# Neural processing of communication signals: The extent of sender-receiver matching varies across species of *Apteronotus*

Simplified neural encoding of social signals

K.M. Allen[1] & G. Marsat[1]

[1]Department of Biology, West Virginia University, Morgantown, WV 26505

Author contributions: K.A.-Designed and performed the research, analyzed data, wrote the paper; G. M.-Designed research, created analytic tools, wrote the paper

Corresponding Author: G. Marsat gary.marsat@mail.wvu.edu

Figures: 7

Tables: 1

Abstract: 238

Significance Statement: 119

Introduction: 683

Discussion: 2186

Acknowledgments: We thank G.T. Smith for discussions about this project, data on chirp properties and feedback on the manuscript. We thank M.J. Chacron for comments on this manuscript.

Conflict of interest statement: The authors report no conflict of interest.

Funding was provided by West Virginia University and the National Science Foundation grant IOS-1557846.

## ABSTRACT

As communication signal properties change, through genetic drift or selective pressure, the sensory systems that receive these signals must also adapt to maintain sensitivity and adaptability in an array of contexts. Shedding light on this process helps us understand how sensory codes are tailored to specific tasks. In a species of weakly electric fish, *Apteronotus albifrons*, we examined the unique neurophysiological properties that support the encoding of electrosensory communication signals that the animal encounters in social exchanges. We compare our findings to known coding properties of the closely related species, *Apteronotus leptorhynchus*, to establish how these animals differ in their ability to encode their distinctive communication signals. While there are many similarities between these two species, we found notable differences leading to relatively poor coding of the details of chirp structure occurring on high-frequency background beats. As a result, small differences in chirp properties are poorly resolved by the nervous system. We performed behavioral tests to relate *A. albifrons* chirp coding strategies to its use of chirps during social encounters. Our results suggest that *A. albifrons* do not exchange frequent chirps in non-breeding condition, particularly when the beat frequency is high. These findings parallel the mediocre chirp coding accuracy in that they both point to a reduced reliance on frequent and rich exchange of information through chirps during these social interactions. Therefore, our study suggests that neural coding strategies in the central nervous system vary across species in a way that parallels the behavioral use of the sensory signals.

**SIGNIFICANCE:**

Sender-receiver matching is a phenomenon commonly observed in the peripheral nervous system. It enables communication production and reception to evolve together so that conspecifics remain sensitive to important signals. In this manuscript we examine this phenomenon in the central nervous system of the weakly electric fish *A. albifrons* and compare its processing of communication signals to a closely related species (*A. leptorhynchus)*. Although some differences across the two species can help tailor the system for processing species-specific signals, our data indicate that encoding of communication signals in *A. albifrons* is not as sensitive as in *A. leptorhynchus* for certain categories of signals. Our data support the idea that the extent of sender-receiver matching can vary as a function of behavioral needs.

## INTRODUCTION

Sender and receiver matching facilitating communication has been demonstrated across diverse groups, including songbirds (Brumm and Slabbekoorn, 2005), anurans (Schul and Bush, 2002), and insects (Neuhofer et al., 2008). Peripheral tuning and call matching are well characterized, and studies have shown evidence for sender and receiver matching in response to auditory coding of courtship song (Gerhardt and Schwartz, 2001; Tootoonian et al., 2012; Woolley and Moore, 2011) but little work has been done in other sensory modalities. We aim to identify species-specific variations in coding properties of central electrosensory neurons and link them to the divergent use of communication signals, showing that the extent of species-specific adaptations results in a variably tight sender-receiver match.

Weakly electric fish are ideal for examining diversification in signal production and reception. Apteronotids share a common mode of communication, modification of their electric organ discharge (EOD), but exhibit huge variety in signal structure. Voluntary modulations of EOD (chirps) vary dramatically in properties such as duration, frequency, and shape, even between closely related species (Turner et al., 2007; Zakon and Smith, 2002). Likewise, chirp reception is influenced by EOD waveform shape (Petzold et al., 2016), chirp features or structure (Benda et al., 2006; Marsat et al., 2012), and social environment (Stamper et al., 2010). The apteronotid electrosensory system could have coding properties generic enough to process these signals efficiently despite differences in structure and use between species; alternatively, differences in chirp processing could reflect adaptations of the electrosensory system to these differences in chirp production.

Relative EOD frequencies of interacting fish greatly influences chirp perception. These fish perceive ongoing amplitude modulations (AM beat) that are the product of two fish with

81 different EOD frequencies interacting at close range (Bastian, 1981). Chirping modulates this
82 beat (Hagedorn and Heiligenberg, 1985; Zupanc and Maler, 1993). In *A. leptorhynchus*, low
83 frequency beats are typical of agnostic encounters between fish of similar sex and size, and fish
84 respond with frequent small chirps (<100Hz increase) (Hupé and Lewis, 2008). Higher
85 frequencies occur between fish of opposite sexes or with large differences in body size and more
86 often elicit big chirps (>100Hz), typical of courtship (Hagedorn and Heiligenberg, 1985;
87 Henninger et al., 2018).

88 Chirp coding has been well characterized in *A. leptorhynchus.* The two categories of
89 signals described above produce different responses in the primary sensory area, the
90 electrosensory lateral line lobe (ELL). Small chirps on low frequency beats cause stereotyped
91 bursting among ELL pyramidal cells. This encoding strategy, and the structure of the signal
92 itself, means variations in small chirps cannot be discriminated (Allen and Marsat, 2018; Marsat
93 et al., 2009). Conversely, both big and small chirps on high frequency beats produce
94 heterogeneous responses and chirp variations are accurately discriminated (Allen & Marsat,
95 2018; Marsat & Maler, 2010).

96 The mechanisms of chirp production are similar in *A. albifrons* and *A. leptorhynchus*, but
97 chirp structure differs in a number of ways. Chirp duration is the most notable difference in these
98 species. *A. leptorhynchus* chirps are typically tens of milliseconds long, whereas *A. albifrons*
99 chirps are generally over 100 milliseconds long (Dunlap et al., 1998; Turner et al., 2007; Zupanc
100 and Maler, 1993). This lengthening is thought to be a recent evolutionary change, with shorter
101 chirps representing the basal state of this branch (Smith et al., 2016). *A. albifrons* show
102 differences in frequency tuning from *A. leptorhynchus* (Martinez et al., 2016) that may be
103 adaptations for coding these long chirps. Additionally, *A. albifrons* chirps typically do not fall

into discreet “small” and “big” chirp categories like those of *A. leptorhynchus* (Turner et al., 2007), and chirps of varying durations and frequency are used in all contexts (Kolodziejski et al., 2007). Therefore, it is unknown whether varying chirps have different neural and behavioral impacts in *A. albifrons*.

In this study, we examine coding of conspecific social signals and their behavioral use to understand if the sensory system is adapted to the specific characteristics of the communication system of *A. albifrons,* and if so, to what extent. We compare our findings to the behavior and physiology of the closely related *A. leptorhynchus* to identify specific neurophysiological adaptations that reflect differences in the structure and behavioral use of chirps in these two species.

115 **METHODS**

116 **Animals**

117 All animals were housed according to WVU IACUC standards, protocol 151200009.2.
118 Wild-caught *Apteronotus albifrons* and *Apteronotus leptorhynchus* were obtained from
119 commercial fish suppliers and housed in small groups (1-10 fish per tank). Tank conductivity
120 was maintained at 200-500 µS. Sex was not confirmed, but adult animals with a wide range of
121 EOD frequencies (600-1300Hz) were used for all experiments, indicating that we likely had
122 multiple animals of both sexes.

123 **Electrophysiology**

124 Surgical techniques were identical to those previously described in (Marsat et al., 2009;
125 Marsat and Maler, 2010). Cells of the LS of the ELL were targeted. *A. albifrons* brain anatomy is
126 very similar to that of *A. leptorhynchus*, so major landmark blood vessels described in Maler et
127 al., (1991) and electrode depth served as an adequate guide to locate LS pyramidal cells (see
128 Histology). *In vivo* recordings were made via metal filled extracellular electrodes (Frank and
129 Becker, 1964) and amplified with an A-M Systems amplifier (Model 1700). Data were recorded
130 (Axon Digidata 1500 and Axoscope software) at a 20kHz sampling rate. ON and OFF cells were
131 identified using known response properties, particularly responses to sinusoidal stimulation and
132 spike triggered average waveforms calculated from responses to 0-60Hz noise (Saunders and
133 Bastian, 1984).

134 **Histology**

135 In N=9 fish, correct electrode placement was confirmed by injection of Dextran Texas
136 Red dye (Thermo Fisher, catalogue # D1829) at recording site using double barreled electrodes,

similar to methods described in Krahe et al., (2008). After recording, dye was pressure injected with a PicoPump (WPI, PV820). Animals were then anaesthetized and respirated with a solution of Tricaine-S (.5g/L, Western Chemicals) and perfused with 4% paraformaldehyde (Electron Microscopy Supply, # 15712) in 1X PBS. Brains were postfixed overnight, sectioned (150 µm), and counterstained with Syto59 nuclear stain (Thermo Fisher, # S11341), which allowed for clear distinction between ELL segments. In all marked sections, correct placement within the LS was observed.

**Stimuli**

All stimuli were created in MatLab (Mathworks, Inc.) and sampled at 20 kHz. Stimulation was provided by a direct modulation of the amplitude (AM) of a carrier artificial EOD phase locked to the fish's own rather than by mimicking a second EOD. This method (described below) is commonly used in similar experiments (Benda et al., 2005; Krahe et al., 2008; Marsat et al., 2009) and allows precise control over the stimulus AM . Baseline EOD was recorded via electrodes near the head and tail of the fish. Each EOD cycle triggered a sine wave generator (Rigol DG1022A) to generate one cycle of a sine wave matched to the animal's own. This signal was then multiplied using a custom-built signal multiplier (courtesy of the Fortune Laboratory, New Jersey Institute of Technology) by the AM stimulus to create the desired modulation of the electric field. Stimuli were played through a stimulus isolator (A-M Systems, Model 2200) into the experimental tank via either two 30.5cm carbon electrodes arranged parallel to the fish's longitudinal axis (global stimulation) or two silver chloridized point electrodes 1 cm apart from each other positioned near the receptive field on the fish's skin (local stimulation). The stimulus strength was adjusted to provide ~20% contrast (the difference

between the maximum and minimum of amplitude modulation divided by the baseline EOD). See Fig1-3 for further comments on stimulus strength.

Random amplitude modulation (RAM) stimuli consisted of 30 seconds of random noise filtered using either a low pass (0-20Hz), band pass (40-60Hz) or broadband (0-60Hz) Butterworth filter. Each stimulus was played for three repetitions in both global and local stimulation configurations. Sinusoidal amplitude modulation (SAM) stimuli were 2 second long stimuli modulated at 2, 5, 15, 30, 60, and 90 Hz with 2 seconds rest between each frequency, repeated at least three times. Step stimulations were 100ms long increases or decreases in amplitude repeated for 30 seconds.

Chirp stimuli were created by using recorded EOD samples (courtesy of Dr. Troy Smith, Electric Fish Signal Library) to create a 1000 Hz template *A. albifrons* EOD shape, thereby accounting for the actual EOD shape and resulting AM beat shape. Chirps of varying durations, frequency increases, and frequency rise/fall time (Table 1) were each embedded in this template EOD by decreasing the template EOD period by the inverse of the desired frequency increase for each EOD cycle in the duration of the chirp. For each 1Hz of frequency increase, the template amplitude was decreased by .11%, based on Dunlap at al. (1998). Chirps were added at the rate of 1 chirp per second. To this chirper EOD, a second EOD, either 990Hz or 900Hz, was added to create a combined EOD signal with a beat AM frequency of 10 or 100Hz. The AM of this stimulus was extracted by rectifying and low-pass filtering the combined EOD signal. The AM signal was delivered during the experiments as described above. Each chirp stimulus was played for at least 30 seconds and up to 60 seconds. Due to time constraints, chirps were only played in the global configuration.

**Data Analysis**

For all analyses spike trains were first binarized into a sequence of 1's (spike) and 0's (no spike) using a bin width of 0.5ms. All analyses described here were performed on these binary sequences in MatLab. Statistical analyses (ANOVA, Student's t-Test, Wilcoxon rank sum) were performed using the MatLab's statistical analysis toolbox.

