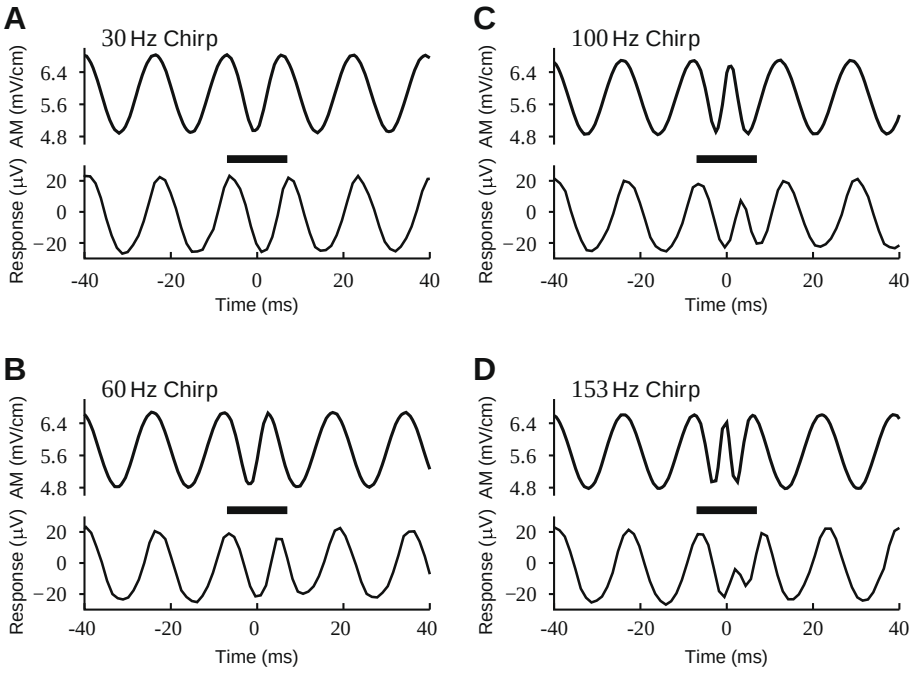


**Fig. 7.** Population response to Type 2 chirps of different sizes. The stimulus is in all four examples a beat with frequency Df = 60 Hz with a chirp of 14 ms duration at time $t = 0$ ms (thick horizontal bars, upper panels). The lower panels show the population response recorded from the trunk lateral line nerve (same fish as in Fig. 6). With increasing size of the chirp (30, 60, 100, and 153 Hz as indicated) the response to the chirp is reduced.

