Simplified neural encoding of social communication reflects lifestyle

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ABSTRACT

As signal production changes through speciation, the sensory systems that receive these signals must also adapt to extract the most relevant information effectively. In a species of weakly electric fish, *Apteronotus albifrons* we examine the unique neurophysiological properties that support the encoding of electrosensory communication signals that the animal would encounter 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 find notable differences leading to relatively poor coding of the details of the chirp structure. 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. These findings parallel the mediocre chirp coding accuracy in that they both point to the sparse reliance on chirps in 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.

INTRODUCTION

Animals interact in complex sensory environments constraining the efficiency of information transfer. Potential limiting factors include environmental noise (Brumm and Slabbekoorn, 2005; Wiley and Richards, 1982), limitations of the transmission medium (Wiley and Richards, 1978) or social group size (Aubin and Jouventin, 2002). Optimization of the sensory system for any one type of signal may result in a trade-off between signal coding efficiency and a loss of sensitivity to a more diverse range of signal types. Therefore, efficiency in processing one type of signal must be balanced with the need also to encode other relevant signals. Thus, sensory systems might enhance or relax accuracy in encoding a signal depending of the ethological importance of the different aspects of that signal.

Mapping sensory tuning and deciphering information content available from neural codes are classic studies in neuroethology, with numerous examples of sender and receiver matching observed across many groups, including songbirds (Brumm and Slabbekoorn, 2005), anurans (Schul and Bush, 2002), and insects (Neuhofer et al., 2008). The majority of this work pertains specifically to sensory tuning and call matching at the periphery, but there is comparatively little research exploring adaptation within the brain as a result of sender-receiver matching. Even in drosophila, where coding of courtship song has been examined in high level auditory processing centers, the role of sender-receiver matching between species with divergent song patterns is still unclear (Tootoonian et al., 2012). This body of literature typically highlights the features that enhance the coding of species-specific signals but rarely identifies instances where coding efficiency is relaxed. Using a comparative approach, we aim to identify species-specific variations in sensory coding properties of central neurons and link them to the behavioral use of the signals.

Weakly electric fish are ideal for examining how signal production and signal reception coevolve and diversify. Apteronotid species share a common mode of communication, the modification of their electric organ discharge (EOD), but the detailed structure of their communication signals are highly diverse (Dunlap et al., 1998; Turner et al., 2007). Signal reception could also be influenced by many socially relevant factors such as EOD waveform shape (Petzold et al., 2016), chirp features (Benda et al., 2006), signal structure (Marsat and Maler, 2012; Marsat et al., 2009; Marsat et al., 2012) and social structure (Stamper et al., 2010). Chirps specifically can vary dramatically in specific properties such as duration, frequency or shape between even closely related species (Dunlap et al., 1998; Turner et al., 2007; Zakon et al., 2002; Zupanc and Maler, 1993). The sensory system of apteronotids could have similar coding properties across species and be generic enough to process these communication signals efficiently despite differences in their structure and use between species. Alternatively, differences in processing of these signals could reflect adaptations of the sensory system to varying signal structures and behavioral use.

In wave-type fish, individual EOD frequency is particularly influential on chirp reception. Fish in this group perceive ongoing amplitude modulations (AM beat) that are the product of two fish with different EOD frequencies interacting at close range (Bastian, 1981). Chirping is a brief shift in EOD frequency that creates a modulation of the beat frequency (Hagedorn and Heiligenberg, 1985; Zupanc and Maler, 1993). In species with sexually dimorphic EODs, low frequency beats, the result of fish with similar EOD frequencies interacting, are often typical of agnostic encounters between fish of similar sex and size. Higher

frequencies are more typical of encounters between fish of opposite sexes or with large differences in body size. *A. leptorhynchus* produce Type 1 (big) chirps most often in high frequency beat contexts (Hagedorn and Heiligenberg, 1985; Hupé and Lewis, 2008), while low frequency interactions elicit the production of frequent Type 2 (small) chirps (Hupé & Lewis, 2008). Chirp coding has been previously well characterized in both *Apteronotus leptorhynchus* and *Eigenmannia viscerens.* In both species, chirps cause significant changes in the synchrony of electroreceptor firing, either increasing, or decreasing synchronicity depending on chirp type and beat frequency (Stöckl et al., 2014; Walz et al., 2014). The two categories of signals described above also produce different neural responses in the electrosensory lateral line lobe (ELL), the primary sensory area. Small chirps on low frequency beats cause a synchronized and stereotyped bursting response among the pyramidal cells of the ELL. Due to the encoding strategy and the structure of the signal itself, small variation in these chirps cannot be discriminated (Allen and Marsat, 2018; Marsat et al., 2009). Big and small chirps on high frequency beat lead to heterogeneous and graded responses among pyramidal cells and variation in the signals are accurately discriminated (Allen & Marsat, 2018; Marsat & Maler, 2010). Chirp production in *A. albifrons* is similar, but the most notable difference between these species' chirps is duration; *A. leptorhynchus* chirps are typically tens of milliseconds long, whereas *A. albifrons* chirps are generally well over 100 milliseconds long (Dunlap et al., 1998; Turner et al., 2007; Zupanc and Maler, 1993). Characterization of frequency tuning in the primary sensory area of *A. albifrons* show differences from *A. leptorhynchus* that may be an adaptation for the coding of these particularly long chirps (Martinez et al., 2016)

In this study, we examine the coding of conspecific social signals and the underlying basic neural properties to understand if, and how, the sensory system is adapted to the specific characteristics of the communication system of *A. albifrons*. Additionally, we examine the behavior of chirp production to help understand how the behavioral use of the signals, rather than just their structure, could influence the way they are processed. We compare our findings to the well-studied 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.