**Synchronization Coefficient**

The strength of phase locking to SAM stimulation was calculated as

$$s = \frac{\sqrt{(\sum x_i)^2 + (\sum y_i)^2}}{n}$$

where $n$ is the number of spikes in the analysis, and $x$ and $y$ are the cosine and sine of the stimulus' phase at which spike $i$ occurs (Goldberg and Brown, 1969; Marsat and Pollack, 2004). The vector strength, $s$, ranges from 0 to 1, with 1 being perfect precision in responding to a given phase of the cycle.

**Coherence**

Lower bound (stimulus-response) coherence is a measure of linear coding of the stimulus and is calculated by comparing the spike train against the RAM stimulus (Borst and Theunissen, 1999). Stimulus-response (S and R respectively) magnitude-squared coherence ($C_{SR}$) was calculated as a function of frequency ($f$) according to:

$$C_{SR}(f) = \frac{|P_{SR}(f)|^2}{P_{SS}(f)P_{RR}(f)}$$

where $P$ indicates the power-spectral or cross-spectral densities.

Upper bound (response-response) coherence measures the total information potentially coded in the response, including linear and non-linear information. It is measured by comparing multiple responses of one neuron to each other to determine response reliability (Borst and Theunissen, 1999). Coherence between responses *Ri* and *Rj* was calculated as:

$$C_{RiRj}(f) = \sqrt{\frac{P_{RiRj}(f)P_{RjRi}(f)}{P_{RiRi}(f)P_{RjRj}(f)}}$$

and averaged across all pairwise combinations of responses.

Envelope responses were determined by computing the lower-bound coherence between the responses and the envelope of the 40-60Hz RAM stimuli. To do so, the stimulus *S(t)* in the lower-bound analysis described above was replaced by the envelope *E(t)* calculated by rectifying and low pass filtering the 40-60Hz noise stimulus AM. Similar results were obtained when using a Hilbert transform to calculate the envelope signal.

**Burst detection**

Bursts in RAM and chirp responses were identified by creating a histogram of all inter-spike intervals (ISIs) in the response. Bursting neurons have a bimodal, non-Poisson distribution of ISIs, allowing us to visually identify a threshold ISI at the upper boundary of intra-burst intervals. Spikes in groups with ISIs below the threshold were classed as occurring in bursts, while all remaining spikes were classed as tonic. This method is similar to that described in (Ávila-Åkerberg et al., 2010)

**Chirp detection and discrimination analysis**

Analysis of chirps for detection and discrimination is based on Allen and Marsat (2018). Our analysis accounts for both the firing rate as well as the temporal pattern of spikes to quantify how similar or dissimilar spiking patterns are (van Rossum, 2001). For detection analysis, a window around the chirp ($R_c(t)$) of length $L$ (50, 105, or 205ms) was extracted from the filtered spike train and compared to a window of beat of the same size ($R_b(t)$). Different window sizes led to qualitatively similar results, and those using 205ms are shown. The responses were convolved with an α filter, $f(t) = t * \exp(-\frac{2.45t}{\tau})$ , with τ being the width of the function at half maximum (3, 10, 30, and 100ms; 30ms shown) (Machens et al., 2003). Detection accuracy is calculated for population responses by averaging multiple spike trains:

$$PR_i(t) = \frac{\sum_{i=t}^{n} R_i(t)}{n}$$

The result ($PR_i(t)$) represents a population of neurons presented with the same stimulus and mimics a neuron integrating postsynaptic potentials with similar weights. Distance ($D$) was calculated for all sets of combined responses, $PR_c(t)$ and $PR_b(t)$, creating an array of response distances for each comparison using the function:.

$$D_{cb} = \frac{\sum_{t=O}^{L} [R_c(t) - R_b(t)]^2}{L}$$

The probability distributions of the values in these arrays ($P(D_{cb})$ or $P(D_{bb})$) were used for analysis. Receiver operator characteristic curves were calculated by varying a threshold distance ($T$) to separate chirp and beat responses. For each threshold the probability of detection ($PD$) was calculated as the sum of ($P(D_{cb} > T)$), and the probability of false alarm ($PF$) as the sum of ($P(D_{bb} < T)$). The error level for each threshold value is $E=PF/2 +(1-PD)/2$. The values shown in Figure 3 are the minimum calculated values of $E$ as increasing numbers of spike trains are included in the calculation.

Discrimination analyses were similar to those for detection, but rather than comparing chirp and beat responses, the distances between two different chirps were compared. Similar to detection analysis, windows of chirp responses ($R(t)$) of length $L$ (50,105, and 205ms) were used for analysis and the results using 205ms are shown. The responses were convolved with an α filter and population averaged as described above. Up to 200 random combinations of spike trains from all recorded neurons were used for all comparisons as more become computationally prohibitive without improving results. Responses to a given chirp against a different chirp (x vs. y) were compared as well as multiple responses to the same chirp (x vs. x). Spike train metric distances ($D_{xy}$ or $D_{xx}$) the ensuing distribution and error rates were calculated as described for the detection analysis.

**Adaptation**

Adaptation to stimuli was measured by using the MatLab Curve fitting toolbox to fit an exponential curve to a plot of instantaneous firing rate for each neuron resulting in a time constant τ. The portion of the response to use for fitting was determined by selecting the time of peak firing rate and the following 500ms.

**Behavior**

Twenty-eight behavior trials were conducted in a small tank (27x27x14cm) containing water with conductivity, pH, and temperature matched to the home system, and one shelter tube. The tank was enclosed to block ambient light, lit with infrared lights and all trials were recorded via infrared camera (Logitech HD Pro Webcam C920). 14cm carbon rod electrodes placed diagonally from each other in each corner of the tank recorded electrical activity, which was then amplified (A-M Systems, Model 1700) and recorded using a computer sound card.

Stranger fish from different home tanks were paired. Defining physical features (size, markings) and EODf were noted to avoid repeatedly testing the same pairing. Individual fish were used no more than three times, with at least seven days between each trial. One fish was selected and allowed to acclimate to the test tank for 20 minutes before the introduction of the second fish. Recording began immediately upon introduction of the intruder fish. Interactions were recorded for five minutes.

To detect chirps, we used a custom MatLab script to create a spectrogram of the electrical recordings to identify individuals and mark chirp times. Chirps were visually identified as >10Hz abrupt frequency increases.

## RESULTS

### Diversity in conspecific chirp responses

In multiple gymnotid species the lateral segment (LS) of the ELL serves as the primary location for encoding of communication and social signals (Marsat et al., 2009; Metzner, 1999; Metzner and Juranek, 1997), thus we targeted our recordings to pyramidal cells in that segment. To characterize the pattern of responses of *A. albifrons* pyramidal cells we played a series of chirps mimicking the natural range of reported *A. albifrons* chirps (Turner et al., 2007). Additionally, for comparison we used a small number of chirps with properties more typical of *A. leptorhynchus* (Dunlap et al., 1998; Zupanc and Maler, 1993). Detailed descriptions of chirp properties used are located in Table 1. In addition to playing chirps with diverse properties, we also varied the frequency of the beat, presenting chirps on both low (10 Hz) and high (100 Hz) frequency signals.

Figure 1 displays a representative selection of the diversity seen in chirp responses for both ON-type and OFF-type pyramidal cells. Qualitatively, responses appear to be heterogeneous with variability between inhibition, excitation, and desynchronization. On high frequency beats (Fig 1A) we see a transient increase in tonic firing rate in OFF-cell while ON-cells are briefly and weakly inhibited during the chirp. On low frequency beats (Fig 1B), the responses also consisted of brief increases in firing rates, but were additionally characterized by periods of inhibition lasting the entire duration of the chirp. We did not observe synchronized burst firing across the population that is typical of *A. leptorhynchus*' responses to small chirps on low frequency beats (Marsat et al., 2009; Marsat and Maler, 2010), the strategy that limits ability to discriminate these chirps (Allen and Marsat, 2018). Therefore, all chirps appear to be coded primarily through increases in firing among OFF-cells, and inhibition and desynchronization and

297 cancelation of the beat (See Figure 1-1) of ON-cells. These results suggest that *A. albifrons*
298 pyramidal cells uses the same coding patterns for all chirp types, but possibly exhibit differences
299 based on beat frequency. Furthermore, a strong adaptation (see Figure 1-2) in firing rate seen
300 between the beginning and end of the chirp response could cause chirps of different length to be
301 poorly discriminated. Therefore, we next quantified how well the neural responses could support
302 both detection and discrimination of chirps.

303 **Detection of chirps varies with beat frequency**

304 We used a spike metric distance to measure the variability in firing patterns and how
305 reliably an ideal observer could distinguish chirp responses from beat responses. The results are
306 presented as an error level as a function of the number of neural inputs pooled in the analysis.
307 More efficient encoding would lead this measure to drop faster from 0.5 (chance level) as a
308 larger population of neurons is used in the detection task.

309 A small number of ON-cells (about eight) are sufficient to accurately detect chirps,
310 comparable to *A. leptorhynchus* (Marsat and Maler, 2010) (Fig 2A). However, this level of
311 accuracy only holds for chirps on the 10 Hz beat. ON-cell performance on 100 Hz is extremely
312 poor, even with high numbers of spike trains included in the analysis (Fig 2B). OFF-cells are
313 also able to detect chirp occurrence on low frequency beats for all chirp types (Fig 2C) but on
314 100 Hz detecting chirps was less reliable and accurate (Fig 2D). The data suggest that with a
315 large enough sample of neurons accurate detection is possible, but less robustly than on low
316 frequencies. Therefore, while chirps in all contexts are potentially detected, detection sensitivity
317 is much higher in low frequency contexts.

318 **Discrimination between chirps also varies**

We assessed the amount of information carried by the response pattern about chirp properties that could support the discrimination of chirp variants. This analysis is similar to that used for chirp detection, but rather than comparing responses of chirps to beats, we compare responses between chirps. While *A. albifrons* chirps are not typically grouped into distinct categories, we still use the terminology used in *A. lepthorhynchus* to describe the two ends of the spectrum of chirp properties (small< 150 Hz increase; big>150 Hz). Figure 3 shows discrimination ability for chirps grouped by size (See Table 1; Small chirps: 5, 6, 7, 8; Big chirps: 1, 2, 3, 4, 9, 10) for both ON and OFF-cells on high and low beat frequencies.

Chirps of all types are more easily discriminable when presented on 10Hz beats (Fig 3A) rather than on 100Hz beats (Fig 3B). Performance for chirps on high frequency beats was variable: accurate discrimination was achieved only for chirps with large differences in properties (Fig 3-1) and both ON and OFF cell responses allow qualitatively similar discrimination accuracy. These results are unexpected, considering that the same analysis in *A. leptorhynchus* shows that chirps on high frequency beats can be discriminated well using as few as six neurons, and conversely exhibit poorer discrimination on low frequencies (Marsat and Maler, 2010) where *A. albifrons* performs best. Furthermore, we do not observe an asymmetry in coding accuracy between ON and OFF cells as observed in *A. leptorhynchus*.

When looking at which chirps are well discriminated, chirp duration and the steepness of the frequency rise seem to be the most discriminable features, while total frequency increase and number of peaks are less influential on coding (Fig 3-1). However, discriminating the most easily separated chirps is prone to error and requires a larger pool of neurons on high frequency beats. These data indicate that while qualitative observations would suggest that chirp coding is similar between *A. albifrons* and *A. leptorhynchus*, there are quantitative differences in the information

content about chirps within the electrosensory system. To determine which aspects of the neural responses could be responsible for changes in chirp coding, we characterized a wide variety of response properties using an assortment of stimulation protocols.

**Frequency tuning and neural coding**

Encoding amplitude modulations (AM) in stimuli is crucial for the perception of social signals. As shown above, *A. albifrons* neurons respond variably to chirps, which consist of both high frequency AM signals and the low frequency changes in the contrast of the AM (i.e. the envelope). We asked whether the accuracy of detection and discrimination of chirps by *A. albifrons* ELL neurons reflects species-specific frequency tuning properties.

Communication signals cause spatially diffuse stimulation as compared to the localized electric fields of prey items. We simulate this difference by altering the geometry of our stimulating dipoles. Using a large dipole (see methods) that simulates diffuse communication signals drives both the feedforward responses from electroreceptor to pyramidal neuron and feedback pathways driven by broad receptive fields. To separate the effects of feedback from the tuning of the feedforward circuit we also stimulated only the receptive field of each cell with a small local dipole simulating localized objects or prey (Bastian, 1986; Chacron et al., 2005).