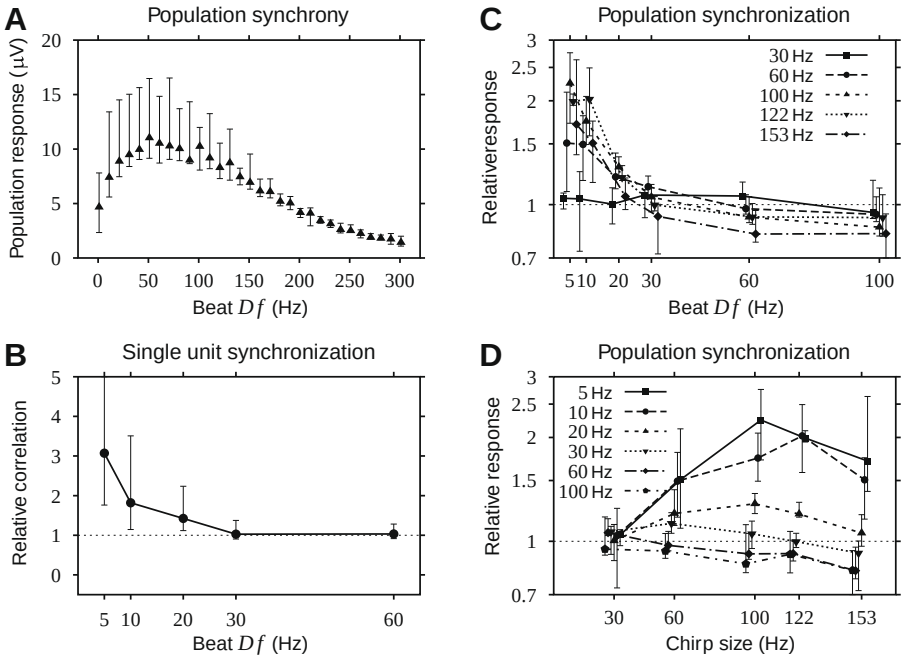


**Fig. 8.** Summary of the P-unit response properties to Type 2 chirps. (A) The standard deviation of the population activity in response to beats with various frequencies Df ranging from 5 to 300 Hz. This measure indicates a high level of synchrony of the P-unit population for beat frequencies between around 60 Hz. (B) Correlation between pairs of spike trains obtained from a single unit recording during a Type 2 chirp relative to the correlation during the beat. A higher relative correlation indicates that the chirps synchronize the P-unit population. This synchronization by the chirps is possible only at beat frequencies below 30 Hz where the population activity is asynchronous as shown in panel A. (C) The relative synchronization response, which is the standard deviation of the population activity measured during the chirp divided by the one during the beat, as a function of Df for different chirp sizes as indicated. Whereas most chirps clearly synchronize the population at Dfs below 30 Hz, they reduce the level of synchrony at higher beat frequencies. (D) Same data as in panel C plotted as a function of chirp size for different beat frequencies as indicated.

maximally synchronized), it will transiently decrease the Df and hence synchrony, following the above arguments. At higher Dfs, however, Type 2 chirps might synchronize the P-unit population since they reduce the beat frequency into a range where the P-units are better synchronized.

Single unit recordings confirm that at low beat frequencies (Df < 30 Hz), the synchrony (the correlation between pairs of spike trains) during the chirp is higher relative to the one during the beat (Fig. 8B, one tailed Wilcoxon test, $p \ll 0.001$, $n = 409$). The expected reversal of the relative correlation at Df = 60 Hz is, however, absent. This could be attributed to the fact that only chirps of size 100 Hz and less (with many 30 Hz chirps) were used in the few experiments where Df = 60 Hz was tested (Benda et al., 2005).

The population activity, on the other hand, shows exactly the expected effect (Fig. 8C and D). At Dfs below 30 Hz the population is clearly more synchronized during the chirp than during the beat (one tailed Wilcoxon test, $p \ll 0.001$, $n = 72$), as in the single unit recordings (Fig. 8B). At higher beat frequencies (Df = 60 Hz) the chirps indeed desynchronize the response (one tailed Wilcoxon test, $p < 0.05$, $n = 65$).

### 3.4. Only larger Type 2 chirps efficiently change population synchrony at high Dfs

This synchronization and desynchronization of the population by the chirps depends strongly on the chirp size (Fig. 8C and D). Very small chirps (30 Hz increase in EOD frequency) fail to synchronize or desynchronize the population (two tailed Wilcoxon test, $p > 0.1$, $n = 8$). Larger chirps easily synchronize the population at beat frequencies below 30 Hz (two tailed Wilcoxon test, $p < 0.01$, $n = 16$). These larger chirps are also able to desynchronize the P-unit population at beat frequencies Df = 60 and 100 Hz (two tailed Wilcoxon test, $p < 0.05$, $n = 57$).

In summary, the reanalysis of the electrophysiological data clearly shows that the P-unit population can respond to Type 2 chirps in high beat frequency contexts, however this response is determined by chirp size.

