Accepted Manuscript

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Abstract

Weakly-electric fish are a well-established model system for neuroethological studies on communication and aggression. Sensory encoding of their electric communication signals, as well as behavioural responses to these signals, have been investigated in great detail under laboratory conditions. In the wave-type brown ghost knifefish, *Apteronotus leptorhynchus*, transient increases in the frequency of the generated electric field, called chirps, are particularly well-studied, since they can be readily evoked by stimulating a fish with artificial signals mimicking conspecifics. When two fish interact, both their quasi-sinusoidal electric fields (called electric organ discharge, EOD) superimpose, resulting in a beat, an amplitude modulation at the frequency difference between the two EODs. Although chirps themselves are highly stereotyped signals, the shape of the amplitude modulation resulting from a chirp superimposed on a beat background depends on a number of parameters, such as the beat frequency, modulation depth, and beat phase at which the chirp is emitted. Here we review the influence of these beat parameters on chirp encoding in the three primary stages of the electrosensory pathway: electroreceptor afferents, the hindbrain electrosensory lateral line lobe, and midbrain torus semicircularis. We then examine the role of these parameters, which represent specific features of various social contexts, for the behavioural responses of *A. leptorhynchus*. Some aspects of the behaviour may be explained by the coding properties of early sensory neurons to chirp stimuli. However, the complexity and diversity of behavioural responses to chirps in the context of different background parameters cannot be explained solely on the basis of the sensory responses and thus suggest that critical roles are played by higher processing stages.

1. Introduction

During social encounters, many animals use communication signals to transmit a variety of

information, such as individual identity and motivational state, that is used to dynamically modulate behavioural strategies. Across taxa, signals involving mechanical (including acoustic and vibrational stimuli; Hill, 2009; Kelley and Bass, 2010), visual (Osorio and Vorobyev, 2008), chemical (Stacey et al., 2003; Johansson and Jones, 2007) and electric modalities as well as a mixture of them (Bro-Jorgensen, 2010) have been characterized. Responding to these signals appropriately can be crucial for reproductive success, as well as the survival of an individual (Kelley and Bass, 2010). Accordingly, understanding why and how signals are produced has been a central goal in animal ethology.

The accurate detection of communication signals depends crucially on signal encoding by the nervous system which can be limited by internal and external noise (Waser and Brown, 1986; Schmidt et al., 2011). In the auditory and electrosensory systems, communication signals can be produced in the presence of an ongoing background signal that is a consequence of the interaction itself (Zupanc and Maler, 1993; Kelley and Bass, 2010). Different aspects of this background signal, including its frequency and contrast also provide behaviourally relevant information about social context, i.e. the identity and proximity of interacting individuals (Engler and Zupanc, 2001; Bastian et al., 2001; Yu et al, in press).

To explore both the meaning of communication signals, and the mechanisms by which they are encoded, it is necessary to consider an integrated description of how sensory stimuli, neural responses, and behaviour change during the social interactions. The study of communication also offers a framework for studying the encoding of sensory stimuli, in that encoding principles and stimulus sensitivities can be inferred directly from behavioural experiments. Behavioural adjustments produced in response to conspecific or simulated communication signals provide evidence that the receiving individual has detected the sensory stimuli. A combined analysis of neuronal encoding and behaviour is therefore profitable for both neurophysiology and ethology.

In this review, our goal is to exemplify this neuroethological approach in the context of electrocommunication among the Gymnotiform weakly electric fish *Apteronotus leptorhynchus*. Environmental conditions involving low-light and low electrosensory signal-to-noise ratio set a premium on efficient detection and processing of electrocommunication signals. For decades, studies examining the neurophysiological systems of weakly electric fish have provided insights into how natural behaviours are generated using relatively simple sensorimotor circuits (for recent reviews see: Chacron et al., 2011; Fortune and Chacron 2011; Marsat et al., 2012). Further, electrocommunication signals are relatively easy to describe, classify and simulate, facilitating quantification and experimental manipulation. Weakly electric fish are therefore an ideal system for examining how communication signals influence sensory scenes, drive sensory system responses, and consequently exert effects on conspecific behaviour.

Electric communication signals can be analyzed by measuring properties of the complex electric field that results from the interaction of nearby fish. In *A. leptorhynchus*, the dipole-like electric field (electric organ discharge, EOD) oscillates in a quasi-sinusoidal fashion at frequencies from 700-1100Hz (Zakon et al., 2002) with males emitting at higher frequencies than females (Meyer et al., 1987). When two fish with different EOD frequencies interact, the combination of their signals results in an amplitude modulation called a "beat"; the beat signal oscillates at the frequency difference between the fish. Beat signals are a direct consequence of social interactions and thus set the background of the electrosensory scene. In addition, through the individual EOD frequencies, information about sex, relative size and individual identities are represented in the beat signal. Physical movements result in slow amplitude modulations of the beat that can encode, among other things, aggressive approach and retreat behaviours (Yu et al., in press). Electrocommunication signals are produced in these social contexts and thus must be detected amidst the resulting complex background.

One type of electrocommunication signal, the chirp, involves brief amplitude and frequency modulations of the EOD and thus induces transient perturbations of the ongoing beat signal (Zupanc and Maler, 1993). Chirp production in this species is sexually dimorphic: males emit chirps at high rates during agonistic encounters, while females do not. Chirp production is strongly influenced by steroid hormones (e.g. testosterone; Dulka & Maler, 1994; Dunlap 2002) and neuromodulators (e.g. serotonin; Maler and Ellis, 1987; Smith and Combs, 2008). Recent physiological results suggest that encoding is influenced by serotonin as well (Deemyad et al., 2011).

Behavioural studies have focused on chirping behaviours under diverse conditions: from stimulating a restrained fish with signals mimicking a conspecific (Zupanc & Maler 1993; Bastian et al., 2001; Engler and Zupanc, 2001) to observing freely-moving fish during social interactions (Dunlap and Larkins-Ford, 2003; Hupé and Lewis, 2008; Triefenbach and Zakon, 2008). The neural encoding of chirps has also been studied at successive stages from electroreceptor afferents (Benda et al., 2005, 2006), through the hindbrain (Marsat et al., 2009; Marsat and Maler, 2010, 2011), and up to the midbrain (Vonderschen and Chacron, 2011), albeit in limited and simplified background contexts. Furthermore, the neural circuitry that controls the production of these signals is well known (Zupanc, 2002).

We here focus on how context-dependent properties of the beat signal influence the neural encoding of chirps and correlate with chirp production and aggression responses to chirp stimuli. We begin with a description of the different beat perturbations that are generated by the interplay of chirps with the different background beat parameters encountered during interactions. Following this, we review how chirps are encoded at successive stages of the electrosensory pathway in different background conditions. We then integrate findings from behavioural studies to reveal how chirp production varies under different social contexts. In the final section, we incorporate

principles from both neurophysiological and behavioural studies, to explore relationships between communication signal encoding and behaviour.

2. Signals and backgrounds in electrocommunication

Weakly electric fish use active electroreception to navigate and communicate under low light conditions (Zupanc et al., 2001). In active electroreception, animals produce an electric field using an electric organ (and this electric field is therefore called the electric organ discharge, EOD) and infer, from changes of the EOD, information about the location and identification of objects and conspecifics in their vicinity (e.g. MacIver et al., 2001; Kelly et al., 2008). However, perturbations result not only from objects and other fish, but also from self-motion and other factors. All of these together make up the electrosensory scene. The perturbed version of the fish's own field on its skin is called the electric image (Caputi and Budelli, 2006) which is sensed via specialized receptors distributed over the body surface (Carr et al., 1982). In the following, we will describe the modulations caused by the superposition of the electric fields of two interacting fish and by the production of specific communication signals.

2.1 Chirps involve transient increases in EOD frequency

Some weakly electric fish, the pulse-type fish, emit EODs in discrete pulses, while wave-type electric fish produce an EOD continuously, with a potential that oscillates with a specific frequency (the EOD frequency, EODf) that remains stable in time (exhibiting a coefficient of variation as low as $2*10^{-4}$; Moortgat et al., 1998). During social encounters, wave-type fish often modulate the frequency as well as the amplitude of their field to communicate (Hagedorn and Heiligenberg, 1985). Several different types of electrocommunication signals have been identified, varying in the type and pattern of frequency and amplitude modulations of the EOD (Zakon et al.,

2002; Zupanc, 2002). Communication signals in *A. leptorhynchus* have been classified into two classes: chirps are transient and stereotyped EODf excursions over tens of milliseconds (Zupanc et al., 2006), while rises are longer duration and more variable modulations of EODf, typically lasting for hundreds of milliseconds to seconds (Hagedorn and Heiligenberg, 1985; Tallarovic and Zakon, 2002). Here, we focus on chirps because chirp encoding in the nervous system, in contrast to that of rises, has been the subject of a number of physiological studies and the behaviour is more stereotyped and is easier to quantify.