In response to simple sine waves, *A. albifrons* exhibit the highest degree of coding fidelity at low frequencies. Firing rate for each sine cycle peaks at 5Hz for all cell types and stimulation configurations (Fig 4A). Maximum phase locking also peaks at low frequencies (Fig 4B). These values closely match previously reported values (Martinez et al., 2016). Temporal coding accuracy is conveniently quantified using random noise stimuli and information theory

364 (Borst and Theunissen, 1999). Lower-bound coherence reflects the amount of information
365 encoded linearly whereas upper bound coherence takes in account both linear and non-linear
366 aspects of the response (Fig 4C). Both ON and OFF cell coherence curves peak at similar
367 frequencies in the 5-20 Hz range (Wilcox Rank Sum, p=.19). However, OFF-cells are clearly
368 low-pass, and exhibit a sharp decline in coherence to higher frequencies, while ON-cells exhibit
369 a slightly broader lower-bound coherence (see Fig 5 and accompanying text for more details).

370 The movement of fish as well as the amplitude changes caused by chirps create low
371 frequency changes in AM contrast, or envelopes (Stamper et al., 2013). During chirps, the AM
372 will be high frequency (tens to hundreds of hertz above the beat frequency) whereas the envelope
373 of the chirp is low frequency (e.g. 100ms chirp will lead to a ~ 10Hz envelope). Therefore, the
374 coding of chirps' low frequency content must be investigated using envelope stimuli. We
375 characterized envelope coding using stimuli consisting of RAMs with AM frequencies between
376 40Hz and 60Hz, containing envelope frequencies of 0-20 Hz (Middleton et al., 2006). Although
377 pyramidal cells do encode the envelope of these stimuli (Fig 4D), the coding accuracy is low
378 compared with the envelope coding observed in *A. leptorhynchus* (Chacron, 2006; Middleton et
379 al., 2006; Fig 5-1). OFF-cells are particularly relevant for chirp discrimination. This is because
380 chirps consist of decreases in envelope amplitude, which are best encoded by OFF-cells (Marsat
381 and Maler, 2010). The observed poor coherence to envelope stimuli, particularly for OFF-cells in
382 receiving global stimulation, may in part cause the poor chirp discrimination we observe.

383 **Burst Firing and Chirp Detection**

384 As described in Marsat et al. (2009) burst firing serves an important role in *A.*
385 *leptorhynchus* for the efficient detection of extremely short chirps. As qualitatively shown above
386 in Figure 1, no conspecific chirps reliably produced a burst response in *A. albifrons*. This implies

387 that *A. albifrons* may not be using this dedicated code for detection of very brief chirp signals. In
388 *A. leptorhynchus* the coding of small chirps is directly related to beat frequency. On low
389 frequencies, small chirps are shorter than the period of one beat cycle. As beat frequency
390 increases and the period becomes shorter, small chirps begin to last longer than one cycle. This
391 change in duration relative to beat period mediates a change in how small chirps are coded (Walz
392 et al., 2014). In *A. albifrons* all chirps are much longer than *A. leptorhynchus* small chirps,
393 spanning more than one beat cycle even on low frequencies, which may eliminate the need for a
394 feature detection code specifically for extremely short chirps. Thus, the observed lack of bursting
395 may result from the change either in signal structure (duration relative to beat cycle) or from
396 underlying changes to physiology and bursting capability of LS neurons.

397 To determine if this change in signal coding is a result of signal structure or underlying
398 physiology, we stimulated *A. albifrons* fish with *A. leptorhynchus* chirps known to elicit a burst
399 response in 73% of *A. leptorhynchus* LS ON-cells (Marsat et al., 2009). We determined burst
400 threshold ISI for each neuron by plotting ISI distribution of chirp and beat responses and
401 manually selecting a threshold that best distinguished between the two (Martinez-Conde et al.,
402 2002) in order to maximize our measure of chirp-specific bursting. The mean burst threshold was
403 8.0ms ±0.7ms (s.e.), comparable to the burst thresholds determined for our RAM analyses and in
404 other reports (Ávila-Åkerberg et al., 2010; Chacron and Bastian, 2008; Marsat et al., 2009).The
405 majority of neurons increased firing rate only slightly more in response to *A. leptorhynchus*
406 chirps than to the beat. There was, however, a small percentage (~ 20%) of ON-cells that did
407 reliably burst more in response to *A. leptorhynchus* small chirps than to the beat (Fig 5A, C).
408 Most importantly, even the bursty neurons did not respond to the shortest *A. albifrons* chirps
409 with bursts (Fig 5B, D). Unlike *A. leptorhynchus* small chirps, which may cause either amplitude

410 increases or decreases, depending on chirp time in relation to beat phase, *A. albifrons* chirps are
411 all long enough to span more than one beat cycle. This chirp length in relation to the beat period
412 means that the AM waveform caused by chirps is less variable and the response they elicit is
413 largely independent of the phase at which chirps start. This may mean that having a chirp-
414 invariant coding mechanism, such as bursting, is not necessary in this species.

415 The low proportion of neurons that burst in response to *A. leptorhynchus* chirps
416 demonstrates that there are physiologic differences between these two species in regards to burst
417 coding of communication signals. We examined the differences between bursty and non-bursty
418 neurons in more detail by analyzing separately their response to RAM stimuli. The subset of
419 bursty neurons have broader AM tuning (Fig 5C) as tested by comparing the ratio of coherences
420 summed over the 0-30Hz vs 30-60Hz (bursty cells mean ratio± s.d.: 0.91 ± 0.12; non-bursty cells
421 mean ratio: 2.35 ± 1.72; t-test not assuming equal variance, p= 0.0047; similar results were found
422 using the upper-bound coherence). While most ON-cells exhibited peak coherence at
423 frequencies between 10-20Hz (non-bursty cells mean peak frequency ± s.d.: 19.6 ± 18), these
424 cells had significantly higher peak frequencies as high as 40-50Hz (mean ± s.d.: 40 ± 13.9 Hz; t-
425 test, p=0.023) and exhibited high coherence across the range of stimulus frequencies. The
426 remainder of the ON cells displayed the low-pass tuning described in Martinez et al. (2016).
427 These data show that while a subset of ELL neurons are capable of bursting in response to short
428 chirp stimuli, they make up a small percentage of the overall ELL population and bursting does
429 not seem to play a role in conspecific chirp coding.

430 **Feature Detection**

431 Burst firing may not be a significant aspect of communication coding in *A. albifrons*, but
432 other uses for burst coding may be conserved. During spontaneous activity, we observed a

433 baseline firing rate of 13.54 Hz (±1.37 s.e.) with 17.01% (±2.55 s.e.) of spikes in bursts. Thus,
434 the pyramidal neurons of *A. albifrons* are physiologically capable of bursting. In *A.*
435 *leptorhynchus* burst firing can reliably signal the presence of spatially localized prey-like stimuli
436 (Gabbiani et al., 1996; Oswald et al., 2004). We examined bursting in response to local RAM
437 stimulation (Fig 6A) to determine if bursting could serve similar prey detection functions in *A.*
438 *albifrons*. The ISI histogram of the responses clearly showed that the neurons burst to these
439 stimuli (Fig 6B). The proportion of spikes occurring in bursts was as high as 63.07% (±5.64 s.e.)
440 for stimuli in the local configuration (Fig 6C). The average stimulus waveform triggering burst
441 vs single spikes follows the pattern observed in other species: slower AM for bursts than for
442 single spikes (Fig 6D). However, in response to RAM stimuli, unlike *A. leptorhynchus* there was
443 not a large difference in coding error between bursting and tonic spiking (ANOVA, p=.10) (Fig
444 6E). These data suggest that while bursts encode low frequency stimuli in the LS of *A. albifrons,*
445 they do not appear to implement a burst-based feature detection code for either prey-like stimuli
446 or chirps.

447 **Chirp production and behavior**

448 Our physiology data suggest that *A. albifrons* are able to detect and discriminate chirps
449 on low frequency beats much more sensitively than on high frequency beats. To test this
450 hypothesis, we recorded electrical behavior from pairs of freely swimming and interacting fish.
451 Chirping behavior did occur but differed from that of *A. leptorhynchus* in a number of ways.
452 Primarily, overall rates of chirping are dramatically lower than numbers reported from similar
453 studies in *A. leptorhynchus* (Hupé and Lewis, 2008; Zupanc et al., 2006). Over 28 trials, we
454 recorded a sum of 133 chirps, with a mean of 4.75 (±0.61 s.e.) chirps per 5-minute trial whereas
455 in a similar context male *A. leptorhynchus* produced approximately of 125 chirps per 5 minute

456 trials (Hupé and Lewis, 2008). The maximum number of chirps observed in one trial was 13.
457 Due to the small number of observed chirps, we did not separate them into multiple categories
458 for analysis, although chirps of varying frequency increases and duration occurred. Chirping
459 frequency is correlated with the difference in EODf of interacting fish. Smaller differences in
460 EODf correspond to higher numbers of chirps (Fig 7A). However, under our conditions chirping
461 does not appear to be sexually dimorphic. Animals used in behavior experiments were not killed
462 to determine sex, but we grouped them by EODf into high (>1100Hz) and low (<1100Hz)
463 frequency groups which can correspond to females and males respectively in many populations
464 of *A. albifrons* (Zakon and Dunlap, 1999). We observed no differences in chirp production
465 between high or low frequency groups (Fig 7B). Further, chirp rate does not vary by pairing
466 type, nor by relative EODf of individuals within each pair under our conditions (Fig 7B). These
467 results confirm previous findings that chirp frequency in this species is not sexually dimorphic
468 (Dunlap et al., 1998).

469 Chirp timing plays an important role in mediating *A. leptorhynchus* interactions as they
470 frequently exchange chirps in an echo response pattern (Henninger et al., 2018; Hupé and Lewis,
471 2008; Zupanc et al., 2006), a behavior where one fish responds to the chirp of another fish by
472 chirping back within <1s . To determine if chirp timing plays a similar role in *A. albifrons*
473 interactions, we analyzed inter-chirp intervals for all trials in which both fish chirped. The
474 resulting distribution of all chirp latencies between fish indicates inter-chirp latencies much
475 longer than are typical of *A. leptorhynchus* echo responses (Fig 7C). Of all recorded chirps, only
476 two occurred within 2.5s of each other, making an echo response unlikely to be an important
477 feature of *A. albifrons* interactions. Furthermore, inter-chirp intervals for a given fish follow a
478 Poisson distribution (7D) indicating that chirp production is not produced in stereotyped

479 sequences. The lack of echoing, the long inter-chirp intervals, and the low rate of chirping in
480 general, suggest that *A. albifrons* do not rely on the detailed decoding of individual chirp
481 characteristics as is the case in *A. leptorhynchus*.

482

## DISCUSSION

We found that neurons in *A. albifrons'* primary sensory area are able to detect chirp occurrence and do encode chirp identities on low frequency beats. However, they discriminate between chirps less accurately when chirps are presented on high frequency beats. Rather than producing discrete burst responses for small chirps and tonic responses for big chirps, all chirps in this species are encoded via graded and heterogeneous increase or decreases in firing rate. We found several adaptations in the basic response properties that may explain the neural responses to conspecific chirps. These adaptations, frequency tuning in particular, may facilitate encoding long chirps in *A. albifrons* as well as enhancing chirp coding in the behavioral context in which chirps are used most often. Therefore, we also asked whether chirping behavior could explain differences in chirp coding efficiency for high and low frequency beats. We found that the pattern of chirp use during common social interactions is very different from that of closely related species: *A. albifrons* produces very few chirps and do not respond to each other with echo chirps. Infrequent chirping is particularly pronounced in the context of high-frequency beats suggesting that certain types of interactions are not mediated by chirps. We suggest that the observed differences in chirp coding accuracy on high frequency beats may reflect a relaxation in demands for exchanging information via chirps in this context.

### Low frequency tuning could be adaptive for chirp coding

Our results confirm that pyramidal cells in the LS of *A. albifrons* are low pass. This finding parallels the low frequency envelopes produced by long *A. albifrons* chirps. However, the impact of this AM tuning on chirp coding depends on the way AMs and envelopes are processed in this system. Whereas the envelopes of chirps are low frequency, the AM are high frequency. Therefore, low frequency tuning to AM is not automatically helpful. We found that

506 the coding of low-frequency envelopes was relatively poor, particularly in OFF cells, which -in
507 *A. leptorhynchus*- are best at coding chirp envelopes. However, if envelope response is
508 synthesized before the pyramidal cells (e.g. at the electroreceptors as could be the case in *A.*
509 *leptorhynchus* (Savard et al., 2011)) low frequency tuning to AM could benefit chirp coding
510 since the input to the pyramidal cells would contain low-frequencies (Metzen et al., 2018).
511 Nevertheless, we show that the neural response does not accurately reflect the chirp envelope
512 and that envelope coding of decreases in amplitude of RAM envelope stimuli is poor. Our data
513 replicate the findings of Martinez et al. (2016) that also show poor coding of envelope stimuli,
514 with coherence values of approximately 0.1. It is possible that without low frequency tuning,
515 chirp and envelope coding would be even worse. Thus, low frequency tuning may compensate
516 for other properties that hinder envelope coding (e.g. amount of rectification performed by the
517 receptors; (Savard et al., 2011)).