## 4. Discussion

### 4.1. Comparison of electrophysiological and behavioural data

It is through a combination of behavioural observations and neurophysiological recordings that we can best explore the relationship between sensory encoding ability and social signalling in this species of weakly electric fish. From Fig. 3A, it is evident that fish produce most chirps when Dfs are optimal for chirp encoding through transient increases in P-unit firing rate and thus synchrony (i.e. when Dfs are small), as was shown by Benda et al. (2005, 2006). Through correlation analysis, it is now clear that Type 2 chirps are authentic communication signals that influence both signal production and aggression in interacting conspecifics (Hupé and Lewis, 2008). Here we have shown that the influence of chirps on conspecific behaviour depends on Df (Fig. 5). Interestingly, the magnitude of chirp influence on conspecifics chirping and attack behaviours is largest for Dfs much higher than the aforementioned optimal range of beat frequencies in which chirps can be coded by increases in synchrony in the P-unit population (Fig. 6). Here we provide evidence that the electrosensory system may use a different code for chirps at high Dfs. A reanalysis of the electrophysiological data (Figs. 6–8), suggests that mechanisms other than increases in P-unit synchrony encode Type 2 chirps at large Dfs, namely that Type 2 chirps with large frequency excursions transiently desynchronize the P-unit population activity that is already synchronized by the beat at large Dfs.

Type 1 chirps that are of similar duration as the Type 2 chirps, but increase the EOD frequency much more (by about 500 Hz) and decrease the EOD amplitude more, have also been shown to desynchronize the P-unit population (Benda et al., 2006). Whereas this desynchronization is probably induced by a strong reduction of the amplitude of the AM, the desynchronization due to Type 2 chirps is due to a shift in AM frequency into a range that is less efficient in synchronizing the P-unit population. Note that Type 2 chirps also induce a sudden phase shift in the beat that could be detected by higher order neurons independently of cues like the synchronization or desynchronization.

Revealing the mechanisms by which stereotyped communication signals, like chirps, are extracted from often noisy sensory stimuli is a behaviourally relevant neuroethological problem (e.g. Carlson and Hopkins, 2004). In *A. leptorhynchus*, it is not completely understood how chirps and other electrocommunication signals are extracted from sensory inputs, and how these signals are processed in higher brain centers based on the activity of the electroreceptor afferent neurons. It is clear that chirp detection pathways must exert a strong influence on the chirp production pathway, demonstrated here by the chirp echo response seen in a number of trials. Future experiments should address how this sensory motor pathway is influenced by Dfs. Our data also show that chirping is related to aggression. This is not surprising, given that chirp production, as well as electroreceptor tuning, are both influenced by levels of circulating androgens (Dulka et al., 1995; Dulka and Maler, 1994; Meyer et al., 1987; Keller et al., 1986). There is also evidence that serotonin, which often mediates aggressive behaviours, may mediate both chirp production and encoding in *A. leptorhynchus* (Smith and Combs, 2008; Telgkamp et al., 2007). The physiological basis for the relationship between chirp production and a decreased propensity to attack in interacting conspecifics is not yet understood, and the neural basis for these relationships should be investigated in the future.

## 5. Conclusions

In this study, we unify two areas of research, electrophysiology and behaviour, in an attempt to better understand and better explain how sensory encoding shapes the associated behaviours. We have provided evidence that both the behavioural relationships and electrosensory encoding of chirps in *A. leptorhynchus* are dependent on the difference in EOD frequency between interacting conspecifics. Although the behavioural data presented in this paper suggests that chirps exert an influence on conspecific behaviours at Dfs outside the range in which chirps can be encoded by increases in P-unit synchrony (i.e. at low Dfs), we also show evidence that a desynchronization of the population response encodes chirps outside of the range in which they can be encoded by increases in synchronization (i.e. at high Dfs).

Our findings demonstrate that chirps can indeed be encoded by P-unit afferents for all Dfs where chirps also elicit a behavioural response. Information about chirps is thus in principle available to higher brain areas. How this information is analyzed and why chirps at high Dfs have a stronger impact on behaviour is, however, still unknown. Future investigations of the communication behaviours of weakly electric fish will certainly reveal many more interesting and unexpected insights that will guide electrophysiological work on the computations performed by the electrosensory system.

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