Several types of chirps have been distinguished (Zupanc et al., 2006, Types 1-6). Under most experimental conditions, the most commonly produced type is the small chirp (Type 2 chirp), with males producing these signals at high rates during agonistic interactions (e.g. Larimer and MacDonald, 1968; Hagedorn and Heiligenberg, 1985; Hupé et al., 2008; Triefenbach and Zakon, 2008). A small chirp is traditionally defined as a short duration (10-20ms) increase in EODf of about 60-150Hz (Fig. 1A; Zupanc and Maler, 1993; Engler and Zupanc, 2001). The only other chirp type observed across a number of experimental contexts and also studied electrophysiologically, is the big chirp (Type 1 chirp), so called because of the much larger increase in EODf (>350Hz, Zupanc and Maler, 1993; Engler et al., 2000; Cuddy et al., 2012). The big chirp is accompanied by a marked decrease in EOD amplitude that is not seen in small chirps. Although the behavioural relevance of chirps remains unclear, researchers are beginning to gain insights about the relationship between chirping behaviours and aggression using a diversity of experimental approaches (see below).

2.2 The beat background and its social context

During the interaction of two wave-type fish, their electric fields superimpose and summate at every point in space. Measured across the skin of each fish, the combined signal consists of a

carrier determined by its own EOD with a periodic amplitude modulation (AM) at a frequency equal to the difference of the two individual EODfs, the beat frequency (Fig. 1C, D). The beat frequency has been suggested to reflect different aspects of the social encounter (Bastian et al., 2001; Kolodziejski et al., 2007). Crucial to this idea is that EODf correlates with identifying characteristics of the emitting fish including sex and dominance status. Given that EODfs are sexually dimorphic in *A. leptorhynchus*, slower beat frequencies are more common in same-sex interactions. In addition, EODf has been found to be correlated with size and dominance (Hagedorn and Heiligenberg, 1985; Dunlap and Oliveri, 2002; Triefenbach and Zakon, 2008; Fugère et al., 2011), suggesting that the beat frequency also provides information about relative size and dominance status.

The depth of an AM signal (its peak to trough distance) is referred to as its contrast. The contrast of the beat, as well as its phase, are determined by the position and orientation of the two fish with respect to each other (Kelly et al., 2008), with contrast decreasing as the distance separating two fish increases (see Fig. 1B, D). During social interactions, fish experience increases and decreases in beat contrast due to their own movements and those of interacting conspecifics. More aggressive interactions involve more frequent and longer-lasting approach behaviours that are associated with similar changes in contrast. The contrast also depends on the amplitude of the EODs of both fish. At a given distance, fish with larger EOD amplitudes produce larger contrasts than do fish with lower amplitude EODs. The beat phase varies spatially along the fish's body in a manner that depends on their orientation (i.e. whether fish are positioned parallel or perpendicular to one another; Kelly et al., 2008; Heiligenberg, 1986).

2.3 Chirps modulate the beat background

Chirps involve brief changes in EOD frequency and thus directly influence the amplitude,

frequency and the phase of the underlying beat (Benda et al., 2005; Zupanc and Maler, 1993). Even chirps of the same duration having identical frequency and amplitude modulations can induce very different effects on the composite signal received by the other fish depending on the specific beat parameters (Fig. 1). Classically, a small chirp has been described in the context of a slow beat and generated by the higher frequency fish (Fig. 1B, for a beat frequency of 20Hz), and in the example shown it causes a fast amplitude upstroke. However, the amplitude modulation looks different if the underlying beat is fast. The chirp still accelerates the beat, but now does so over multiple beat cycles (Fig 1C, frequency difference of 100Hz). Because the distance between the two fish influences the contrast, the AM caused by the chirp is smaller when fish are farther apart (compare Fig. 1B, D). However, the position of the chirping fish relative to the other fish also plays a critical role: the beat phase is 180° out of phase between the right and left sides of the receiving fish, so the same chirp will occur at two different phases on each side of the body (Fig. 1B, E). In all these cases, the chirp is produced by the fish with the higher EODf. A different picture emerges if the chirping fish emits the lower EODf because under these conditions, a chirp transiently decreases the beat frequency and decelerates the beat (Fig. 1F). In summary, the beat signal is not simply a static background noise source over which a chirp must be detected, but rather, it dynamically interacts with the chirp signal in a way that depends on social context. Thus, reliably detecting and encoding chirps presents a significant challenge for the electrosensory system.

3. Electrosensory pathways and principles of chirp encoding

Central to the detection and discrimination of a chirp is its representation in the nervous system. Chirp encoding has been studied in electroreceptor afferents called P-units (Benda et al., 2005, 2006; Hupé et al., 2008), and in primary electrosensory nuclei called the electrosensory lateral line lobe, ELL (Marsat et al., 2009; Marsat and Maler, 2010; Marsat and Maler, 2012) and torus semicircularis, TS (Vonderschen and Chacron, 2011). In this section, we summarize how beat

frequency, contrast and phase influence the processing of chirps at these different stages.

3.1 Electrosensory pathways

As all Gymnotiform fish, *A. leptorhynchus* possesses two kinds of electroreceptors on its skin that are activated by electric signals with different properties. Ampullary receptors are tuned to the low frequencies and DC signals associated with the passive electric sense, while tuberous receptors are tuned to the EOD frequency and comprise the active electric sense. In contrast to those in other species (*Eigenmannia*, see Hopkins, 1974; Metzner and Heiligenberg, 1991; Naruse and Kawasaki, 1998), *A. leptorhynchus* chirps do not contain DC components and are thus thought to be encoded by tuberous receptors.

Each electroreceptor organ is made up of several electroreceptor cells and innervated by afferents that make up the octavolateralis nerve (Zakon, 1986) projecting to the brain. Among the tuberous receptor afferents, two subpopulations can be discriminated (Scheich et al., 1973): P-type electroreceptor afferents called P-units respond by phase-locking to the EOD, firing an action potential with a probability that depends on the amplitude of the EOD received at the skin surface (Bullock, 1969; Nelson et al., 1997), while T-type electroreceptor afferents fire in response to every EOD cycle at a particular phase in the cycle. Electroreceptor afferents project to the ELL of the hindbrain, the first stage in which electrosensory information is processed in the central nervous system (see Fig. 2A). Here, the axons of P-unit afferents trifurcate to connect to pyramidal neurons in three different maps of the electroreceptive body surface (Heiligenberg and Dye, 1982; Carr et al., 1982), represented in regions called the centromedial segment (CMS), centrolateral segment (CLS) and lateral segment (LS), respectively. A fourth segment, the medial segment (MS) processes information carried by ampullary receptors and will not be described in detail here. ELL pyramidal neurons can be further categorized as superficial, intermediate and deep cells based on their

morphology and physiology (Bastian and Courtright, 1991; Harvey-Girard et al., 2007).

Pyramidal ELL neurons then project to higher processing areas including the nucleus praeeminentialis (nP) and torus semicircularis (TS, an inferior colliculus homologue, Fig. 2A; Metzner and Heiligenberg, 1991; Maler et al., 1991; Rose, 2004). nP provides direct and indirect (via the eminentia granularis pars posterior, EGp) feedback that is involved in reafference suppression and enhanced feature detection (Berman and Maler, 1998; Bastian et al., 2004; Lewis et al., 2007; Bol et al., 2011). In the TS, the pyramidal cells of the lateral segment converge together with cells of other types and all four ELL maps (Maler et al., 1982).

The TS projects to the tectum, to the diencephalic nucleus electrosensorius (nE), as well as back to nP (Maler et al., 1991; Rose, 2004). The sensorimotor nE integrates convergent electrosensory information and sends projections to two prepacemaker nuclei: the sublemniscal prepacemaker nucleus (sPPn) and the diencephalic prepacemaker nucleus (PPn) that are responsible for controlling the frequency of the EOD set by the medullary pacemaker nucleus (Pn). Spatially specific stimulation of the nE by glutamate iontophoresis results in EODf modulations (rises and chirps) via distinct inputs to the PPn (Rose, 2004). The sPPn and PPn project to the medullary pacemaker nucleus (Pn). The Pn contains electrotonically-coupled pacemaker neurons, whose endogenously oscillating membrane potential sets the EODf, and relay cells which propagate these signals to the electric organ (Smith and Zakon, 2000; Smith, 2006).