518 **The role of burst coding**

519 Bursts also often play an important role in coding specific features of communication
520 signals (Creutzig et al., 2009; Fujimoto et al., 2011) as it is the case for *A. leptorhynchus* where
521 bursting enhances detectability of small chirps on low frequency beats (Marsat et al., 2009). *A.*
522 *leptorhynchus* small chirps are unusual in that, for low frequency beats, they span less than a full
523 cycle of the beat. Consequently, the same chirp can be perceived as a sharp decrease in
524 amplitude, a sharp increase, or a mix of the two, depending on the phase of the beat at which the
525 chirp starts (Benda et al., 2005). It may be because of this aspect of chirp structure and its
526 shortness, that the sensory system of *A. leptorhynchus* uses bursts to enhance detectability rather
527 that optimizing the coding of these chirps' properties. *A. albifrons* is not subject to the same
528 constraints. The perceived AM of a chirp is largely independent of beat phase since chirps span

529 several cycles of the beat and their long duration might make them more conspicuous (Petzold et
530 al., 2016). Using noise stimuli, we showed that burst coding in the LS is different in *A. albifrons*.
531 Since bursts are not used to encode chirps, and because LS primarily focuses on processing
532 communication signals, it is possible that these neurons do not need to use burst as a feature
533 detection signaling mechanism as in other species (Gabbiani et al., 1996; Oswald et al., 2004).

534 **Chirp coding and behavior**

535 Our chirp response analysis shows that all chirps are coded with graded increases and
536 decreases in firing rate containing some information about chirp properties but, for high
537 frequency beats, coding accuracy is inefficient at supporting both chirp detection and
538 discrimination. Our behavioral data might shed some light on this apparent inefficiency at coding
539 chirps across all social contexts. We show that, in our conditions, *A. albifrons* chirp relatively
540 infrequently to meditate dyad interactions. Furthermore, we show that there is a tendency to
541 chirp less when the beat frequency is higher. This is supported by previous findings showing that
542 chirp production is more frequent in interactions involving low frequency beats (Kolodziejski et
543 al., 2007) reiterating the relevance of chirp coding on low-frequency beats rather than high
544 frequency interactions.

545 **Comparison to *A. leptorhynchus* and other electric fish**

546 Our physiology and behavioral findings are markedly different than those observed in the
547 closely related *A. leptorhynchus.* Primarily, our data show *A. albifrons* exhibit poor chirp coding
548 on high beat frequencies, and efficient coding on low beat frequencies. This is directly in
549 contrast with *A. leptorhynchus* that encode chirps on high frequency stimuli well and exhibit
550 poorer coding of chirp identity on low frequency beats (Allen and Marsat, 2018; Marsat and

551 Maler, 2010). Furthermore, we find that *A. albifrons* do not rely on the same coding strategy as
552 used in *A. leptorhynchus* since feature detection through bursts is not involved in chirp coding.
553 Our data clearly demonstrate that despite being so closely related (Smith et al., 2016) the sensory
554 coding properties of the neurons encoding communication signals have diverged between *A.*
555 *leptorhynchus* and *A. albifrons.* Chirps have been suggested to be short in the ancestors basal to
556 the two species (Smith et al., 2016) and longer chirps a development of the *A. albifrons* branch.
557 Therefore, it is possible that the differences in frequency tuning we observed in *A. albifrons* may
558 be adaptive for coding these long chirps.

559 Our behavioral results suggest that *A. albifrons* also use chirp exchanges very differently
560 than *A. leptorhynchus*. Unlike the complex chirp interactions observed in *A. leptorhynchus*,
561 where chirps are omnipresent and play a central role in various type of interactions (Hagedorn
562 and Heiligenberg, 1985; Henninger et al., 2018; Hupé, 2012) *A. albifrons* chirp infrequently and
563 generally only in low frequency difference interactions (Kolodziejski et al., 2007). While *A.*
564 *leptorhynchus* have dedicated uses for different chirp types (Henninger et al., 2018), *A. albifrons*
565 use all types of chirps interchangeably for low beat frequency interactions and may not possess
566 distinct chirp categories (Dunlap et al., 1998; Kolodziejski et al., 2007; Turner et al., 2007). The
567 link between chirp properties and behavior in *A. albifrons* is also less clear than in *A.*
568 *leptorhynchus* (Dunlap and Larkins-Ford, 2003). Therefore, we suggest that rather than having
569 distinct coding strategies for different chirp types, they have adopted context specific coding to
570 better encode chirps on low frequency beats where chirps are most likely to be produced.

571 Chirping behavior has been investigated across many species of wave-type gymnotiforms
572 (Hagedorn and Heiligenberg, 1985; Petzold et al., 2018, 2016; Turner et al., 2007). Details of
573 chirp encoding, however, have only been investigated in one other species, *Eigenmannia*

574 *viscerens* (Metzner and Heiligenberg, 1991; Metzner and Viete, 1996; Stöckl et al., 2014). In this
575 species, similarly to *A. leptorhynchus*, different chirp types are used to mediate agonistic and
576 courtship encounters (Hagedorn and Heiligenberg, 1985). In this species, unlike the apteronotids,
577 chirp coding appears to be unaffected by beat frequency, at least at the level of electroreceptors
578 (Stöckl et al., 2014). However, the coding strategy observed in the ELL pyramidal cells is that
579 the duration of the chirp is the primary feature encoded. This feature is coded via brief excitation,
580 then inhibition of both ON and OFF-cells (dependent on sign of the DC component of the chirp)
581 that signals the duration of the chirp (Metzner and Heiligenberg, 1991). This strategy is similar
582 to the coding observed in *A. albifrons* in low frequency interactions, suggesting that perhaps *A.*
583 *leptorhynchus* represent the outlier group, and burst detection of chirps is a specialization for
584 their small chirps.

585 **Conditions affecting chirp coding**

586 Our study did not test for the effects of neuromodulation on neural tuning and chirp
587 coding. Neuromodulation can change cell response properties for different behavioral states
588 (Harris-Warrick and Marder, 1991). Previous work in *A. leptorhynchus* shows that serotonin
589 enhances pyramidal cell excitability and responsiveness to small chirps on low frequency beats
590 (Deemyad et al., 2013). While we worked on adult animals, we did not determine sex or
591 breeding status, both of which have large effects of the frequency and quality of chirps produced
592 (Smith, 2013). It is likely that the effects of neuromodulation due to behavioral state could affect
593 the reception and encoding of these chirps as well, altering sensitivity to chirps and possibly even
594 coding accuracy in response to behavioral need. This may particularly influence the coding of
595 chirps on high frequency beats, which we observed was surprisingly poor. This kind of
596 interaction is more likely to occur in breeding contexts, so it is possible that animals in breeding

condition could be better able to detect and discriminate these signals than the results we show here. We also did not investigate other environmental factors that could influence the sensory specializations of both species, such as microhabitats, prey capture, and general sociality, all of which could drive some of the adaptations seen.

Our analysis technique is widely used to quantify the encoding performance of sensory neurons (Itatani and Klump, 2014; Mouterde et al., 2017; Neuhofer and Ronacher, 2012), and is biologically realistic (Larson et al., 2009). Nevertheless, other analysis technics could be devised to improve the discrimination accuracy estimate. Weighting the contribution of the different neurons, or weighting them differently across time could be envisioned (Larson et al., 2010). Keeping the different neurons as separate dimensions in the analysis (Houghton and Sen, 2008) or combining ON and OFF-cell responses (Aumentado-Armstrong et al., 2015) could also improve discrimination, as could accounting for neural correlation in response variability (Hofmann and Chacron, 2017). Finally, various more complex decoding methods (e.g. multilayered neural net or PCA-type dimensionality reduction) could be used for analysis. Nevertheless, we do not expect the neural mechanism to differ widely across apteronotid species so our analysis provides a meaningful comparison. The strength of our measure is that it is conservative: it gives a clear account of the information available in ELL pyramidal cells with few assumptions about what the downstream networks use for decoding.

**Trade-offs between specialization and generalization**

Classical neuroethology dictates that the mode of signal production and mode of signal reception must evolve in synchrony so that senders and receivers never lose the ability to

619 exchange information (Bradbury and Vehrencamp, 2011). There are many examples of
620 specialization of particular aspects of sensory systems to accomplish a highly specialized tasks
621 (Endler, 1992). In the case of communication, sensory tuning for sender-receiver matching has
622 been shown repeatedly. However, the converse may also be true. Over-specialization may come
623 at the cost of reduced sensitivity to more general environmental signals. In such a case, it may be
624 more beneficial to favor sensory generalization over specialization in animals that engage in
625 social behaviors less often than their more gregarious relatives do. Indeed, there are several
626 examples of peripheral sender-receiver mismatching that may be explained by gains in
627 sensitivity to prey or predator signals to (Mason, 1991; Römer, 2016). While we see in *A.*
628 *leptorhynchus* dedicated codes for communication signals with distinct meaning and in distinct
629 contexts (Allen and Marsat, 2018; Marsat and Maler, 2010), maintaining that level of specificity
630 for conspecific communication may be costly both metabolically (Niven and Laughlin, 2008)
631 and in regards to detecting environmental stimuli apart from communication signals.

632 In *A. albifrons* we show a general match between signal characteristics, low frequency
633 chirp envelopes, and CNS sensitivity to low frequency signals, but a lack of detailed coding that
634 would allow for efficient discrimination of chirp identity in all contexts. Additional changes that
635 would lead to a more accurate chirp coding could come at the expense of the ability to encode
636 other stimuli. Therefore, for this species, limiting the resources dedicated to the coding of social
637 signals in certain contexts (e.g. aggressive encounters with a fish with a high difference in EOD
638 frequency) may allow the system to preserve or enhance sensitivity to other stimuli.

639

640

641 **BIBLIOGRAPHY**


642 Allen KM, Marsat G (2018) Task-specific sensory coding strategies matched to detection and
643 discrimination behaviors in Apteronotus leptorhynchus. J Exp Biol In Press.

644 Aumentado-Armstrong T, Metzen MG, Sproule MKJ, Chacron MJ (2015) Electrosensory
645 Midbrain Neurons Display Feature Invariant Responses to Natural Communication Stimuli.
646 PLoS Comput Biol 11:1–28.

647 Ávila-Åkerberg O, Krahe R, Chacron MJ (2010) Neural heterogeneities and stimulus properties
648 affect burst coding in vivo. Neuroscience 168:300–313.

649 Bastian J (1986) Gain control in the electrosensory system: a role for the descending projections
650 to the electrosensory lateral line lobe. J Comp Physiol A 158:505–515.

651 Bastian J (1981) II. The effects of moving objects and other electrical stimuli on the activities of
652 two categories of posterior lateral line lobe cells in Apteronotus albifrons. J Comput Physiol
653 144:481–494.

654 Bastian J, Chacron MJ, Maler L (2004) Plastic and nonplastic pyramidal cells perform unique
655 roles in a network capable of adaptive redundancy reduction. Neuron 41:767–779.

656 Benda J, Longtin A, Maler L (2006) A synchronization-desynchronization code for natural
657 communication signals. Neuron 52:347–358.

658 Benda J, Longtin A, Maler L (2005) Spike-frequency adaptation separates transient
659 communication signals from background oscillations. J Neurosci 25:2312–21.

660 Borst A, Theunissen FE (1999) Information theory and neural coding. Nat Neurosci 2:947–957.

661 Bradbury JW, Vehrencamp SL (2011) Principles of animal communication, 2nd ed. Sunderland,

662 Mass., Mass.: Sinauer Associates.

663 Brumm H, Slabbekoorn H (2005) Acoustic Communication in Noise. Adv Study Behav.

664 Chacron MJ (2006) Nonlinear Information Processing in a Model Sensory System. J
665 Neurophysiol 95:2933–2946.

666 Chacron MJ, Bastian J (2008) Population coding by electrosensory neurons. J Neurophysiol
667 99:1825–1835.

668 Chacron MJ, Maler L, Bastian J (2005) Feedback and feedforward control of frequency tuning to
669 naturalistic stimuli. J Neurosci 25:5521–5532.