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. 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 highly heterogeneous with variability between inhibition, excitation, and desynchronization. Some patterns are in line with expectations from *A. leptorhynchus* coding strategies. On high frequency beats in particular (Fig 1A) we see a graded increase in firing rate in OFF-cell while ON-cell are inhibited during the chirp similar to responses of *A. leptorhynchus* pyramidal cells. On low frequency beats (Fig 1B), the responses also consisted of variable increses and decreases in firing rate and we did not observe synchronized burst firing across the population of pyramidal as is present in *A. leptorhynchus*' responses to small chirps on low frequency beats (Marsat and Maler, 2010; Marsat et al., 2009). Therefore, all chirps appear to be coded primarily through increases in firing among OFF-cells, and inhibition and desynchronization of ON-cells. Since *A. leptorhynchus* uses two different coding strategies to encode different categories of signal and support different perceptual abilities (discrimination vs. detection only) but *A. albifrons* uses the same coding patterns for all signals, we next quantify how well the neural responses could support detection and discrimination of chirps.

Detection of chirps varies with beat frequency

To quantify the ability of LS neurons to signal chirp occurrence (i.e. detection) we created ROC curves to determine the ability of an ideal observer to distinguish chirp responses from beat responses based on firing patterns (i.e. spike trains metric distances; see Methods for details). The results quantify the proportion of error that an ideal observe would make as a function of the number of neurons it monitors. An error of .5 would indicate chance level performance, while zero indicates perfect detection.

ON-Cells can efficiently detect all chirps to a high level of accuracy with about 8 neurons, comparable to *A. leptorhynchus* (Marsat and Maler, 2010) (Fig 2A). However, this level of accuracy only holds for chirps on the 10 Hz beat. ON-cell performance on 100 Hz is extremely poor, even with high numbers of spike trains included in the analysis (Fig 2B). OFF-cells are able to detect chirp occurrence on low frequency beats for all chirp types, although less efficiently than ON-cells (Fig 2C). On 100 Hz OFF-cells can detect the longest, most intense chirps, but perform poorly detecting smaller chirps (Fig 2D). Therefore, while chirps on low frequencies are reliably detected, detection sensitivity is poor in high frequency contexts.

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. 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 rather than on 100Hz beats (Fig 3). On low frequencies, the all chirps are well discriminated by both ON and OFF cells. Performance for chirps on high frequency beats was more varied (Fig 3B); accurate discrimination was achieved only for chirps with big difference in properties (Fig 3B and Fig S1) and both ON and OFF cell response allows similar discrimination accuracy. These results are unexpected considering that *A. leptorhynchus* chirps on high frequency beats can be discriminated well using as few as six neurons, and, conversely,exhibit poor discrimination coding 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 S1). However, discriminating the most easily separated chirps is still prone to error, even when recruiting up to 17 neurons. 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 extracted from chirps within the electrosensory system. To determine which aspects of the neural responses could be responsible for these surprising coding differences, we characterized a wide variety of response properties using an assortment of stimulation protocols.

Frequency tuning and information coding

Encoding amplitude modulations (AM) in stimuli is crucial for the recognition 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 tuning properties. Communication signals cause spatially diffuse stimulation and we simulate this configuration using a large dipole (see methods) that drives both the feedforward and feedback pathways. To separate the effects of feedback from the tuning of the feed forward circuit we also stimulated only the receptive field of each cell with a small local dipole. (Bastian, 1986).

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). These peak frequencies are lower than those reported for *A. leptorhynchus*, especially for ON-cells, which exhibit broad- to high- frequency tuning in the LS (Krahe et al., 2008; Fig S2). Temporal coding accuracy is conveniently quantified using random noise stimuli and information theory (Borst

and Theunissen, 1999). Lower-bound coherence reflects the amount of information encoded linearly whereas upper bound coherence takes in account both linear and non-linear aspects of the response (Fig 4C). All coherence curves peak at similar frequencies in the 5-20 Hz range (Wilcox Rank Sum, p=.19). However, OFF-cells are clearly low-pass, and exhibit a sharper decline in coherence to higher frequencies, while ON-cells exhibit a broader lower-bound coherence.

The movement of fish as well as the amplitude changes caused by chirps create low frequency changes in AM contrast, or envelopes (Stamper et al., 2013). During chirps, the AM will be high frequency (tens to hundreds of Hz above the beat frequency) whereas the envelope of the chirp is low frequency (e.g. 100 ms chirp will lead to a ~ 10Hz envelope). Therefore, the coding of chirps' low frequency content must be investigated using envelope stimuli. We probed envelope coding using stimuli consisting of RAMs with AM frequencies between 40Hz and 60 Hz, containing envelope frequencies of 0-20 Hz (Middleton et al., 2006). Although pyramidal cells do encode the envelope of these stimuli (Fig 4D), the coding accuracy is low compared with the envelope coding observed in *A. leptorhynchus* (Chacron, 2006; Middleton et al., 2006; Fig S2). This poor envelope coding, particularly for OFF-cells in response to global stimuli, may help to explain the poor chirp discrimination we observe.