The most direct route that information can flow from sensory input to motor output is from electroreceptors to ELL, TS, nE, prepacemaker nuclei and then to the pacemaker nucleus. This direct route is indeed thought to form the basis of the jamming avoidance response (Heiligenberg, 1986; Rose, 2004), a behaviour that involves the fish changing their EOD frequency when stimulated with an EOD of similar frequency.

3.2 Chirps are encoded by electroreceptor afferents

To date, the afferent encoding of chirps has exclusively been studied in the tuberous P-unit receptors. In response to a step increase in EOD amplitude, P-units exhibit pronounced spike frequency adaptation (Xu et al., 1996; Nelson et al., 1997; Benda et al., 2005). Spike-frequency adaptation involves a strong peak in firing response to the onset of a constant stimulus, followed by a decrease to a lower steady state response. Thus, adaptation acts as a high-pass filter, reducing the response to low stimulus frequencies, such as beat frequencies lower than about 25Hz (Xu et al., 1996; Nelson et al., 1997; Benda et al., 2005). When produced by the higher frequency fish, chirps transiently increase the frequency content of the beat signal such that adaptation is transiently overcome. The result is a strong response similar to those evoked by the onset of a constant stimulus – provided the chirp is emitted during a sufficiently slow beat background (see Fig. 2C). The increase in firing rate is accompanied by an increase in P-unit population synchrony (Benda et al., 2006). The degree of synchrony between P-units is maximal for an intermediate range of beat frequencies (30Hz to 80Hz) and decays for higher beat frequencies. Small chirps at beats faster than 30Hz accelerate the beat frequency into a regime in which the synchrony between P-units decreases relative to their response to the beat. Hence, while P-units are synchronized by chirps occurring at beats slower than approx. 30Hz, they are desynchronized by the same chirps presented in conjunction with faster beats (Hupé et al., 2008; Walz et al., 2010).

The increase of the EOD frequency associated with big chirps is so large that they decrease the rate as well as the synchrony of P-units regardless of the underlying beat frequency (although there seems to be an increase in single unit reliability at beats < 10Hz, Benda et al., 2006); this effect is enhanced by the concomitant decrease in EOD amplitude typical of big chirps. The enhanced response to small chirps at slow beats, as well as the decrease in response to small and big

chirps at fast beats, are seen in measures of the firing rate as well as in measures of synchronization (Benda et al., 2006).

3.3 Chirp encoding in the electrosensory lateral line lobe

The next processing stage is the electrosensory lateral line lobe (ELL). There are two main classes of pyramidal neurons in each segment of the ELL. E-cells receive direct input from P-units and are excited when P-units increase their rate (i.e. during EOD amplitude increases), while I-cells receive the P-unit input via disynaptic connections from interneurons and are inhibited by an increase in afferent rate (Maler, 1979; Shumway and Maler, 1989).

As a consequence of differential ion channel distributions (Ellis et al., 2007; Mehaffey et al., 2008) as well as different connectivity to the afferent neurons (Maler, 2009), E-cells of all three segments exhibit very different response properties to P-unit inputs. From the CMS to the LS, neurons are increasingly responsive to higher frequency AMs (Krahe et al., 2008) and have larger receptive fields. Both characteristics, high-pass frequency tuning and large receptive fields, make neurons of the LS most responsive to communication signals (Marsat et al., 2009); compared to signals encountered during navigation and hunting, communication signals are much higher in frequency and more spatially broad. Not surprisingly, the LS has been shown to be crucial for communication behaviour (Metzner and Juranek, 1997).

Feedback to ELL from nP and EGp plays an important role in chirp encoding. Superficial E-cells of the LS respond with a highly reliable and synchronous burst of spikes to small chirps emitted at slow beats (Fig. 2D; Marsat et al., 2009). The second spike of the burst is not phase-locked to the EOD, indicating that it is not caused by input from P-units. The bursting mechanism relies on a depolarizing after potential (DAP) that stems from backpropagating action potentials

from the dendrites (Turner et al., 2002; Marsat and Maler, 2012). In these cells, the indirect feedback from EGp provides a negative image of a low frequency beat (Bastian et al., 2004). During an ongoing beat, feedback and input are antiphase, but the chirp shifts the phase of the beat stimulus. When this occurs, the feedback coincides with the DAP and a spike in response to a chirp is more likely to be followed by a second one (Marsat and Maler, 2012). Such bursts may facilitate chirp detection, similar to many systems where bursts enhance signal detection by increasing the signal to noise ratio (for a review, see Krahe and Gabbiani, 2004). The feedback, however, is only present in response to beats of frequencies up to 20Hz (Bol et al., 2011, Bastian et al., 2004). The enhancement of the ELL response by feedback to small chirps is therefore likely to be even more confined to low beat frequencies than the P-unit response.

Big chirps are encoded by a strong increase in firing rate in I-cells of all maps and types (superficial, intermediate and deep, Marsat et al., 2009). This is expected since they cause a decrease in the response of P-units and because, in contrast to E-cells, I-cells of different maps and morphology do not show strong differences in frequency tuning (Krahe et al., 2008).

3.4 Higher level processing of chirps

The main target area of the ELL for further information processing is the TS. TS cells can be grouped into two categories according to their baseline firing rate and selectivity to different chirp stimuli (Vonderschen and Chacron, 2011; Chacron et al., 2011). One category, the densely coding neurons, produce responses that resemble those of ELL pyramidal cells (Fig. 2E, left), while cells in the other category respond much more sparsely, i.e. with a higher selectivity (Fig. 2E, right). Compared to the dense coding TS cells and ELL pyramidal cells, sparsely coding TS cells do not respond during the beat and respond similarly to chirps with certain attributes, but not at all to those with others (see also Fig. 2 in Vonderschen and Chacron, 2011). This population of TS cells can

thus, in principle, detect the presence of certain categories of chirps and differentiates between them. How this selectivity arises is currently unknown. The synapses between ELL pyramidal cells and TS neurons show pronounced short-term synaptic plasticity that can act as a temporal filter passing low or high frequencies (Fortune and Rose, 2000, 2001, shown for *Eigenmannia*). This synaptic plasticity has been shown to create direction selectivity to moving electrosensory images in TS neurons (Chacron et al., 2009; Chacron and Fortune, 2010). Whether synaptic plasticity sharpens responses to chirps is unknown. Cells that respond selectively to chirps are not direction selective and vice versa (Vonderschen and Chacron, 2011).

3.5 Large contrasts enhance the encoding of beats and chirps

So far, we have primarily considered the effect of beat frequency on chirp encoding. We will now turn to the influence of beat phase and contrast. The encoding of beats and chirps at different contrasts has been studied only in P-units. P-units respond linearly to increasing contrasts within a certain range (Gussin et al., 2007). The responses of P-units to chirps and beats are greater for larger contrasts, however, the relative response to chirps is qualitatively independent of the contrast (Benda et al., 2006). At higher contrasts, the responses of P-units become nonlinear (due to rectification) suggesting that responses to chirps will change (Savard et al., 2011). For example, at beat frequencies in which the chirp elicits a stronger response than the underlying beat, the response is cut off and both chirp and beat elicit responses that are equally strong.

Heterogeneity in the characteristics of P-units (Gussin et al., 2007) as well as in spatial properties of the signal (Kelly et al., 2008), however, result in saturation of their responses at different levels of beat contrast. Using whole-nerve recordings, Benda et al. (2006) showed that the population response begins saturating at contrasts around 20%. Whether or not saturation effects make the detection of chirps more difficult for interacting *A. leptorhynchus* remains to be tested

with behavioural experiments.

3.6 The phase of the beat influences chirp encoding at low frequencies

The timing within the beat cycle at which a chirp is produced strongly affects the shape of the AM at beat frequencies lower than 30Hz (Fig. 1), causing either transient upstrokes or downstrokes. In response to a chirp that causes an upstroke, a strong increase in firing rate is seen in P-units, whereas in response to a chirp that results in a downstroke, P-units cease firing. However, when chirps occur at times between a trough and peak, the resulting AM contains parts of an upstroke as well as a downstroke and the response is similar to the case of a pure upstroke (Benda et al., 2005).