670 Creutzig F, Wohlgemuth S, Stumpner A, Benda J, Ronacher B, Herz AVM (2009) Timescale-
671 Invariant Representation of Acoustic Communication Signals by a Bursting Neuron. J
672 Neurosci 29:2575.

673 Deemyad T, Metzen MG, Pan Y, Chacron MJ (2013) Serotonin selectively enhances perception
674 and sensory neural responses to stimuli generated by same-sex conspecifics. Proc Natl Acad
675 Sci U S A 110:19609–14.

676 Dunlap KD, Larkins-Ford J (2003) Diversity in the structure of electrocommunication signals
677 within a genus of electric fish, Apteronotus. J Comp Physiol A 189:153–161.

678 Dunlap KD, Thomas P, Zakon HH (1998) Diversity of sexual dimorphism in
679 electrocommunication signals and its androgen regulation in a genus of electric fish,
680 Apteronotus. J Comp Physiol A Sensory, Neural, Behav Physiol 183:77–86.

681 Endler JA (1992) Signals, Signal Conditions, and the Direction of Evolution. Am Nat 139:S125–
682 S153.

683 Frank K, Becker MC (1964) Microelectrodes for recording and stimulation In: Physical
684 Techniques in Biological Research (Nastuk WL ed), pp23–84. New York: Academic Press.

685 Fujimoto H, Hasegawa T, Watanabe D (2011) Neural Coding of Syntactic Structure in Learned
686 Vocalizations in the Songbird. J Neurosci 31:10023.

687 Gabbiani F, Metzner W, Wessel R, Koch C (1996) From stimulus encoding to feature extraction
688 in weakly electric fish. Nature 384:564–567.

689 Gerhardt HC, Schwartz JJ (2001) Auditory tuning and frequency preferences. Anuran Commun
690 Smithson Inst Press Washingt.

691 Goldberg JM, Brown PB (1969) Response of binaural neurons of dog superior olivary complex
692 to dichotic tonal stimuli: some physiological mechanisms of sound localization. J
693 Neurophysiol 32:613–636.

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

696 Harris-Warrick RM, Marder E (1991) Modulation of neural networks for behavior. Annu Rev
697 Neurosci 14:39–57.

698 Henninger J, Krahe R, Kirschbaum F, Grewe J, Benda J (2018) Statistics of natural
699 communication signals observed in the wild identify important yet neglected stimulus
700 regimes in weakly electric fish. J Neurosci 0350-18.

701 Hofmann V, Chacron MJ (2017) Differential receptive field organizations give rise to nearly
702 identical neural correlations across three parallel sensory maps in weakly electric fish. PLoS
703 Comput Biol 13:e1005716.

Houghton C, Sen K (2008) A New Multineuron Spike Train Metric. Neural Comput 20:1495–1511.

Hupé GJ (2012) Electrocommunication in a Species of Weakly Electric Fish Apteronotus Leptorhynchus: Signal Patterning and Behaviour. Université d'Ottawa/University of Ottawa.

Hupé GJ, Lewis JE (2008) Electrocommunication signals in free swimming brown ghost knifefish, Apteronotus leptorhynchus. J Exp Biol 211:1657–1667.

Itatani N, Klump GM (2014) Neural correlates of auditory streaming in an objective behavioral task. Proc Natl Acad Sci 111:10738 LP-10743.

Kolodziejski J a, Sanford SE, Smith GT (2007) Stimulus frequency differentially affects chirping in two species of weakly electric fish: implications for the evolution of signal structure and function. J Exp Biol 210:2501–2509.

Krahe R, Bastian J, Chacron MJ (2008) Temporal processing across multiple topographic maps in the electrosensory system. J Neurophysiol 100:852–67.

Larson E, Billimoria CP, Sen K (2009) A biologically plausible computational model for auditory object recognition. J Neurophysiol 101:323–331.

Larson E, Perrone BP, Sen K, Billimoria CP (2010) A Robust and Biologically Plausible Spike Pattern Recognition Network. J Neurosci 30:15566 LP-15572.

Machens CK et al. (2003) Single auditory neurons rapidly discriminate conspecific communication signals. Nat Neurosci 6:341–342.

Maler L, Sas E, Johnston S, Ellis W (1991) An atlas of the brain of the electric fish Apteronotus

725 leptorhynchus. J Chem Neuroanat 4:1–38.

726 Marsat G, Maler L (2010) Neural heterogeneity and efficient population codes for
727 communication signals. J Neurophysiol 104:2543–55.

728 Marsat G, Pollack GS (2004) Differential temporal coding of rhythmically diverse acoustic
729 signals by a single interneuron. J Neurophysiol 92:939–48.

730 Marsat G, Proville RD, Maler L (2009) Transient signals trigger synchronous bursts in an
731 identified population of neurons. J Neurophysiol 102:714–723.

732 Martinez-Conde S, Macknik SL, Hubel DH (2002) The function of bursts of spikes during visual
733 fixation in the awake primate lateral geniculate nucleus and primary visual cortex. Proc Natl
734 Acad Sci U S A 99:13920–13925.

735 Martinez D, Metzen MG, Chacron MJ (2016) Electrosensory processing in Apteronotus
736 albifrons: implications for general and specific neural coding strategies across wave-type
737 weakly electric fish species. J Neurophysiol 116:2909–2921.

738 Mason AC (1991) Hearing in a primitive ensiferan: the auditory system of Cyphoderris
739 monstrosa (Orthoptera: Haglidae). J Comp Physiol A 168:351–363.

740 Metzen MG, Huang CG, Chacron MJ (2018) Descending pathways generate perception of and
741 neural responses to weak sensory input. PLOS Biol 16:e2005239.

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

744 Metzner W, Heiligenberg W (1991) The coding of signals in the electric communication of the
745 gymnotiform fish Eigenmannia: From electroreceptors to neurons in the torus semicircularis

746 of the midbrain. J Comp Physiol A 169:135–150.

747 Metzner W, Juranek J (1997) A sensory brain map for each behavior? Proc Natl Acad Sci 94.

748 Metzner W, Viete S (1996) The Neuronal Basis of Communication and Orientation in the
749 Weakly Electric Fish, EigenmanniaI. Communication Behavior or: Seeking a Conspecific?s
750 Response. Naturwissenschaften 83:6–14.

751 Middleton JW, Longtin A, Benda J, Maler L (2006) The cellular basis for parallel neural
752 transmission of a high-frequency stimulus and its low-frequency envelope. Proc Natl Acad
753 Sci U S A 103:14596–601.

754 Mouterde SC, Elie JE, Mathevon N, Theunissen FE (2017) Single Neurons in the Avian
755 Auditory Cortex Encode Individual Identity and Propagation Distance in Naturally
756 Degraded Communication Calls. J Neurosci 37:3491 LP-3510.

757 Neuhofer D, Ronacher B (2012) Influence of Different Envelope Maskers on Signal Recognition
758 and Neuronal Representation in the Auditory System of a Grasshopper. PLoS One
759 7:e34384.

760 Neuhofer D, Wohlgemuth S, Stumpner A, Ronacher B (2008) Evolutionarily conserved coding
761 properties of auditory neurons across grasshopper species. Proc R Soc B Biol Sci 275:1965–
762 1974.

763 Niven JE, Laughlin SB (2008) Energy limitation as a selective pressure on the evolution of
764 sensory systems. J Exp Biol 211:1792 LP-1804.

765 Oswald A-MM, Chacron MJ, Doiron B, Bastian J, Maler L (2004) Parallel processing of sensory
766 input by bursts and isolated spikes. J Neurosci 24:4351–4362.

Petzold JM, Alves-Gomes JA, Smith GT (2018) Chirping and asymmetric jamming avoidance responses in the electric fish <em>Distocyclus conirostris</em> J Exp Biol 221:jeb178913.

Petzold JM, Marsat G, Smith GT (2016) Co-adaptation of electric organ discharges and chirps in South American ghost knifefishes (Apteronotidae). J Physiol.

Römer H (2016) Matched Filters in Insect Audition: Tuning Curves and Beyond BT - The Ecology of Animal Senses: Matched Filters for Economical Sensing In: (von der Emde G, Warrant E eds), pp83–109. Cham: Springer International Publishing.

Saunders J, Bastian J (1984) The physiology and morphology of two types of electrosensory neurons in the weakly electric fish Apteronotus leptorhynchus. J Comp Physiol A 154:199–209.

Savard M, Krahe R, Chacron MJ (2011) Neural heterogeneities influence envelope and temporal coding at the sensory periphery. Neuroscience 172:270–284.

Schul J, Bush SL (2002) Non-parallel coevolution of sender and receiver in the acoustic communication system of treefrogs. Proc R Soc London Ser B Biol Sci 269:1847 LP-1852.

Smith AR, Proffitt MR, Ho WW, Mullaney CB, Maldonado-Ocampo JA, Lovejoy NR, Alves-Gomes JA, Smith GT (2016) Evolution of electric communication signals in the South American ghost knifefishes (Gymnotiformes: Apteronotidae): A phylogenetic comparative study using a sequence-based phylogeny. J Physiol 110:302–313.

Smith GT (2013) Evolution and hormonal regulation of sex differences in the electrocommunication behavior of ghost knifefishes (Apteronotidae). J Exp Biol 216:2421

LP-2433.

Stamper SA, Carrera-G E, Tan EW, Fugère V, Krahe R, Fortune ES (2010) Species differences in group size and electrosensory interference in weakly electric fishes: Implications for electrosensory processing. Behav Brain Res 207:368–376.

Stamper SA, Fortune ES, Chacron MJ (2013) Perception and coding of envelopes in weakly electric fishes. J Exp Biol 216:2393 LP-2402.

Stöckl A, Sinz F, Benda J, Grewe J (2014) Encoding of social signals in all three electrosensory pathways of Eigenmannia virescens. J Neurophysiol 112:2076–2091.

Tootoonian S, Coen P, Kawai R, Murthy M (2012) Neural Representations of Courtship Song in the Drosophila Brain. J Neurosci 32:787 LP-798.

Turner CR, Derylo M, de Santana CD, Alves-Gomes J a, Smith GT (2007) Phylogenetic comparative analysis of electric communication signals in ghost knifefishes (Gymnotiformes: Apteronotidae). J Exp bBology 210:4104–4122.

van Rossum MC (2001) A novel spike distance. Neural Comput 13:751–763.

Walz H, Grewe J, Benda J (2014) Static frequency tuning properties account for changes in neural synchrony evoked by transient communication signals. J Neurophysiol 112:752–765.

Woolley SMN, Moore JM (2011) Coevolution in communication senders and receivers: vocal behavior and auditory processing in multiple songbird species. Ann N Y Acad Sci 1225:155–165.

Zakon HH, Dunlap KD (1999) Sex steroids and communication signals in electric fish: a tale of two species. Brain Behav Evol 54:61–9.

809 Zakon HH, Smith GT (2002) Weakly electric fish: behavior, neurobiology, and
810 neuroendocrinology. Horm brain Behav 3:349–375.

811 Zupanc GKH, Maler L (1993) Evoked chirping in the weakly electric fish Apteronotus
812 leptorhynchus : a quantitative biophysical analysis. Can J Zool 71:2301–2310.

813 Zupanc GKH, Sirbulescu RF, Nichols A, Ilies I (2006) Electric interactions through chirping
814 behavior in the weakly electric fish, Apteronotus leptorhynchus. J Comp Physiol A
815 192:159–173.