Burst Firing and Chirp Detection

As described in Marsat et al. (2009) burst firing serves an important role in *A. leptorhynchus* for the efficient detection of extremely short chirps. As qualitatively shown above in Figure 1, no conspecific chirps reliably produced a burst response in *A. albifrons*. This implies that *A. albifrons* may lack a dedicated code for detection of very brief chirp signals. In *A. leptorynchus* the coding of small chirps is directly related to beat frequency. On low frequencies, small chirps are shorter than the period of one beat cycle. As beat frequency increases and the period becomes shorter, small chirps begin to last longer than one cycle. This change in duration relative to beat period mediates how small chirps are coded (Walz et al., 2014). In *A. albifrons* all chirps are much longer than *A. leptorhynchus* small chirps, spanning more than one beat cycle even on low frequencies, which may eliminate the need for a feature detection code specifically for extremely short chirps. Thus, the observed lack of bursting may result from the change either in signal structure (duration relative to beat cycle) or from underlying changes to physiology and bursting capability of LS neurons.

To determine if this change in signal coding is a result of signal structure or underlying physiology, we stimulated *A. albifrons* fish with *A. leptorhynchus* chirps known to elicit a burst response in *A. leptorhynchus* (Marsat et al., 2009). We determined burst threshold ISI for each neuron by plotting ISI distribution of chirp and beat responses and manually selecting a threshold that best distinguished between the two (Martinez-Conde et al., 2002) in order to maximize our measure of chirp-specific bursting. The mean burst threshold was 8.0ms ±0.7ms (s.e), comparable to the burst thresholds determined for RAM analyses and in other reports (Ávila-Åkerberg et al., 2010; Chacron and Bastian, 2008; Marsat et al., 2009).The majority of neurons increased firing rate only slightly more in response to *A. leptorhynchus* chirps than to the beat. There was, however, a small population (n=5) of ON-cells that did reliably burst more in response to *A. leptorhynchus* small chirps than to the beat (Fig 5A, C). The shortest *A. albifrons* chirps are most similar to the *A. leptorhynchus* chirps that elicit bursts, but are still long enough

to span more than one beat cycle. Even the bursty neurons did not respond to the shortest *A. albifrons* chirps with bursts (Fig 5B, D).

The low numbers of neurons that burst in response to *A. leptorhynchus* chirps suggests that there are physiologic differences between these two species in regards to burst coding of communication. We examined the differences between bursty and non-bursty neurons in more detail. The subset of bursty neurons also tend to have broader AM tuning (Fig 5C). While most ON-cells exhibited peak coherence at frequencies between 10-20Hz, these cells had peak frequencies as high as 40-50Hz and exhibited extremely high coherence across the range of stimulus frequencies. The remainder of the ON cells displayed the low-pass tuning described in Martinez et al. (2016) therefore the coherence for ON cells displayed in Fig 4C are broader than previously described because of this small subset of broadly tuned neurons. These data show that while a subset of ELL neurons are capable of bursting in response to short, high frequency chirp stimuli, they make up a small percentage of the overall ELL population and bursting does not seem to play a role in conspecific chirp coding.

Feature Detection

Burst firing may not be a significant aspect of communication coding in *A. albifrons*, but other uses for burst coding may be conserved. During spontaneous activity, we observed a baseline firing rate of 13.54 Hz (±1.37 s.e.) with 17.01% (±2.55 s.e.) of spikes in bursts. Thus, the pyramidal neurons of *A. albifrons* are able to burst although these *A. albifrons* LS neurons are less prone to bursting than those of *A. leptorhynchus* (S2). In *A. leptorhynchus* burst firing is not used solely for chirp coding, but also for detection of prey like stimuli (Gabbiani et al., 1996; Oswald et al., 2004). We examined bursting in response to local RAM stimulation (Fig 6A) to determine if bursting could serve similar prey detection functions in *A. albifrons*. The ISI histogram of the responses clearly showed that the neurons burst to these stimuli (Fig 6B). The proportion of spikes occurring in bursts was as high as 63.07% (±5.64 s.e.) for stimuli in the local configuration (Fig 6C). The average stimulus waveform triggering burst vs single spikes follow the pattern observed in *A. leptorhynchus*: slower AM for burst than for single spikes (Fig 6D). However, in response to RAM stimuli, unlike *A. leptorhynchus* there was not a large difference in coding error between bursting and tonic spiking (ANOVA, p=.10) (Fig 6E). These data suggest that while bursts encode low frequency stimuli in the LS of *A. albifrons*, the role of bursting in coding for specific stimulus features is not clear.

Stimulus adaptation

As seen in the examples of conspecific chirp responses (Fig 1), pyramidal cells often demonstrated a rapid adaptation to chirp stimuli. For example, the responses to chirp 9 or 4 embedded in high frequency beats (Fig 1) are stronger in the first half of the chirp but weaker in the second half. This adaptation maybe hinder the accurate encoding of chirp properties since two chirps with different length could elicit identical responses if they adapt shortly after chirp onset. Therefore, we characterize adaptation rate using standard step-up and -down stimuli. Both ON and OFF cells' responses decreased throughout the 100ms stimulus without plateauing in

sharp contrast *with A. leptorhynchus* responses to global stimuli (S2, Krahe et al., 2008 see their figure 8C&F) that adapt within 40-50ms and then plateau.