One way to characterize whether a certain attribute of a chirp significantly influences its encoding, is to analyze whether a response is sufficient to differentiate between chirps of different values of this attribute. This is traditionally done in a discrimination analysis (Green and Swets, 1974) and such an analysis has been conducted using responses in the ELL and TS (Marsat and Maler, 2010; Vonderschen and Chacron, 2011). At both processing stages, responses to the same chirp presented at different phases of the beat are significantly different. The beat phase selectivity of ELL and TS neurons to chirp stimuli suggests that there may be a behavioural relevance of chirps produced at different times in the beat phase cycle and that fish could therefore control chirp production to influence perception by the other fish. If this were the case, one would expect a nonuniform distribution of chirps over the beat cycle (see behavioural section for further discussion).

As discussed earlier, the amplitude modulation of a chirp depends on whether the emitting fish carries the higher or lower EODf (the sign of the frequency difference, see Fig. 1E). The results

from electrophysiological studies about the encoding mechanisms suggest that the responses of Punits, ELL and TS to chirps will also depend on whether the chirping fish has the lower or higher EODf. However, this remains to be shown as all electrophysiological studies thus far have been conducted using positive difference frequencies.

4. Behavioural Responses to Chirp Stimuli

In the previous section we reviewed how electrophysiological responses to chirps in electrosensory afferents and primary integration centers are influenced by parameters of the background signal, including beat frequency, contrast, and beat phase. In this section, we will review evidence from the literature that describes how behavioural responses to chirps are influenced by these same parameters. We will begin with a brief discussion of *A. leptorhynchus* responses to different chirp stimuli characterized using a simplified experimental design, addressing how chirp delivery influences chirp production rates depending on stimulus parameters (the beat frequency and contrast, and the rates and pattern of chirps delivered). following this, we will examine how chirping and aggressive responses to conspecific chirps are influenced by beat parameters under more natural contexts. We focus primarily on small chirps, with some discussion of big chirps at the end of the section. Throughout, we consider whether what is known about sensory encoding is sufficient for explaining the relationships between behavioural responses to chirps and the background beat parameters.

4.1 Chirping in chirp chambers

Stimulus specific behavioural responses have been characterized using "chirp chamber" experiments, wherein the EOD modulations produced by individual fish restrained in tubes are recorded in response to electrical stimuli (sinusoidal or EOD mimics) of varying frequency and

amplitude (Dulka et al., 1995; Zupanc and Maler 1993; Engler and Zupanc, 2001). In these conditions, chirp production rates of males decrease with increasing beat frequency (Engler and Zupanc, 2001; Bastian et al., 2001) regardless of the sign of the frequency difference. This selective behavioural response corresponds well with the range of beat frequencies over which chirps lead to the greatest increases in P-unit synchrony and enhancement of chirp encoding in the ELL by feedback mechanisms. Beat contrast also influences the chirp production rates of fish in chirp chambers. These experiments have suggested stimulus intensities greater than 50μV/cm are required to elicit chirp responses in *A. leptorhynchus* (Dunlap et al., 1998; Engler and Zupanc, 2001; Zupanc et al., 2006). Further, chirp production rates of males increase with increasing stimulus intensity i.e. increasing contrast (Zupanc and Maler, 1993; Engler and Zupanc, 2001).

Chirp chambers have also been used to characterize the occurrence of chirps relative to the phase of the beat. In chirp chamber experiments using slow beat stimuli, chirps were produced at all beat phases (Zupanc and Maler, 1993). To investigate whether there might be effects of beat phase on chirp production at other beat frequencies, we performed chirp chamber experiments using various stimulation frequencies (Fig. 3). The data shows that chirps were produced at equal rates across all beat phases (Fig. 3C). Thus, fish will likely be exposed to chirps at all different beat phases. Marsat and Maler (2010) suggested that the high variability in the response of pyramidal cells, resulting from chirps at different beat phases, hinders the fish's ability to distinguish between chirps of different parameters. However, fish often emit chirps in bursts and the electric image evoked by the chirp is heterogeneous along the body (Kelly et al., 2008). This means that receiver fish likely have access to information about a chirp at multiple beat phases. The absence of a pronounced relationship between chirp production times and beat phase does not necessarily imply that beat phase is not behaviourally relevant. Even if the fish might not control the production at certain beat phases, chirps emitted at certain beat phases could still be represented better in the nervous system and evoke stronger behavioural responses. In free-swimming conditions, the exact

phase of the beat at the skin of a fish is hard to infer and no such analysis has been done so far. However, such experiments could give important information in this direction.

Chirp chamber experiments have also been performed using playback stimuli containing chirps. In these experiments, chirping also decreases with increasing beat frequency similar to the response to stimulus EODs that do not contain chirps (Engler and Zupanc, 2001; Triefenbach, 2005). Interestingly though, overall chirping rates are lower when playbacks contain chirps, suggesting that in these conditions, chirp reception inhibits the chirp production rates of receiving fish (Dunlap and Larkins-Ford, 2003; Triefenbach, 2005). Chirp production rates in response to playbacks with chirps approximately 3cm and 10 cm from the receiving fish (resulting in field intensities of 0.5mV/cm and 0.075mV/cm, respectively, near the receiving fish) also increase with higher intensity stimuli (Dunlap and Larkins-Ford, 2003). However, as will be discussed in the following section, these relationships are more complicated during natural interactions.

4.2 Behavioural responses to chirps under more natural experimental conditions

The stimuli presented and experimental conditions used in chirp chamber experiments are similar to those used in electrophysiological experiments, but both lack many features common to natural interactions. While many of the observations found in chirp chambers carry over to more natural experimental conditions involving staged social interactions (Dunlap and Larkins-Ford, 2003; Zupanc et al., 2006; Triefenbach and Zakon, 2008; Hupé et al., 2008), there are also important differences, suggesting that the complexity of chirping behaviours produced under increasingly naturalistic conditions involves the integration of multiple features of a social interaction. As described earlier, the beat background during conspecific interactions can be quite complex because fish constantly change position relative to one another and produce rapid frequency and amplitude modulations in varying temporal patterns.

4.2.1 Chirp response rates and patterns are influenced by the experimental setting and behaviour of interacting conspecifics

When two fish confined to separate tubes interact electrically (but not physically), the chirp production pattern of one fish is correlated with that of the other fish (Zupanc et al., 2006). Correlation analyses of the instantaneous chirp rates of fish responding to chirps suggest that following chirp reception there is a short-term inhibition of chirping (~100-200ms) which precedes a subsequent period of chirp rate enhancement (Zupanc et al., 2006; Hupé and Lewis, 2008; Gama-Salgado and Zupanc, 2011).

From a sensory coding perspective, this so-called "echo response" implies that conspecific (or artificial) chirps are discriminated by the sensory system of a receiving individual amongst various background beat modulations. It is thus a convenient measure of sensory detection at the behavioural level. Using EOD playbacks, Gama-Salgado and Zupanc (2011) found that 20ms-long chirp mimics with a frequency increase of just 1.2%, delivered with an interchirp interval of 0.6s, were sufficient to induce a robust echo response. This indicates that the typical frequency excursion associated with small chirps (~50-100Hz) is at least five times greater than the behavioural threshold for chirp detection. These results were characterized with beat background conditions optimal for chirp encoding: in response to a signal delivered at a high stimulus intensity (mimicking an inter-individual distance of approximately 1-2cm) with an EODf similar to that of the stimulated fish (±10Hz) (Gama-Salgado and Zupanc, 2011).

The pattern of chirp stimuli also influences both the chirping and aggressive responses of free-swimming fish. With EOD playbacks containing chirps delivered in a random sequence, the chirp and aggressive responses of male *A. leptorhynchus* decrease with the number of chirps

delivered (Hupé, 2012). In addition, fish echo more often in response to higher randomly patterned stimulus chirp rates, produce fewer chirps and are less aggressive towards stimulus mimics (Hupé, 2012). This inhibition of chirping was not observed in fish responding to playbacks during which chirps are delivered interactively (stimulus chirps echo those produced by the real fish with a latency of 200ms). These observations suggest that both the rate and pattern of chirps delivered differentially influence behaviour and provide evidence that chirps received are temporally integrated in electrosensory systems.

4.2.2 The influence of beat frequency

The relationship between chirp rate and beat frequency characterized in chirp chamber studies persists across a number of behavioural scenarios (Dunlap and Larkins-Ford, 2003; Zupanc et al., 2006; Hupé et al., 2008). These results imply that chirps are produced at high rates during stimulus conditions that represent more aggressive same-sex contexts. Given that EODf is related to indicators of dominance among males, increased chirping and physical escalation are expected between more closely matched individuals (see section 2, Fugère et al., 2011).