816

## FIGURE LEGENDS

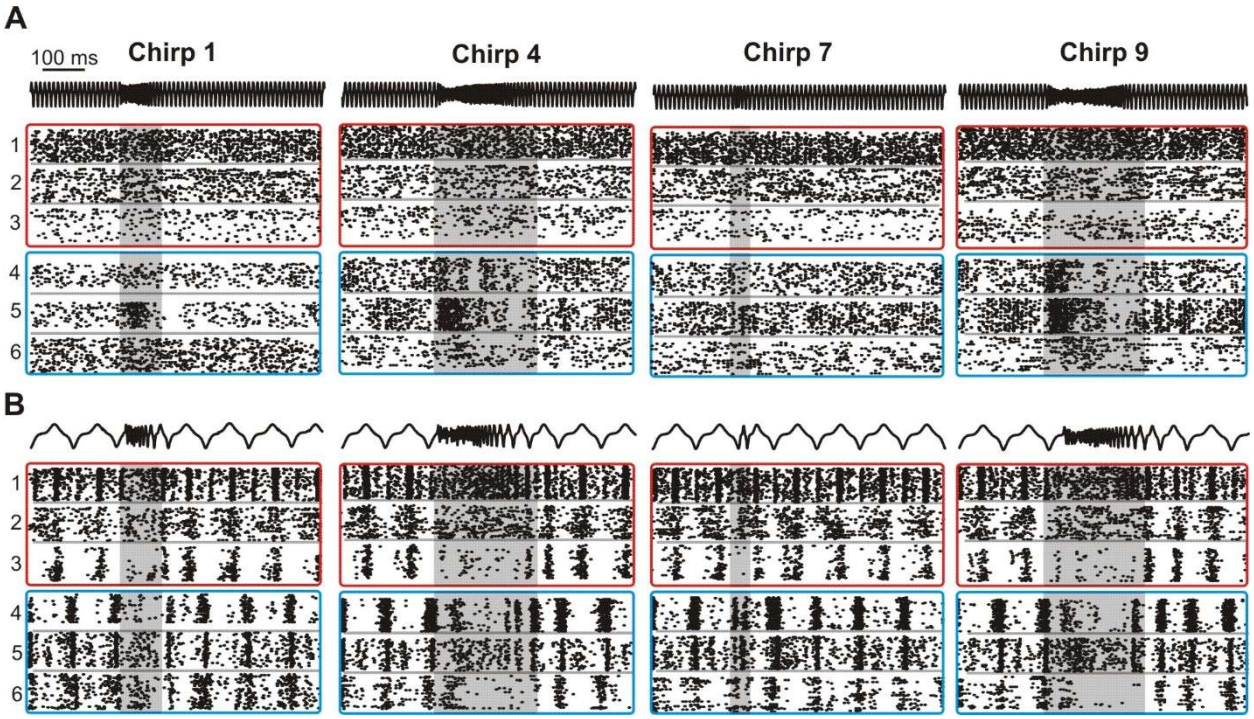



**Figure 1: ELL Pyramidal cell responses to conspecific signals. A.** Raster plots of chirp responses on a high frequency (100Hz) beat. AM of the EOD stimulus is shown in black; 3 representative OFF-cells are shown in cyan boxes while 3 ON-cells responses are displayed in red boxes. The same 6 neurons are used for all panels. **B.** Responses to chirps on a low frequency (10Hz) beat. For a detailed description of all chirps used, see Table 1. The shaded area highlights the duration of the chirp. See Figure 1-1 and 1-2 for comments and analysis of specific response properties (adaptation time and biphasic responses to low frequencies).

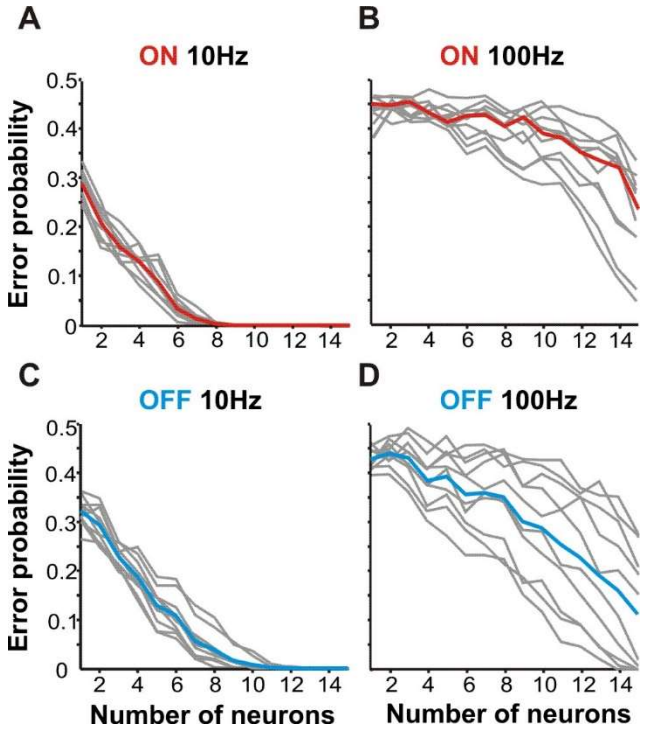



**Figure 2: Detection efficiency depends on beat frequency. A.** Detection of chirps on 10Hz beat by ON-cells as a factor of neurons included in the analysis (n=17). Error probability is the probability of an ideal observer to correctly assign a spike train as a chirp or beat response. Detection error levels for individual chirp identities are shown in gray. Red line indicates mean detection error for all chirps. ON-cells can reliably detect the occurrence of all chirps. **B.** ON-cell performance is worse on 100Hz beats. **C, D** Mean OFF-cell performance (cyan, n=16) is also more efficient on a 10Hz beat.

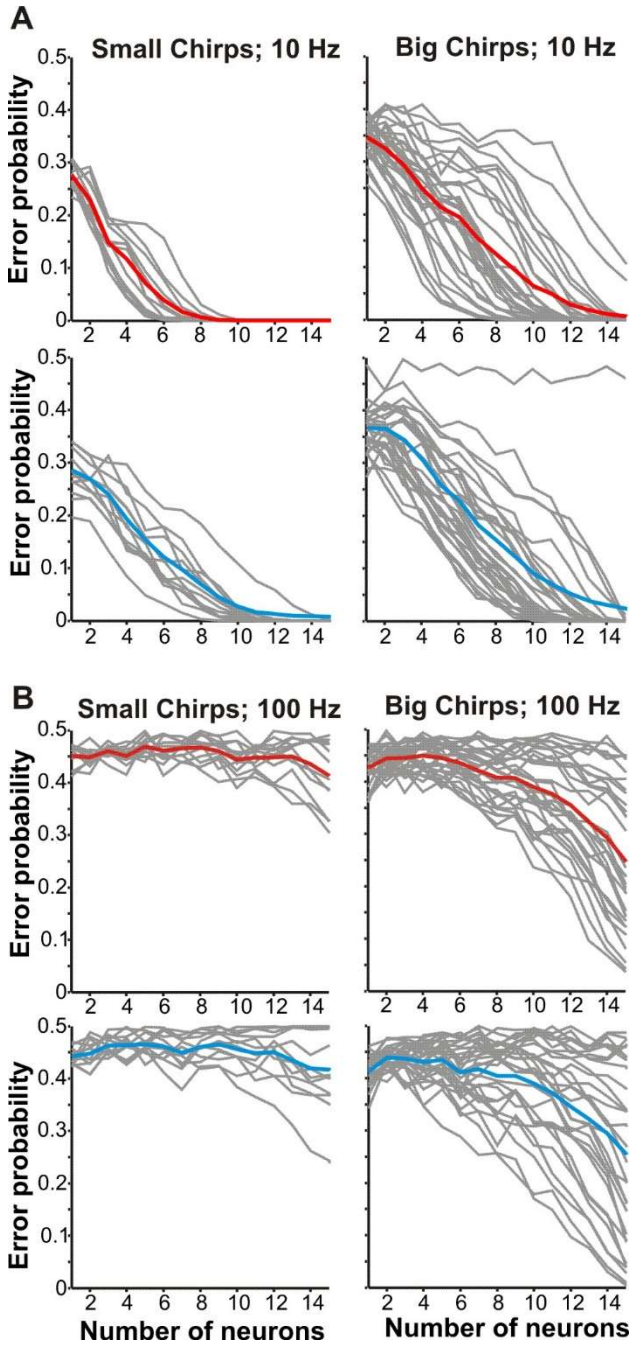



**Figure 3: Discrimination of chirps on high frequency beats is poor. A.** Discrimination for small chirps (Chirps 5,6,7,8, right) and big chirps (Chirps 1,2,3,4,9,10, left) on 10Hz beat. Mean ON-cell chirp discrimination shown in red, OFF-cells in cyan, and discrimination for individual chirp pairs shown in gray. **B.** Discrimination of small chirps on a 100Hz beat is relatively inefficient for both ON (red) and OFF-cells (cyan). Discrimination of big chirps varies with chirp identity, but is still poor. For chirp-by-chirp discrimination comparisons see Figure 3-1.

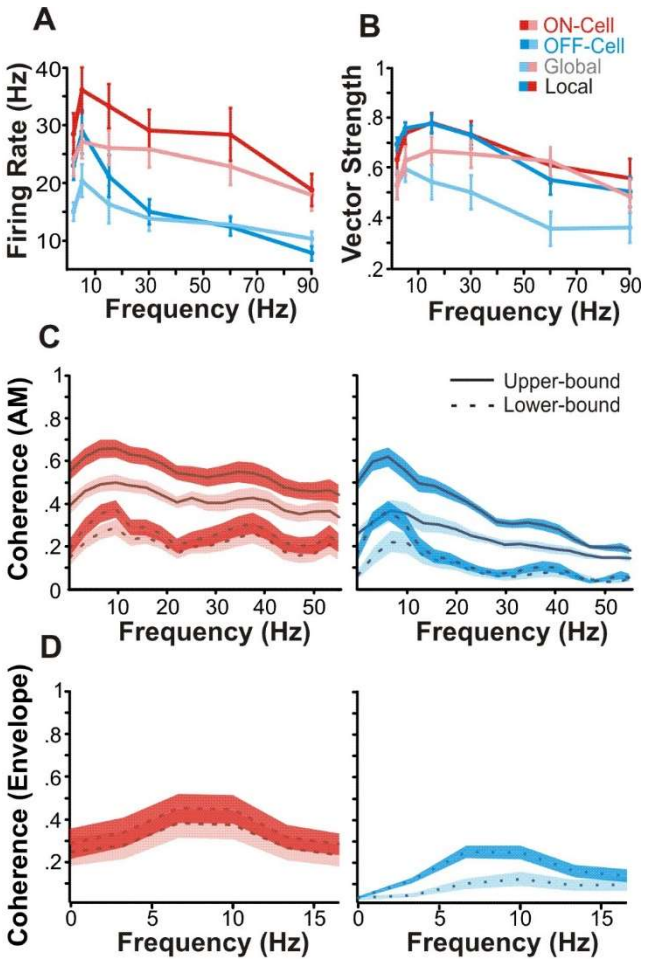



**Figure 4: Temporal coding properties of pyramidal cells A.** Mean firing in response to SAM stimuli. Mean firing rate (±s.e.) for both ON (red, n=19) and OFF (cyan, n=15) cells in both stimulation configurations (global: pink, pale blue; local: red, cyan) peaks at 5Hz. **B.** Phase locking to AM sinusoids is also best at low frequencies. Maximum phase locking is seen at 15Hz, with the exception of locally stimulated OFF-cells, which peak at 5Hz. **C.** Mean coherence to noise stimulation is also low-pass. ON-cell coherence is shown in red, OFF-cell in cyan. The upper bound coherence measure (solid line) is based on the response-response correlations between multiple presentations of the stimulus, while the lower bound (dashed line) is based on stimulus-response correlations. Shaded areas indicate standard error; darker shading indicates local stimulation. Mean global lower bound maximums: ON-cells: 23.1Hz (±.86 s.e.); OFF-cells: 11.6Hz (±.75 s.e.), (Wilcoxon rank-sum test p=.04). **D.** Coding of low frequency envelopes is poor. Mean (± s.e.) lower bound coherence between responses and the low-frequency (0-20Hz) envelope of a bandpass RAM stimulus (40-60Hz) are displayed for both local and global stimulus configurations. Both ON and OFF cells exhibit peak envelope tuning at 10.10Hz (±1.52 s.e.) (Wilcoxon rank-sum test, p=.21). OFF-cells have noticeably lower coherence to low envelopes than ON cells.