Chirp production and behavior

Our physiology data suggest that while *A. albifrons* are able to detect and discriminate chirps they might not be as efficient or accurate as *A. leptorhynchus*. These observations suggest a behavioral difference in chirp usage. To test this hypothesis, we recorded electrical behavior from pairs of freely swimming and interacting fish. Chirping behavior did occur but differed from that of *A. leptorhynchus* in a number of ways. Primarily, overall rates of chirping are dramatically lower than numbers reported from similar studies in *A. leptorhynchus* (Hupé and Lewis, 2008; Zupanc et al., 2006). Over 28 trials, we recorded a sum of 133 chirps, with a mean of 4.75 (±0.61) chirps per 5-minute trial. The maximum number of chirps observed in one trial was 13. Due to the small number of observed chirps, we did not separate them into multiple categories for analysis, although chirps of varying frequency increases and duration occurred. Like *A. leptorhynchus*, per trial chirp count is correlated with the difference in EODf of interacting fish. Smaller differences in EODf correspond to higher numbers of chirps (Fig 7A). Unlike *A. leptorhynchus*, however, chirping does not appear to be sexually dimorphic. Animals used in behavior experiments were not sacrificed to determine sex, but grouped by EODf into high (>1100Hz) and low (<1100Hz) groups which can correspond to females and males respectively in many populations of *A. albifrons* (Zakon and Dunlap, 1999). This allowed us to compare chirp rates among higher or lower frequency individuals, as well as by large or small differences in EODf. We observed no differences in chirp production between high or low frequency groups (Fig 7B). Further, chirp rate does not vary by pairing type, nor by relative EODf with each pair under our conditions (Fig 7B). These results replicate previous findings that chirp frequency in this species is not sexually dimorphic (Dunlap et al., 1998).

Chirp timing plays an important role in mediating *A. leptorhynchus* interactions, particularly during interactions involving echo response (Henninger, 2015; Hupé and Lewis, 2008; Zupanc et al., 2006), a behavior where one fish quickly responds to the chirp of another fish. To determine if chirp timing plays a similar role in *A. albifrons* interactions, we analyzed inter-chirp intervals for all trials in which both fish chirped. The resulting distribution indicates chirp latencies much longer than are typical of *A. leptorhynchus* echo responses (Fig 7C). Of all recorded chirps, only six occurred within 2.5s of each other, making an echo response unlikely to be an important factor in *A. albifrons* interactions. This lack of echoing, the long interchirp intervals and the low rate of chirping in general, suggest that *A. albifrons* relies relatively little on chirps to mediate social interaction (although see the discussion about courtship interactions).

DISCUSSION

We find that *A. albifrons* exhibit many notable differences in neurophysiology that correspond to communication strategies particular to this species. Compared to the closely related *A. leptorhynchus*, *A. albifrons* exhibit low frequency tuning, corresponding to the low frequency envelopes produced by their long chirps. However, despite this increased sensitivity to low frequency signals, coding of chirp identity is surprisingly poor. This may be due to the usage of chirps in this species. Infrequent chirping and the lack of complex bouts of back-and-forth chirping may reduce the need to encode details about chirp properties and chirper identity. Therefore, less complex chirp coding may represent a relaxation rather than optimization of the sensory system for communication.

Low frequency tuning could be adaptive for chirp coding

Our results confirm that pyramidal cells in the LS of *A. albifrons* are low pass in contrast with the ON-cells of the *A. leptorhynchus*'s ELL (Krahe et al., 2008). This difference parallels the much slower time course of *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. Indeed, whereas the envelope of chirps are low frequency, the AM is high frequency. Therefor the low frequency tuning to AM is not automatically helpful and we found that the coding of low-frequency envelopes was relatively poor, particularly in OFF cells which are -in *A. leptorhynchus*- best at coding chirp envelopes. However, if envelope response is synthesized before the pyramidal cells (e.g. the electroreceptors as could be the case in *A. leptorhynchus* (Savard et al., 2011) although see low frequency tuning to AM could benefit chirp coding since the input to the pyramidal cell would thereby contain low-frequencies (Metzen et al., 2018). Nevertheless, we show that the neural response does not accurately reflect the chirp envelope and that envelope coding of decreases in amplitude of RAM envelope stimuli is poor. Our data replicate the findings of Martinez et al. (2016) that also show poor coding of envelope stimuli, with coherence values of approximately 0.1. The long-lasting adaptation process we observed further supports this result. It is possible that without the low frequency tuning, chirp and envelope coding would be worse and thus compensate for other properties that hinder envelope coding.

The role of burst coding

Bursts also often play an important role in coding specific features of communication signals (Creutzig et al., 2009; Fujimoto et al., 2011). This is particularly true for *A. leptorhynchus* in which bursting enhances detectability of small chirps on low frequency beats (Marsat et al., 2009). *A. leptorhynchus* small chirps are unusual in that, for low frequency beats, they span less that a full cycle of the beat. Consequently the same chirp can be perceived as a sharp decrease in amplitude, an increase or a mix of the two, depending on the phase of the beat at which the chirp starts. It may be because of this aspect of chirp structure, and its shortness, that the sensory system of *A. leptorhynchus* uses bursts to enhance detectability. *A. albifrons* is not subject to the same constrains: chirp perceived AM is largely independent of beat phase since chirps span several cycles of the beat and their long duration might make them more conspicuous (Petzold et al., 2016) .We showed that burst coding in the LS is different between

A.leptorhynchus and *A. albifrons* and we suggest that it might reflect their different role in coding of communication signals.