Along these lines, it follows that chirp rates should be asymmetrical with respect to the sign of the frequency difference. Contrary to this prediction, results from chirp chamber studies reveal no significant dependence of chirp rates on the sign of the frequency difference (Engler and Zupanc, 2001; Bastian et al 2001). However, contrary to the results from chirp chambers, there is growing evidence that under more natural experimental conditions, fish respond in a way that depends on the sign of the frequency difference. During experiments in which one fish is restrained in the center of a tank in an electrically transparent hammock, and another fish swims freely around it, the chirp rates of the free-swimming fish correlate significantly with the magnitude and the sign of the frequency difference (Hupé, 2012), Additionally, playbacks of lower frequency EODs without

chirps elicit more approach behaviours from fish than do playbacks of an EOD of the same frequency with chirps, or playbacks of higher frequency EODs regardless of whether they contain chirps or not (Triefenbach, 2005).

Although small chirps are produced infrequently in response to large beat frequencies, analysis of the chirp echo response has demonstrated that free-swimming fish reciprocate chirps at rates significantly greater than chance even during social pairings that result in high beat frequencies (Hupé et al., 2008), evidence that small chirps can be encoded across the range of all beat frequencies encountered.

4.2.3 The effect of beat contrast

Chirp rates of pairs of males, each confined to a separate tube, change significantly only when the tubes are positioned within 10cm of one another, suggesting that the dependence of chirp production on contrast characterized in chirp chambers extends to dyadic interactions (Zupanc et al., 2006). When one or both of two interacting fish are unrestrained, contrasts change dynamically throughout the interaction and can provide fish with information about conspecific motion and proximity (Yu et al., in press). During such free swimming interactions, there are significant correlations between measures of aggression (associated with large increases in contrast) and average chirping rates (Triefenbach and Zakon, 2008; Hupé and Lewis, 2008).

To further characterize the temporal relationship between chirping and contrast, we quantified, over time, the relationship between chirp production and the distance separating a chirping fish and the playback mimic to which it is responding (Methods described in Hupé, 2012). Figure 4 shows the mean distances centered at the time of chirp production (Fig. 4A), and centered at the time of delivered chirps (Fig. 4B), in one free-swimming fish responding to a low beat

frequency EOD playback with chirps delivered to echo those of the real fish, calculated for every minute of a ten minute trial (Hupé, 2012). At the onset of the trial (within the first couple of minutes), the fish remains at a distance from the mimic, and during this time chirps are produced when the distance separating the fish and mimic is largest (when contrasts are small). Further into the trial (from 3-4 minutes onward), the fish spends more time in close proximity to the mimic and produces its chirps during, or slightly following, times when mean distances are the smallest (corresponding to large contrasts that occur during approach behaviours). This suggests that the timing of chirps produced does not depend only on the absolute contrast or on specific types of contrast changes. Further, it is expected that the distance relationships in Fig. 4A and 4B should be similar, because chirps delivered echo those produced by the fish. However, the relationship between chirp time and distance is more pronounced for chirps produced than for chirps delivered, suggesting that chirp production may be influenced more strongly by contrast than by conspecific chirps. The strength of these relationships may also depend on the experimental and social conditions under which the behaviours are examined. Future studies should address how aggressive behaviours are differentially patterned with chirping under conditions that more closely represent natural interactions.

4.3 Big and small chirps: Differential chirp production and associated behaviours

Up to now we have exclusively considered the behavioural responses to stimulation with EODs containing small chirps. However, behavioural results from playback experiments suggest that the chirp types are differentially encoded. Playbacks with big chirps increase the production of big chirps relative to small chirps, and evoke approach behaviours in both male and female fish (Triefenbach, 2005). This is consistent with the idea that big chirps are an attractive signal, as predicted from observations of interacting fish during reproductive contexts (Hagedorn and Heiligenberg, 1985), chirp chamber studies (Bastian et al., 2001; Engler and Zupanc, 2001), and

freely interacting *A. leptorhynchus* (Hupé, 2012).

The relative production rates of small chirps and big chirps are also influenced by the frequency and contrast of the beat background, chirp stimulus parameters, as well as experimental setting (Triefenbach, 2005; Hupé and Lewis, 2008). Under a variety of conditions, males preferentially produce big chirps in response to stimulation with high beat frequencies (Engler and Zupanc, 2001; Bastian et al., 2001). High beat frequencies occur during opposite sex-interactions and during interactions between same sex individuals with large differences in EODf, providing additional evidence that big chirps function in attraction, reproduction (Engler and Zupanc, 2001; Zakon et al., 2002) and/or to signal subordinance (Cuddy et al., 2012). This proposed function is further supported by results from dyadic experiments in which less aggressive males produce significantly more big chirps than do more aggressive males (Hupé, 2012).

If two communication signals convey different meaning, they must be discriminated in sensory systems. Signals that convey opposite behavioural states (i.e. aggression and submission) often comprise opposite extremes of some variable in signal space (i.e. frequency, duration) presumably to reduce receiver error (Morton, 1977; Hurd et al., 1995; Triefenbach and Zakon, 2003). As described in the previous sections, responses to big and small chirps can be discriminated at all stages in the early electrosensory pathway when they are emitted on a slow beat background. During fast beats, both small and big chirps desynchronize P-unit electroreceptors, so how these two signals are distinguished in subsequent processing stages is not clear.

4.4 Behavioural responses to chirps depend on the interplay of individual propensities and stimulus condition

Pronounced individual differences in chirp responses have been observed in multiple

contexts (Dunlap and Larkins-Ford, 2003; Gama-Salgado and Zupanc, 2011); and interestingly, the chirp rates of fish to EOD playbacks with and without chirps are correlated (Dunlap and Larkins-Ford, 2003). Some variation is explained by body size, as larger males chirp more overall, maintain higher chirp rates, and are less likely to decrease chirp rate in response to chirp containing EOD playbacks compared to smaller males (Triefenbach, 2005). This is consistent with the idea that the chirping by more dominant males is less affected by threatening stimuli than that of less dominant (smaller, low EODf) males (Triefenbach and Zakon, 2008; Hupé, 2012). These selective responses to different chirp stimuli suggest that responses to chirps can be influenced by the threat potential and the condition of the receiver (Triefenbach, 2005). Individual differences could at least in part be a consequence of differential chirp encoding and processing by electrosensory pathways.

A complex behavioural repertoire is revealed through a comparison of behavioural responses to chirp stimuli presented under different experimental conditions. The information contained in the beat frequency and beat contrast influence both chirp encoding and chirp production behaviours. While differences in chirp encoding under specific beat background conditions may account for some of this variation in behavioural response, it appears that higher processing of conspecific chirps may be categorical (small versus big chirp) and subject to modification by a number of influences.

5. Integration of Encoding and Behaviour

Characterizations of chirping behaviours in male and female *A. leptorhynchus* have revealed that chirp production patterns can be very complex, influenced by a variety of internal and external factors. As demonstrated, some of the complexity of chirping behaviour may be explained by features of chirp encoding in early sensory pathways. Many aspects of chirping behaviour, however, might only be reflected in higher processing stages, downstream from the primary integration

centers that have been studied so far.

5.1 What encoding can tell us about behaviour

Describing the physical properties of the sensory environment of an animal provides information about the nature of the stimuli that activate the receptor cells of a certain modality. However, the internal representation of these stimuli is ultimately responsible for the information an animal has access to about the outside world. Here we have examined how conspecific signals are encoded when presented in conjunction with different background parameters and discuss which aspects of encoding may influence behavioural responses. Small chirps are particularly well-encoded when they are emitted at slow beats (Benda et al., 2005; Marsat et al., 2009; Marsat and Maler, 2010). Behavioural investigations show, however, that they are detected by the fish even when occurring on faster beats, during which chirps have an opposite effect on the response of receptor cells (Hupé et al., 2008). Although the ELL responses have only been studied in a limited context, i.e. a chirp on a 5Hz beat (Marsat and Maler, 2010), and the question of how chirps are encoded in conjunction with different beat frequencies has not been analyzed in detail in the TS, the distinct responses to a chirp at a slow and a fast beat suggest that chirp encoding at low and high beat frequencies is routed through different streams. In the ELL, for example, we would expect E cells to respond to small chirps occurring at low beat frequencies (as shown by Marsat and Maler, 2010) and I cells to be responsive to small chirps occurring at high beat frequencies. Furthermore, the way small chirps are encoded by P-units at high beat frequencies seems to be similar to the encoding of big chirps at these frequencies.