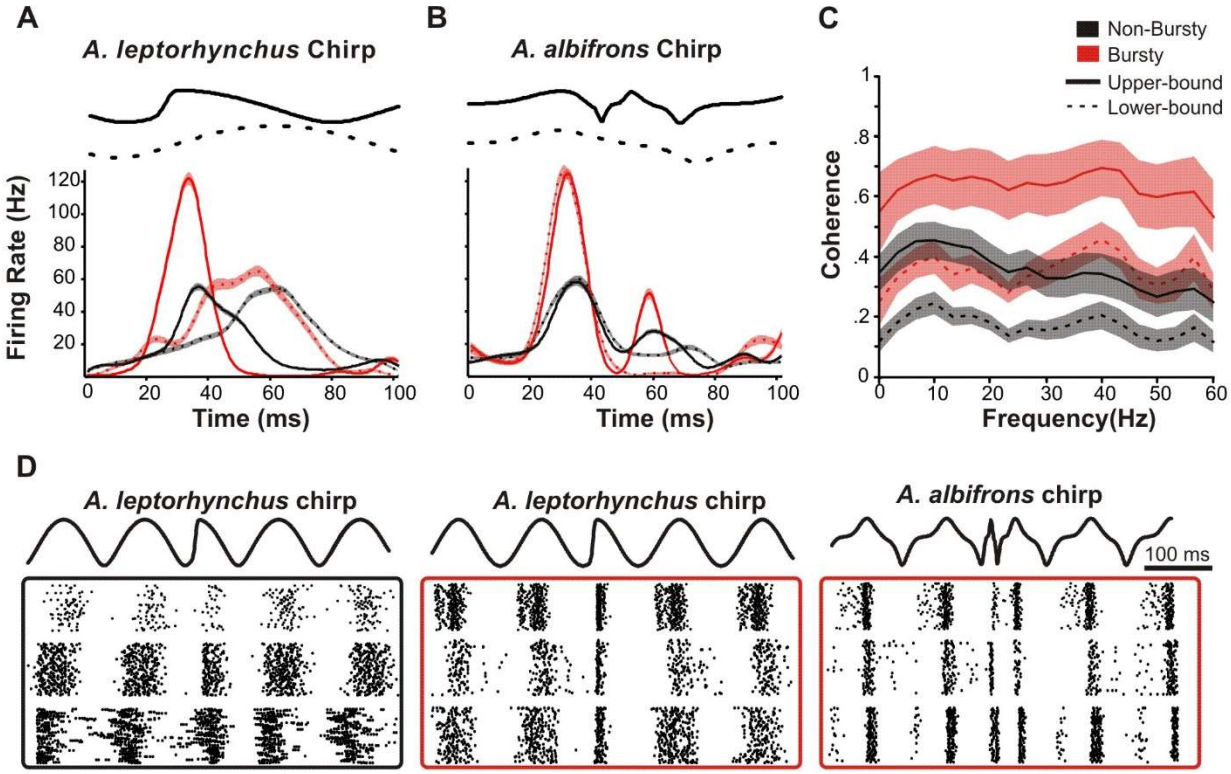



**Figure 5: Coding of chirps by a small population of higher-pass neurons. A.** The mean instantaneous firing rates of ON-cells over the time course of chirp and beat stimuli. The difference in peak firing rate characterize a small population (n=5, red) that bursts in response to *A. leptorhynchus* small chirps (Beat responses, dashed lines: Peak FR 77.67 HZ ± 7.11; Chirp responses, solid lines: Peak FR 159.57 Hz ± 8.68; Wilcoxon ranked sum test ($p<0.001$). The majority of the ON-cell population (n=16, black) only showed a modest increase in firing (Beat Response: peak FR 62.68 Hz ±1.27; chirp response: peak FR 66.56 Hz±4.21, Wilcoxon ranked sum test p=.002). Shaded area represents standard error. **B.** Even the bursty population fires less in response to *A. albifrons* smallest chirps compared to the beat (beat response: peak FR 155.45 Hz ± 8.82; chirp response: peak FR 117.75Hz ± 9.40, Wilcoxon ranked sum test (p=.04)). **C.** Mean (±s.e.) upper-bound and lower-bound coherences for the bursting (red) and non-bursting

(black) populations. . **D.** Example raster plots of chirp responses used for A and B. The non-bursty population (black box) responds similarly to both *A. leptorhynchus* chirp and beat. The bursty population (red box) bursts to *A. leptorhynchus* chirps, but not *A. albifrons* chirps.). For comparable data obtained from *A. leptorhynchus* see Figure 5-1.

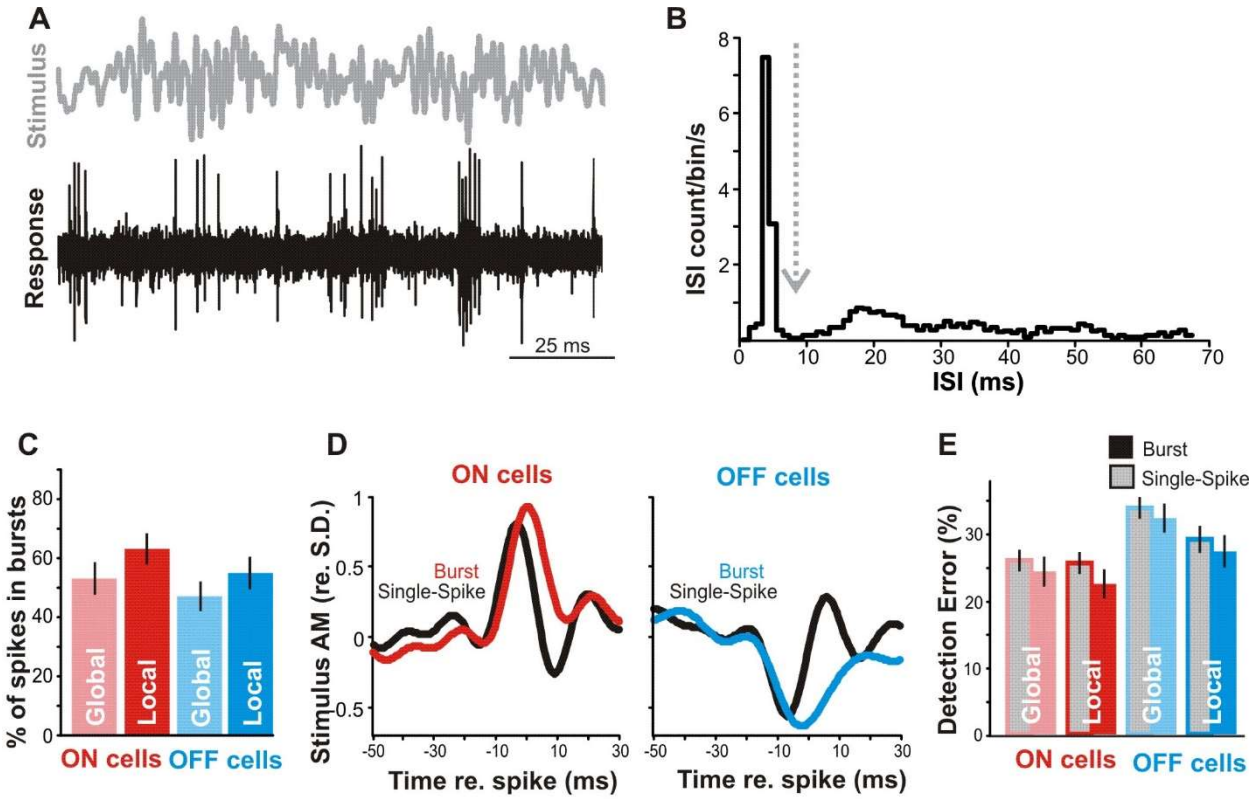



**Figure 6: Coding of AM by bursts. A.** Sample of noise stimulus (0-60Hz, gray) and representative spike train (black) from an OFF-cell during local stimulation. **B.** Example of ISI distribution used to determine burst threshold (dashed line). **C.** ON-cells (red) burst more than OFF-cells (blue) (ANOVA, p=.02), and local stimulation produced more bursting than global (ANOVA, p=.01). Error bars show standard error **D.** Mean burst triggered averages (red/blue) and single spike triggered averages (black) from ON and OFF-cells show that bursts are triggered by wider (lower-frequency) stimulus features than single spikes. **E.** Feature detection performance for burst and isolated spikes. In both ON and OFF cells bursts (blue/red -filled bars) tend to have lower error rates (percentage of events signaling false positives or false negatives) in detecting optimal stimulus features than single spikes (gray-filled bars) but this trend is not significant (ANOVA, p=.10).

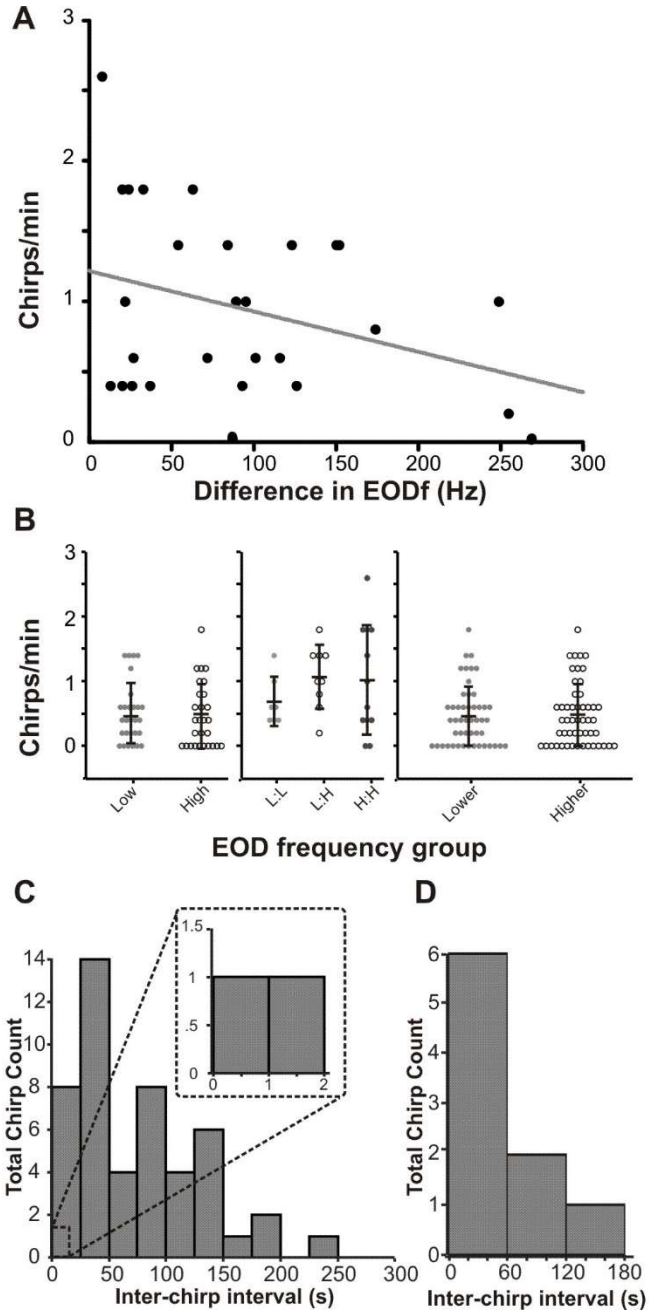



**Figure 7: Chirping behavior in freely swimming pairs** (n=28). **A.** The number of chirps produced during interaction is correlated with difference in EODf ($r^2$=.1093) **B.** Chirping does not differ by absolute EODf, EODf pair type or relative EODf. Mean chirp rate for low frequency (<1100Hz) and high frequency fish (>1100) was similar (Student's t-test, p=.86). Mean chirps per trial based on EODf of pairing (Low: Low, Low: High, High: High) (ANOVA, p=.47), and by relative frequency of individuals within the pairing (Student's t test, p=.55) were all extremely low and similar in all groupings. Error bars indicate standard deviation. **C.** Inter-chirp intervals between pairs binned by time fall show no echoing chirp exchanges. Inset: Enlarged section show that very few chirps occur within 2s of

each other. **D.** Inter-chirp intervals distribution for individual fish follow a Poisson distribution ($R^2$= 0.9).