Chirp coding and behavior Our analysis presents a result in stark contrast with the coding strategy observed in *A. leptorhynchus*: all chirps are coded with graded increases and decreases in firing rate containing some information about chirp properties but, for high frequency beats, coding accuracy is ineffective at supporting both chirp detection and discrimination. Our behavioral data might shed some light on this apparent inefficiency at coding chirps, in particular when the beat is high frequency. We showed that *A. albifrons* seldom uses chirp to meditate dyad interactions. Furthermore we show that there a tendency (weak but significant) to chirp even less when the beat frequency is higher. This is supported by previous findings showing that chirp production is more frequent in same-sex contexts involving low frequency beats (Kolodziejski et al., 2007) therefor reinterating the relevance of chirp coding on low-frequency beats. These behavioral results suggest that chirp exchange is not central in mediating these interactions since they chirp less than once a minute, especially in the context of high frequency beats. This is unlike the complex chirp interactions observed in *A. leptorhynchus*, where chirps are omnipresent and play a central role in various type of interactions (Hagedorn & Heiligenberg, 1985; Henninger, 2015; Hupé, 2012).

Our study did not test for the effects of neuromodulation on tuning and chirp coding. Neuromodulation can change cell response properties as a result of behavioral state (Harris-Warrick and Marder, 1991). Previous work in *A. leptorhynchus* shows that serotonin enhances pyramidal cell excitability and responsiveness to small chirps on low frequency beats (Deemyad et al., 2013). This work suggests a role for modulation of chirp coding in the context of same-sex interactions, but there are likely similar effects related to other behavioral states. While we worked on adult animals, we did not determine sex or breeding status, both of which have large effects of the frequency and quality of chirps produced (Smith, 2013). It is likely that the effects of neuromodulation due to behavioral state could affect the reception and encoding of these chirps as well, altering sensitivity to chirps and possibly even coding accuracy in response to behavioral need. This may particularly influence the coding of chirps on high frequency beats, which we observed was surprisingly poor. This kind of 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.

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 exchange information (Bradbury et al., 2011). There are many examples of specialization of particular aspects of sensory systems to accomplish a highly specialized tasks (Endler, 1992). In the case of communication, sensory tuning for sender-receiver matching has been shown repeatedly. However, the converse may also be true. Over-specialization may come at the cost of reduced sensitivity to more general environmental signals. In such a case, it may be more beneficial to favor sensory generalization over specialization in animals that engage in social behaviors less often than their more gregarious relatives do. Indeed, there are several examples of peripheral sender-receiver mismatching that may be explained by gains in sensitivity to prey or predator signals to (Mason, 1991; Römer, 2016). Maintaining specificity for conspecific

communication may be costly both metabolically (Niven and Laughlin, 2008) and in regards to detecting environmental stimuli apart from communication signals.

In *A. albifrons* we show a general match between signal characteristics, low frequency chirp envelopes, and CNS sensitivity to low frequency signals, but a lack of complex coding that would allow for efficient discrimination of chirp identity in all contexts. For this species, investing fewer resources into the coding of social signals may allow the electrosensory system to focus more broadly. This low frequency tuning may be particularly adaptive for prey location and navigation, both tasks that the electrosensory system must perform, and both tasks that require coding of low frequencies. In such a case, our data argue for a match between neural coding and behavior via a relaxation of sender-receiver matching.

METHODS

Animals

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

Electrophysiology

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

Histology

In N=9 fish, correct electrode placement was confirmed by injection of Dextran Texas 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 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 (S3).

Stimuli

All stimuli were created in Matlab (Mathworks, Inc.) and sampled at 20 kHz. Stimulation was provided by a direct modulation of a carrier frequency matching the fish's own rather than by mimicking a second EOD, based on methods commonly used in similar experiments (Benda et al., 2005; Krahe et al., 2008; Marsat et al., 2009). 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).

Random amplitude modulation (RAM) stimuli consisted of 30 seconds of random noise filtered using a Butterworth filter to be either low pass (0-20Hz), high pass (40-60Hz) or broadband (0-60Hz). Each stimulus was played for three repetitions in both global and local stimulation configurations. Sinusoidal amplitude modulation (SAM) stimuli were 2 second long periods of regular sinusoids of 2, 5, 15, 30, 60, and 90 Hz with 2 seconds between each frequency, repeated at least three times. Step stimulations were 100 ms long increases and decreases in amplitude, attenuated to the fish's own signal strength as described above, 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 template *A. albifrons* EOD shape. Chirps of varying durations, frequency increases, and frequency rise/fall time (Table 1) were each embedded in this template EOD, at a rate of 1chirp/second. For each 1Hz of frequency increase, there was an associated .11% amplitude decrease, based on Dunlap et al. (1998). To this chirp EOD we added a second EOD either 10Hz or 100Hz lower to create an AM envelope. This envelope was extracted via spline interpolation and played using a carrier EOD as described above. Each chirp stimulus was played for at least 30 seconds, up to 60 seconds. Due to time constraints and recording stability, 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) representing spike timings using a bin width of .5ms. All analyses described here were performed on these binary sequences in Matlab. Additional analyses were as described (ANOVA, Student's T Test, Wilcoxon rank sum) and performed in Matlab using the statistical analysis toolbox.