Chirp encoding in the early electrosensory pathway suggests two aspects that future behavioural investigations should consider. First, since the effect or relevance of a chirp might depend on whether they are emitted at low or high beat frequencies, a more careful analysis of

behavioural responses at different beat frequencies is warranted. Second, the categorical distinction between small and big chirps might depend on beat frequency and should be examined further. If behavioural studies confirm a clear distinction between big and small chirps at high beat frequencies, the encoding of big and small chirps at these frequencies might rely on mechanisms and effects that have not been examined in physiological studies so far.

The encoding principles investigated so far in the early electrosensory pathway can only provide hints to the overall representation of the stimulus. Processing at the neural population level could lead to enhanced detection or discrimination in successive stages. Even at the initial stage from P units to the ELL, there is a high degree of convergence (by a factor of 1:1000 in LS; Maler 2009); this is also most likely occurring between ELL and TS. Additionally, in the TS, the information encoded by P-units converges with information about low frequencies and phase differences in the EOD signal that is encoded by ampullary receptors and in T-units, respectively (Metzner and Heiligenberg, 1991; Kawasaki, 1986). Beats as well as the amplitude modulations caused by chirps generate no low-frequency signals that might be detected by the ampullary system. However, Dunlap et al. (2010) demonstrated that *A. leptorhynchus* also chirps in response to the low frequency signals preferred by ampullary receptors. This behaviour suggests that information from the ampullary system could be used to trigger chirp production. Chirp encoding in T-units has not been studied to date, but could provide a complementary stream of information.

5.2 What behaviour can tell us about encoding

Studies of the encoding of sensory stimuli shed light onto the mechanisms by which sensory information may be represented in a nervous system. However, only behavioural studies can ultimately show whether a signal is detected and differentiated by the animal.

The complex temporal patterning observed between chirp production and physical aggression, occurs over subsecond timescales (Triefenbach and Zakon, 2008; Hupé et al., 2008; Gama-Salgado and Zupanc, 2011). This provides evidence that the electrosensory system is able to encode and respond to chirps occurring at rates as high as 3 to 5 chirps per second (Hupé and Lewis, 2008; Gama-Salgado and Zupanc, 2011). In many systems, antiphonal exchanges such as the chirping echo response, mediate mutual assessment of individual status. Coordinated signalling behaviours are often exchanged during confrontations as a means to prevent the costs associated with escalation (Triefenbach and Zakon, 2008) and necessitate that signal timing and quality are rapidly and faithfully represented in sensory pathways.

An even faster control of chirp production time than observed in behavioural experiments seems, however, not possible or necessary. Although at early electrosensory stages the phase in the beat at which a chirp occurs strongly influences chirp encoding, chirps do not appear to be produced with any phase preference. This does not necessarily imply that beat phase is irrelevant. Chirps emitted at certain beat phases could still be represented better in the nervous system and therefore potentially evoke stronger behavioural responses. In free-swimming conditions, the exact phase of the beat at the skin of a fish is hard to infer and no such analysis has been done so far. However, experiments investigating the influence of beat phase on the echo response, for example, could give important information in this direction.

Under various experimental conditions, fish tend to produce chirps in bursts (Zupanc et al., 2006; Hupé and Lewis, 2008). Bursts of chirps might allow for neural responses to integrate over successive chirps in higher processing stages, leading to a larger signal to noise ratio. Up to the level of the TS, this is clearly not the case. All time scales of the responses are still fast and chirps separated by 400 ms will be processed as separate signals. Alternatively, emitting chirps in bursts might simply increase the chance of some chirps occurring at beat phases at which they are

perceived best.

The difference between chirping responses to playback chirps and to those produced by two physically interacting fish suggests that spatiotemporal electric field complexities resulting from relative motion significantly influence chirping and aggressive responses to chirps (Dunlap and Larkins-Ford, 2003). Furthermore, during dyadic interactions, chirps are produced preferentially when fish are positioned in a head-to-tail orientation compared to when oriented head-to-head (Triefenbach and Zakon, 2008). Future studies should characterize the electric image modulations produced during chirping in each of these orientations, and electrosensory responses to these different stimuli.

In other systems, signal attributes such as maximal frequency excursion and duration provide information about the identity and attractiveness of conspecifics: individual identity in damselfish (Myrberg and Riggio, 1985) and attractiveness in crickets (Hennig 2003). This could also be the case for chirping in *A. leptorhynchus* (Dulka et al., 1995). However, the great variability involved in encoding one chirp at different beat phases at the level of the ELL has led to the suggestion that the differentiation between chirps of different attributes is impossible for the fish (Marsat and Maler, 2010). To ultimately evaluate this possibility, the whole parameter space of chirp patterning and beat backgrounds must be taken into account. Also, other parameters such as EODf, beat frequency and chirp production rates already convey redundant information about identity and dominance status, suggesting that specific chirp attributes may be less important. Evidence from choice experiments in which females prefer males with higher EODf (Bargeletti, Gogarten and Krahe, personal communication) show that this information seems to be relevant in reproductive contexts as does the observation that fish increase their EODf in breeding conditions (Cuddy et al., 2012).

However, negative results from behavioural experiments do not necessarily mean that a chirp has not been detected by a receiving fish. Chirping is not a reflexive behaviour and whether or not a fish chirps in response to a stimulus chirp or EOD does not only depend on signal detectability but also on the receiver's motivation and behavioural strategy, as well as the experimental context and various other factors. Carefully designed experiments are required to tease out the relative effects of these different factors on behavioural thresholds.

5.3 The complexity of chirp encoding and behaviour: future directions

The diversity and context specificity of behavioural responses to chirps under more realistic experimental conditions demonstrate that many factors are integrated to influence these responses. Despite the extensive description of chirp encoding in the first three stages of electrosensory processing and the growing body of behavioural characterizations, many open questions about chirp encoding remain. There is a need for a description of electrosensory responses to chirping in higher brain areas as well as behavioural and physiological experiments performed under increasingly natural conditions.

Male and female *A. leptorhynchus* behave very differently to chirp stimuli, with only males producing chirps (Dulka and Maler 1994; Dulka et al., 1995). These behavioural differences are likely a consequence of hormonal modulation of chirp production pathways (Telgkamp et al., 2007; Smith and Combs 2008). Recent evidence suggests that encoding pathways are sensitive to neuromodulation by circulating hormone levels (Deemyad et al., 2011). This *in vitro* study showed that serotonin increases the excitability and the burst firing of the ELL E-cells that are responsible for encoding chirps. The effects of neuromodulation on chirp encoding is an exciting finding that should be investigated *in vivo* and in more detail in future studies.

So far, physiological experiments have characterized responses to stimuli containing chirps on a beat with a constant contrast, presented in conditions similar to those used in chirp chamber behavioural experiments. Certain aspects of movement that are reflected in contrast changes of the beat are correlated with chirping (Hupé, 2012). Whether or not contrast changes will influence chirp encoding is another important question for future physiological or modeling studies (see Yu et al, in press), in particular at higher processing stages. Future studies should also examine electrophysiological responses to stimulus chirps that incorporate elements of the spatiotemporal electric field complexities generated during conspecific interactions, and compare these to the responses to self-generated chirps. Clearly, behavioural responses to chirps are influenced by the context under which they are characterized, and stimulus paradigms that represent more natural electric scenes should be a priority.