Table 1

Table 1: Frequency and duration properties for all chirps used. “Other” indicates differences in chirp shape not due to peak frequency or duration, such as shape of frequency rise and fall, and phase of the beat on which the chirp occurred.

| Chirp ID | Frequency Rise (Hz) | Duration (ms) | Other |
|---|---|---|---|
| 1 | 200 | 100 | α shape |
| 2 | 200 | 200 | α shape |
| 3 | 350 | 100 | α shape |
| 4 | 350 | 200 | α shape |
| 5 | 50 | 50 | α shape |
| 6 | 100 | 50 | α shape |
| 7 | 50 | 50 | Antiphase to 5 |
| 8 | 100 | 50 | Antiphase to 6 |
| 9 | 350 | 200 | Two frequency peaks |
| 10 | 350 | 200 | Ramp Shaped |
| 11 | 60 | 10 | *A. lept.* small chirp |
| 12 | 122 | 15 | *A. lept.* small chirp |

Extended Data

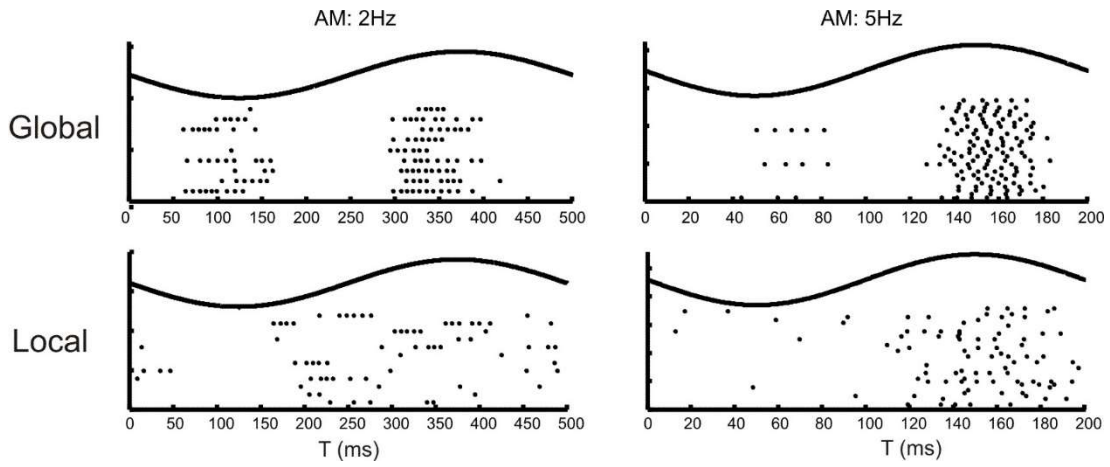



Figure 1-1: Cancellation observed in an LS ON cell. In some recordings a biphasic response to stimuli is observed. While this could indicate an overly strong stimulus, it could also indicate cancellation via feedback from the midbrain (Bastian et al., 2004). Cancellation is less prominent in the LS of *A. leptorhynchus*, but still occurs. The biphasic responses to global SAM stimuli but not to local stimulation indicate that our observed responses likely represent an increased cancellation response in the LS as a result of low frequency tuning.

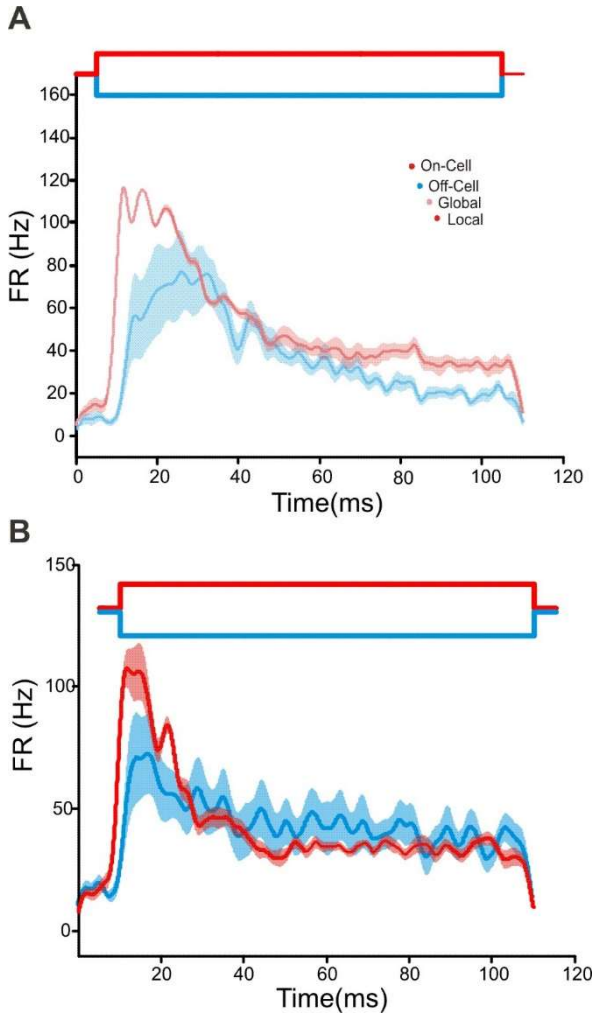
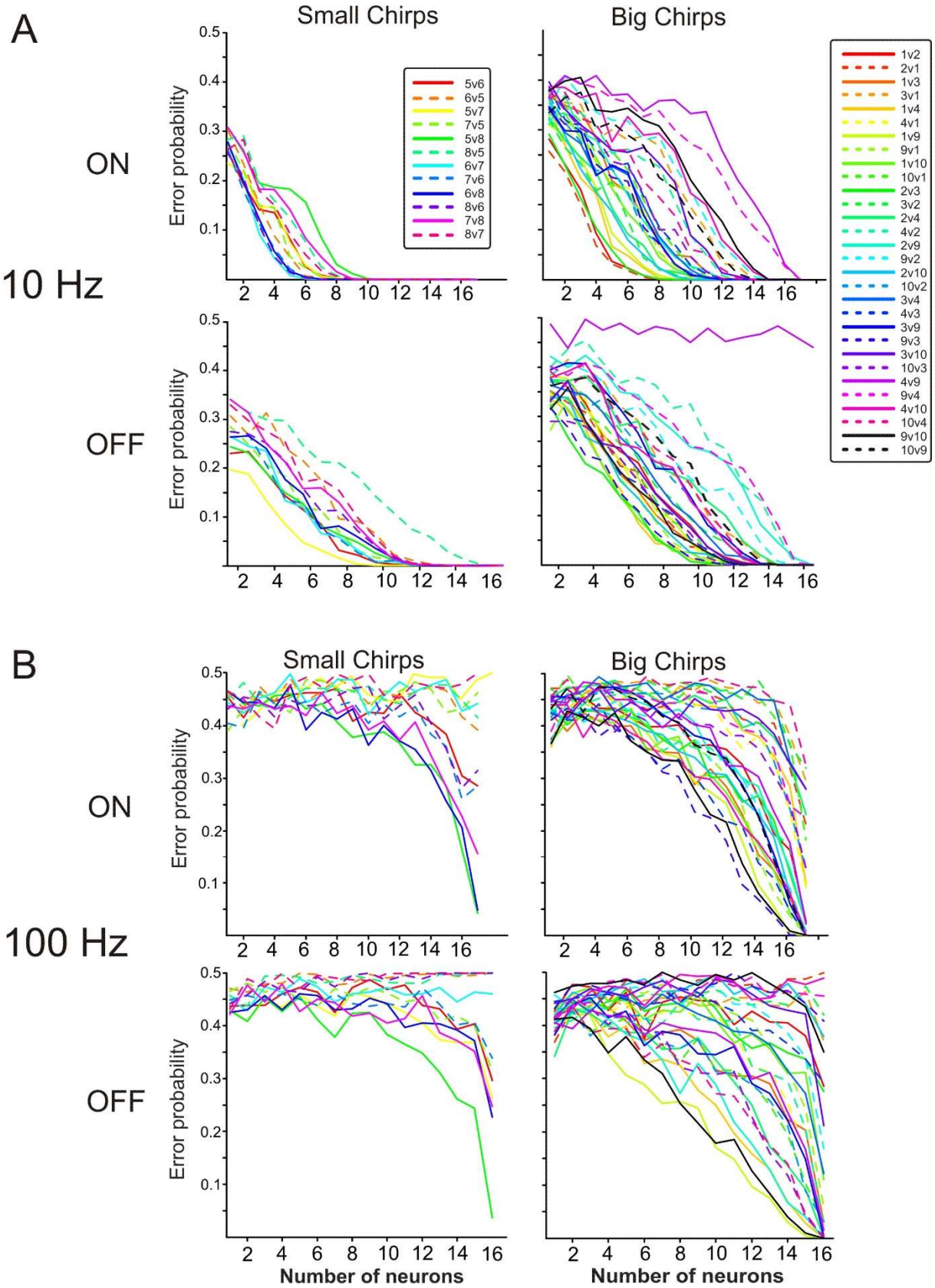



Figure 1-2: *A. albifrons* adaptation to step stimulation. A. Both ON and OFF cells' responses decreased throughout the 100ms stimulus without plateauing in sharp contrast with B. *A. leptorhynchus* responses to global stimuli (S2, Krahe et al., 2008 see their figure 8C&F) that adapt within 40-50ms and then plateau.


A
Small Chirps
Big Chirps
ON
10 Hz
OFF
Error probability
5v6
6v5
5v7
7v5
5v8
8v5
6v7
7v6
6v8
8v6
7v8
8v7
1v2
2v1
1v3
3v1
1v4
4v1
1v9
9v1
1v10
10v1
2v3
3v2
2v4
4v2
2v9
9v2
2v10
10v2
3v4
4v3
3v9
9v3
3v10
10v3
4v9
9v4
4v10
10v4
9v10
10v9
B
100 Hz
Number of neurons

954

Figure 3-1: Detailed discrimination traces indicating results of testing specific chirps against each other. For ease of analysis, chirps were grouped by duration frequency and duration as either big or small. Duration appears to be the feature that is most discriminable, so big and small chirps were not directly compared. Descriptions of chirp properties are located in Table 1. A. Chirp discrimination on a 10 Hz beat. B) Chirp discrimination on a 100 Hz beat.

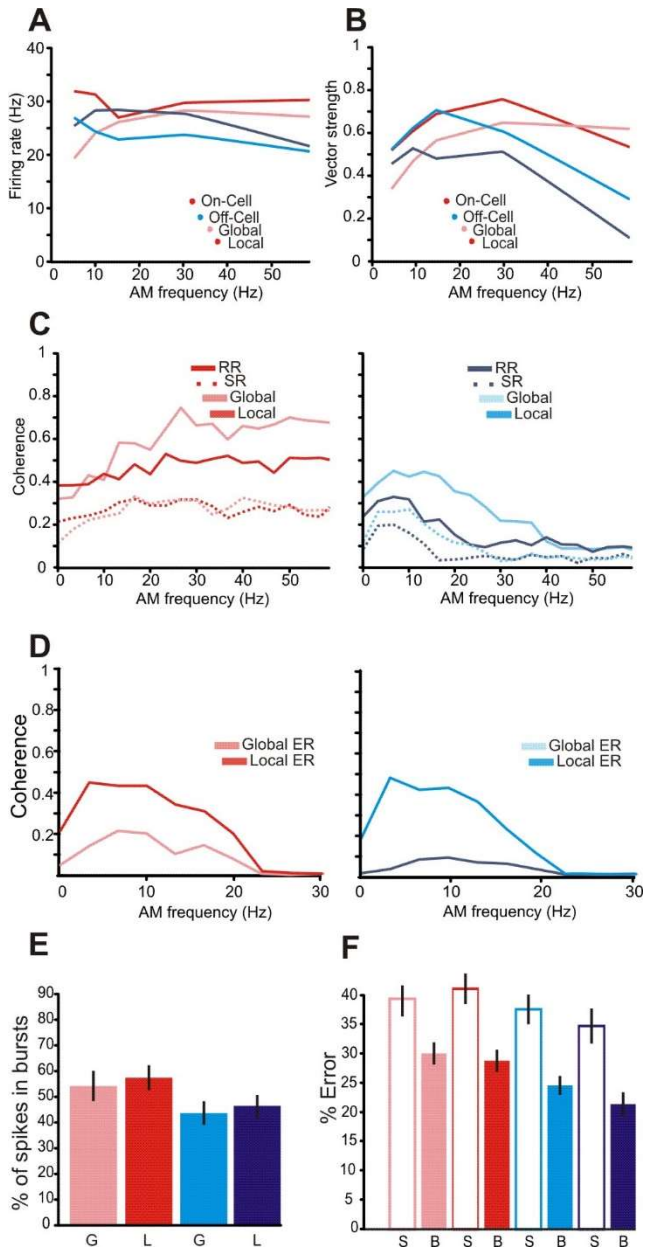



Figure 5-1: *A. leptorhynchus* data for stimulation protocols matched to those used in the main text. This data is comparable to what has already be extensively published in Krahe et al. (Krahe et al., 2008), and thus not highlighted in the main text, but indicate that our methods reproduce previously published results and indicate differences between *A. albifrons* and *A. leptorhynchus*. A. Coherence to 0-60Hz RAM stimulation. Mean coherence to noise stimulation by neuron type. ON-cell coherence is shown in red, OFF-cell in cyan. The upper bound coherence measure is shown with solid lines, lower bound in dotted lines B. Phase locking to sinusoids across 0-60Hz is best at 20-30Hz, while firing rate is relatively constant across frequencies C. Coherence to low frequency envelopes. Both ON and OFF cells perform well at coding low frequency stimulus components.