Synchronization Coefficient

The strength of phase locking to SAM stimulation was calculated as $\frac{\sqrt{(\Sigma x_i)^2 + (\Sigma y_i)^2}}{n}$, where *n* is the number of spikes in the analysis, and *x* and *y* are the cosine and sine of the stimulus at which spike *i* occurs(Goldberg and Brown, 1969; Marsat and Pollack, 2004). Values range from 0 to 1, with 1 being perfect precision in responding to a given phase of the sine cycle.

Coherence

Lower bound coherence is a measure of linear coding of the stimulus and is calculated by comparing the spike train against the stimulus (Borst and Theunissen, 1999). The stimulus (S(t)) and the binarized neural response (R(t)) were expressed as variations around their respective means (S'(t) and R'(t)). These were converted into frequency domain representations of the stimulus (S'(f)) and responses (R'(f) using a fast Fourier Transformation. A linear filter for each response (H_i (f)) was constructed from R_i (f) as: $H_i(f) = R'_i(f) * \frac{S'_i(f)}{R'_i(f)} * R'_i(f)$. ($H_i$ (f)) was

converted back to the time domain using the inverse Fourier transform and the result H_i (t) was convolved with R_i (t) to create an estimate of the stimulus, $esti_i$ (t). Noise, n_i (t), was computed as $S_i(t) - esti_i(t)$. Signal-to-noise ratio, SNR_i (f), was computed as the power spectrum of $esti_i$ (t) divided by that of n_i (t). Lower bound coherence presented is the average *SNR* (f) from all repetitions (Marsat and Pollack, 2004).

Upper bound coherence measures the total information potentially coded in the response, including linear and non-linear information. This information potential is measured by comparing multiple responses of one neuron to each other to determine response reliability (Borst and Theunissen, 1999). Again, frequency domain representations of the responses were created (R(f)) and correlated then divided by an autocorrelation to yield coherence as : $Coh = \sqrt{\langle R_i(f) * R_j(f)\rangle\langle R_j(f) * R_i(f)\rangle / \langle R_i(f) * R_i(f)\rangle\langle R_j(f) * R_j(f)\rangle}$. Pairwise response correlations were performed for all repetitions for each neuron and averaged for an average upper bound coherence ($\overline{Coh}$). Information was calculated as $I(f) = -\log_2(1 - \overline{Coh})$ (Borst and Theunissen, 1999).

Burst detection

Bursts in RAM and chirp responses were determined by creating a histogram of all interspike intervals (ISIs) in the response. Generally, there was an obvious bimodal, non-Poission distribution of ISIs, allowing us to visually identify a threshold between burst and tonic firing. 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 Analysis

Analysis of chirps for detection and discrimination is based on Marsat and Maler (2010). 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, $f(t) = t^{-2.45t/\tau}$, with τ being the width of the function at half maximum (3, 10, 30, and 100ms; 30ms shown) (Machens et al., 2003). Detection becomes more accurate as a function of additional neurons contributing to the response, so we looked at population responses by summing the responses of *n* neurons using the function created population responses, in this case by averaging multiple spike trains using the function: $PR_i(t) = \sum_{i*t}^{n} \frac{R_i(t)}{n}$. 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_{cb} or D_{bb}) 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:. $D_{cb} = \frac{1}{L\sum_{t*O}^{L}[R_c(t) - R_b(t)]^2}$. 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=1/2PF +1/2(1-PD). 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 chirps against different chirps (x vs. y) were compared as well as multiple responses to the same chirp (x vs. x). Distance (D_{xy} or D_{xx}) was calculated for all sets of combined responses, $PR_x(t)$ and $PR_y(t)$, creating an array of response distances for each comparison using the function: $D_{xy} = \frac{1}{L\sum_{t*0}^{L}[R_x(t)-R_y(t)]^2}$. Similarly to the detection analysis, threshold values between distributions were varied. For each threshold value, the probability of discrimination between chirps (PD) is calculated, rather than detection. The sum of $P(D_{xy} > T)$ being the probability of discrimination(PD) and the sum of $P(D_{xx} > T)$ being the probability of false discrimination (PF). The error level for each threshold value is $E = 1/2PF + 1/2(1 - PD)$. The error in discrimination reported in the figures are the minimum values of E.

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 500 ms.

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 selected semi-randomly. Defining physical features (size, markings) and EODf were noted to avoid repeatedly testing the same pair. 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.