Figure Captions

Figure 1. **Beat modulations induced by chirps during representative encounters between different pairs of fish.** (A) shows one example of a small chirp as measured in a chirp chamber (for method descriptions see Fig. 3). When the instantaneous EOD frequency is plotted over time (upper panel), an increase from around 710Hz to 810Hz is seen. The amplitude is almost unchanged during the chirp, as seen when the EOD waveform is plotted over time (lower panel). (B-F) In each scenario, one fish emits the chirp shown in Fig. 1A, but under different simulated background conditions. The sketches of the fish demonstrate the encounter, with the chirping fish shown in red and the size of each fish reflecting its EODf (a higher EODf is indicated by a bigger size). (B) shows the encounter with a beat frequency of 20Hz and a contrast of about 40%; (C) with a beat frequency of 100Hz and 40% contrast; (D) shows the same encounter as in B but with a contrast of 20%; (E) shows an encounter similar to B but at a beat phase shifted 180 ; (F) as in C, but the fish with the smaller EODf emits a chirp. (The fish sketches are modified from Hagedorn and Heiligenberg, 1985)

Figure 2. **The electrosensory processing stages and their response to a chirp**. (A) Connectivity between the different brain nuclei involved is indicated by arrows, with black arrows depicting ascending projections and red arrows feedback. ELL, electrosensory lateral line lobe; EGp, eminentia granularis pars posterior; nP, nucleus praeeminentialis; TS, torus semicircularis; nE, nucleus electrosensorius; SPPn, sublemniscal prepacemaker nucleus; Ppn, prepacemaker nucleus. (B). The same chirp stimulus was used to stimulate cells of the different processing stages. It consisted of a chirp with a frequency excursion of 60Hz and a beat frequency of 20Hz. The responses of P-unit electroreceptor afferents (C), pyramidal cells of the hindbrain electrosensory lateral line lobe (ELL; D) and of two types of neurons in the midbrain torus semicircularis (TS; the

dense and sparse coding cells in the left and right column, respectively) to this chirp stimulus are shown as raster plots. The data from p-units was recorded by H. Walz following the methods described in Benda et al. 2005; data from ELL and TS were kindly provided by M. Chacron (for methods see Vonderschen & Chacron, 2011).

Figure 3**. Beat phase and chirp production.** (A) shows a histogram of all chirps over beat phase recorded in 66 chirp chamber experiments with a stimulation of 4Hz above the fish's own EODf. Fish were placed in a tube and stimulated with mimics of conspecifics using two carbon electrodes, one on either side of its body. The fish's field was measured with silver chloride electrodes placed near the head and the tail of the fish and chirps were detected as frequency increases of more than 10Hz of the EODf using custom made software. To exclude effects of an overall higher chirp rate of individual fish, we normalized the histograms with the overall chirp rate for each fish. Shown are the number of chirps in each phase bin (of 36°) divided by the number of all emitted chirps of this fish, then summed over all experimental conditions. For a more detailed description of chirp chamber experiments see Bastian et al., 2001. (B) shows the results from the same experiments under a stimulation with 48Hz above the fish EODf. (C) For each stimulation frequency we calculated the vector strength of the histogram. The vector strength is a measure for phase locking and ranges from 0 to 1. As we find values of 0.1 for all stimulation frequencies, this shows that chirp production rates do not depend on beat phase.

Figure 4. **Chirp patterning over time.** Chirps are patterned with contrast changes that result from physical movements in a manner that changes over time. The mean distance separating a free-swimming fish and a playback mimic calculated over twenty seconds centered at the time of (A) chirp production and (B) chirp delivery. Distances are depicted in the colour of each 100ms bin centered at the time of chirp production or delivery, averaged over one minute bins for every minute of a ten minute interactive chirp playback trial. The colour bar denotes the linearly distributed

representation of distances. Playback stimuli EODs were delivered through a mimic at a frequency slightly higher (+10Hz) than that of the real fish, with an amplitude matching that of the real fish, and chirps were delivered to echo those produced by the real fish with a latency of 200ms (Methods described in Hupé, 2012).