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FIGURE LEGENDS

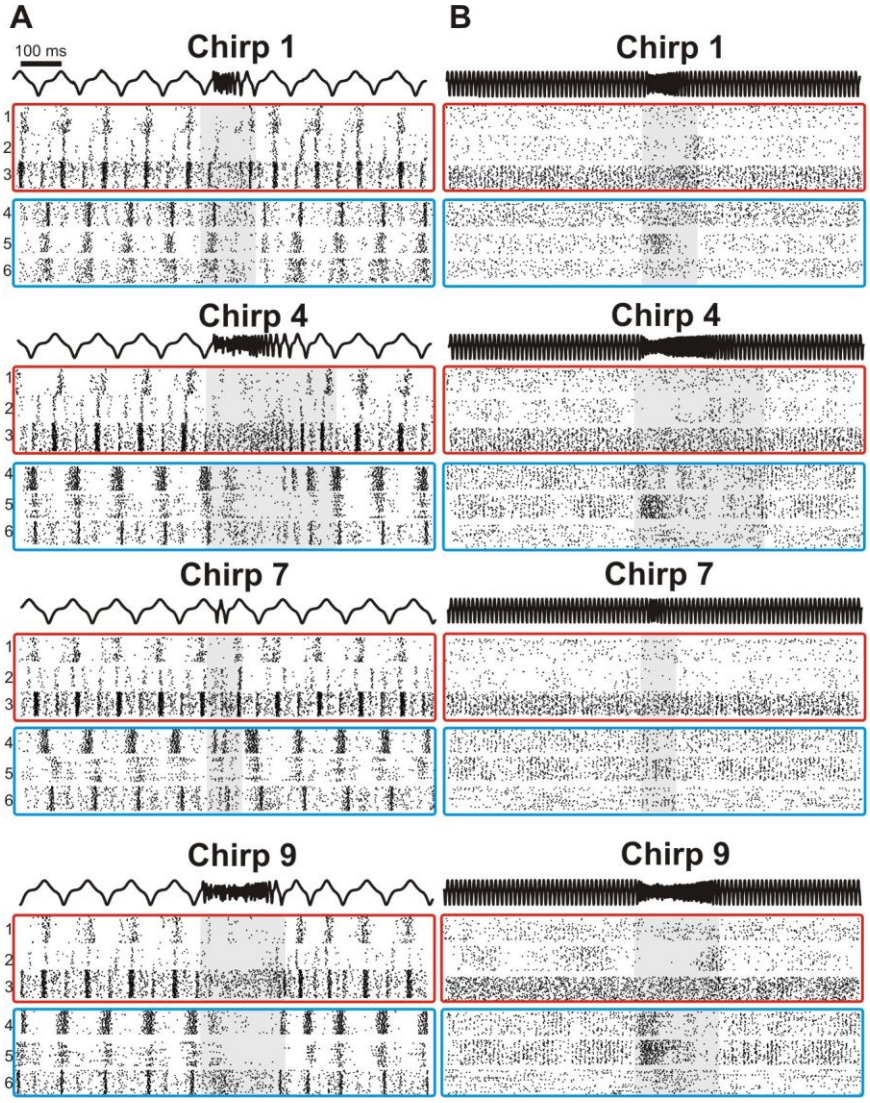


Figure 1: ELL Pyramidal cells respond to conspecific signals. A. Representative Raster plots of chirp responses on a low frequency (10Hz) beat. AM stimulus is shown in black; OFF-cells are shown in cyan boxes, ON-cells in red boxes. Three different ON-cells and three different OFF-cells are shown. The neurons displayed are consistent throughout. B. Identical chirps presented on high frequency (100Hz) beat. For a detailed description of all chirps used, see Table 1.

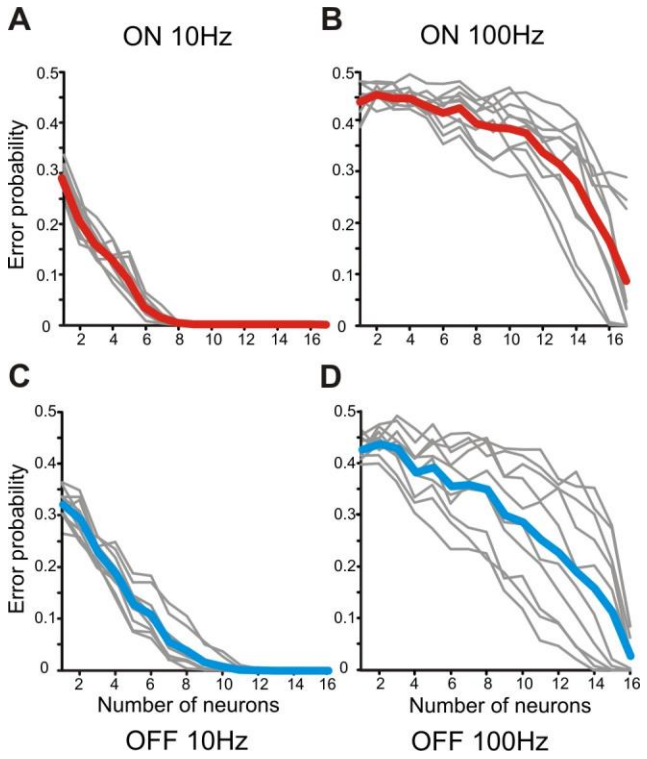


Figure 2: Detection efficiency is mediated by beat frequency. A. Detection of chirps on 10Hz beat by ON-cells as a factor of neurons included in the analysis (n=17). ON-cells can reliably detect the occurrence of all chirps. Detection error levels for individual chirp identities are shown in gray. Red line indicates mean detection error for 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.