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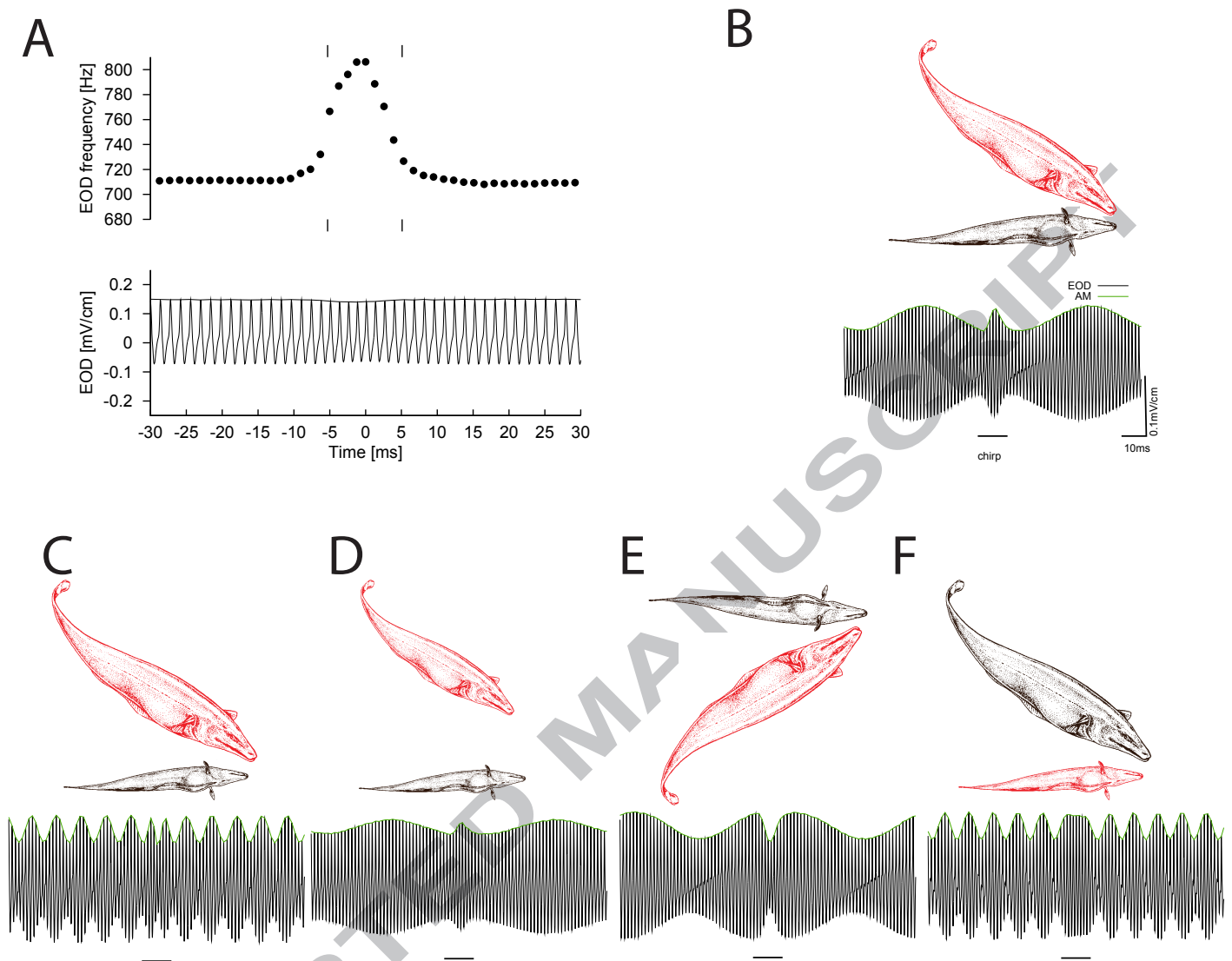
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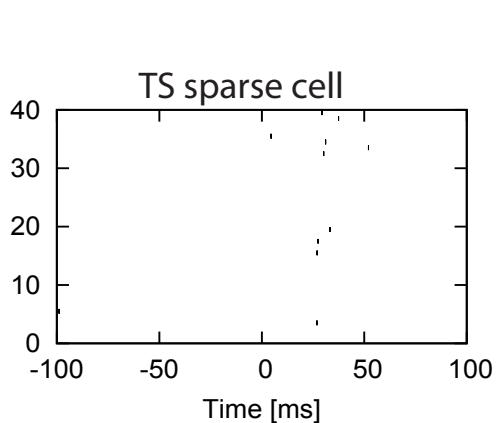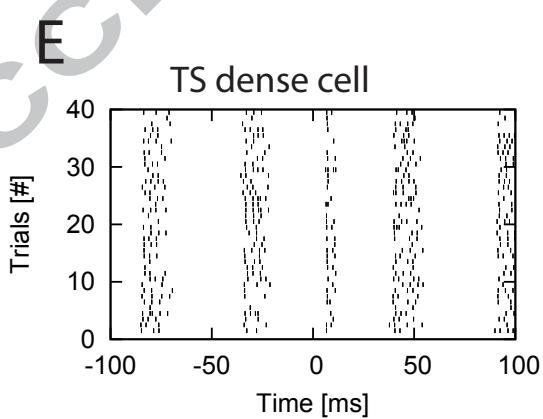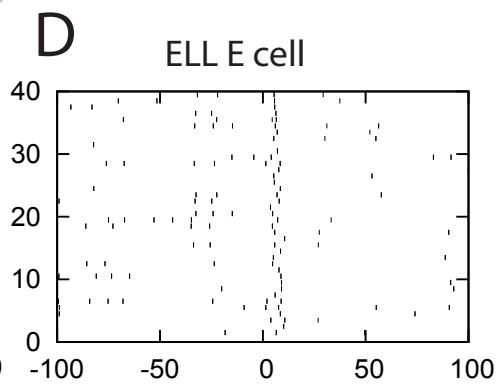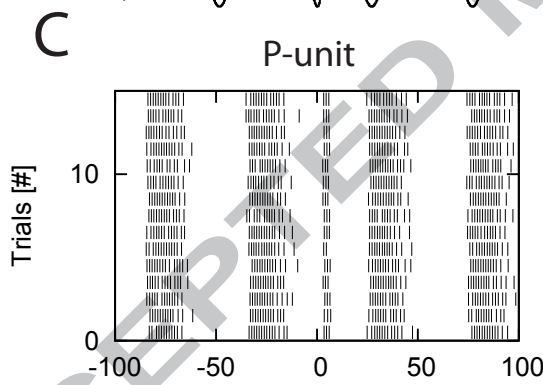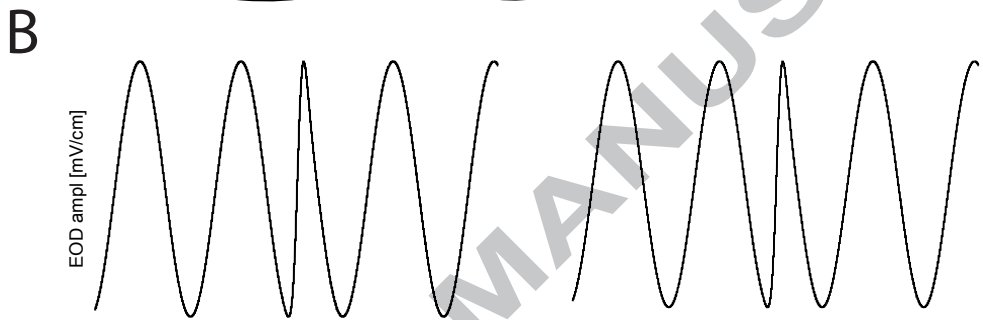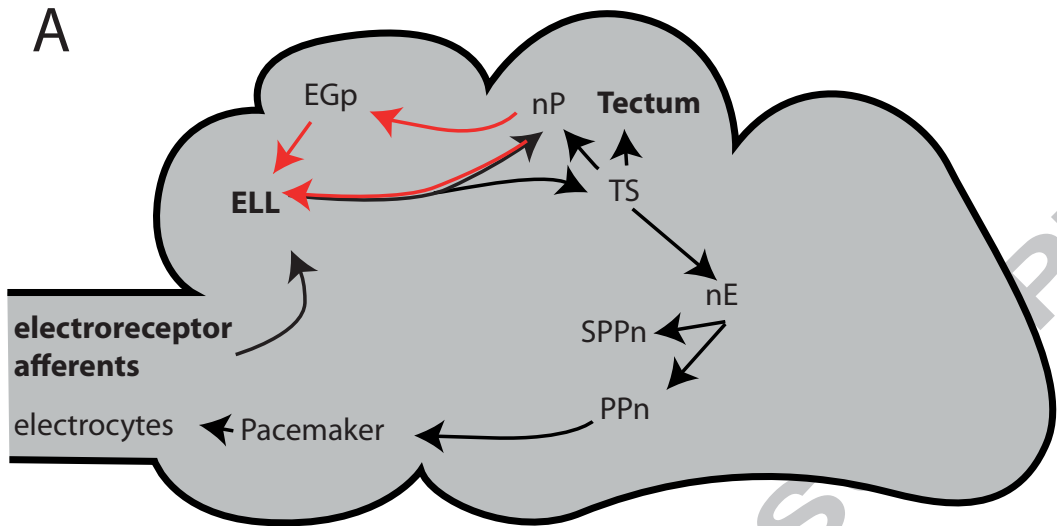
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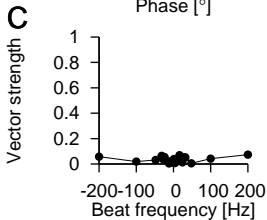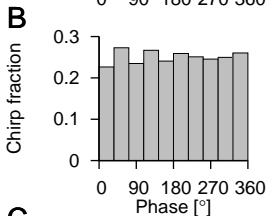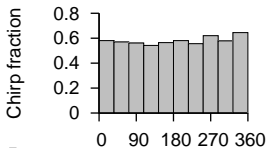Zupanc MM, Engler G, Midson A, Oxberry H, Hurst LA, Symon MR, Zupanc GKH (2001) Lightdark changes in spontaneous modulations of the electric organ discharge in the weakly electric fish, *Apteronotus leptorhynchus. Animal Behaviour* 62, 1119-1128
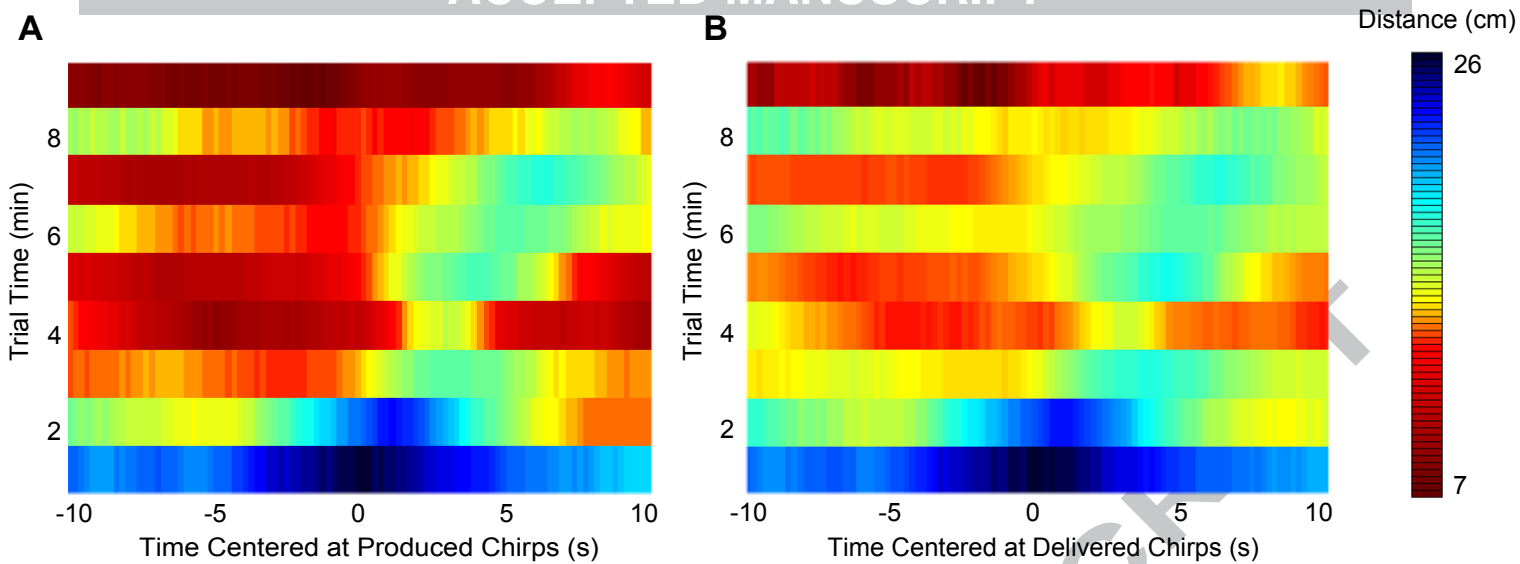
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A

B

C

D

E

F

A

B

C P-unit

D ELL E cell

E TS dense cell

TS sparse cell

A

B

C

Figure with panels A, B (Chirp fraction vs Phase [°]) and C (Vector strength vs Beat frequency [Hz])

A

B

Distance (cm)

- ⅄ Wave-type electric fish produce communication signals called chirps. We review chirping behaviour as well as how sensory neurons encode these signals.

- ⅄ We consider the electrosensory background resulting from the interaction of two fish and discuss how various social interactions shape both behaviour and sensory encoding.

- ⅄ We show how the encoding properties can explain some aspects of the behavior, but, to fully understand the complexities of the behavior will require more detailed knowledge of higher processing stages.