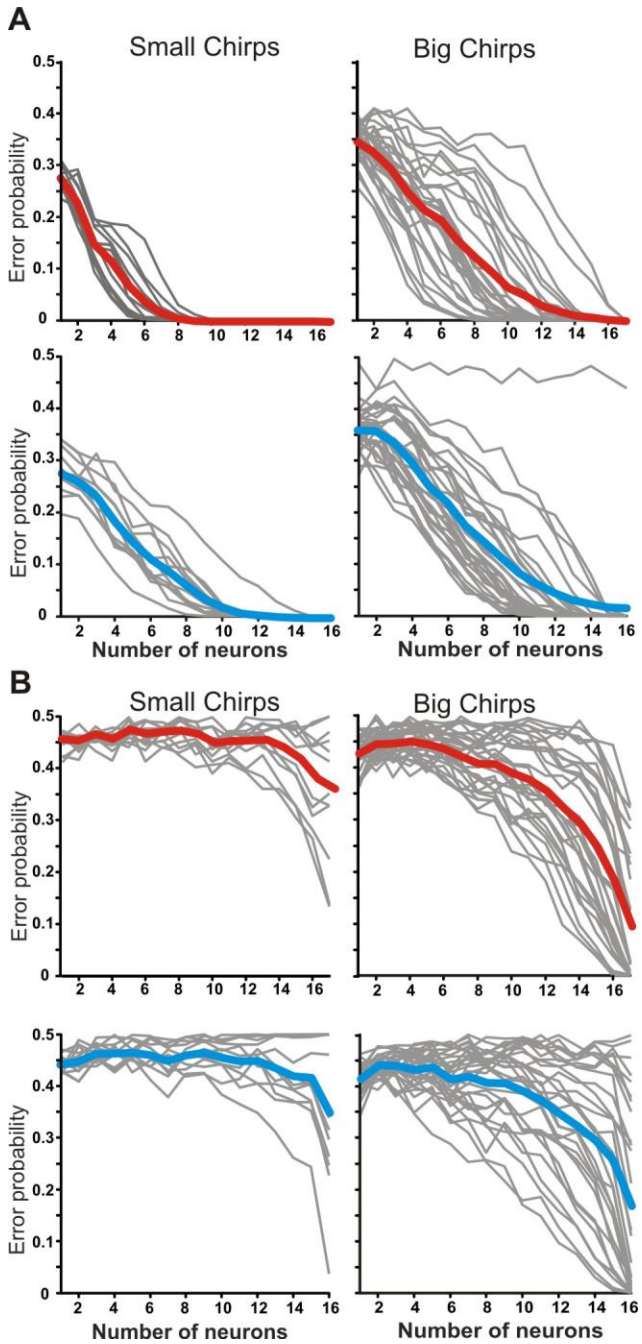


Figure 3: Discrimination of chirps on high frequency beats is poor. A. Discrimination for small chirps (50-100Hz, 50-100ms, Chirps 5,6,7,8, right) and big chirps (200-350Hz, 100-200ms, Chirps 1,2,3,4,9,10, left) on 10Hz beat. Mean ON-cell chirp discrimination shown in red, OFF-cells in cyan, and discrimination for individual chirps shown in gray. B. Discrimination of small chirps on a 100Hz beat is little better than chance for both ON (red) and OFF-cells (cyan). Discrimination of big chirps varies with chirp identity, but is still poor.

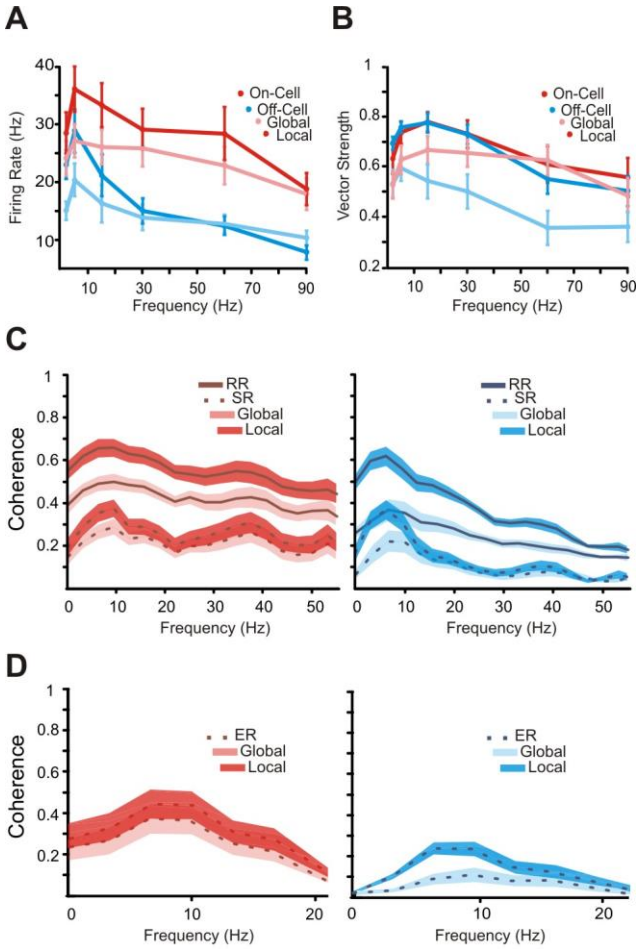


Figure 4: Low frequency coding by pyramidal cells A. Sinusoidal stimulation indicates low frequency tuning. Firing rate for both ON (red, n=19) and OFF (cyan, n=15) cells in both stimulation configurations (global: pink, pale blue; local: red, cyan) peaks at 5Hz. Error bars indicate standard error. B. Phase locking to 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 (RR, solid line) is the response-response autocorrelation between multiple presentations of the stimulus, while the lower bound (SR, dashed line) is stimulus-response correlation. 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. OFF-cell coding of low frequency envelopes is poor. Envelope responses to low frequency contrast changes (ER, solid line) are also low tuned . Both ON and OFF cells exhibit peak envelope tuning at 10.10Hz (±1.52 s.e.) (Wilcoxon rank-sum test, p=.21). OFF-cells are noticeably poorer at coding envelopes.

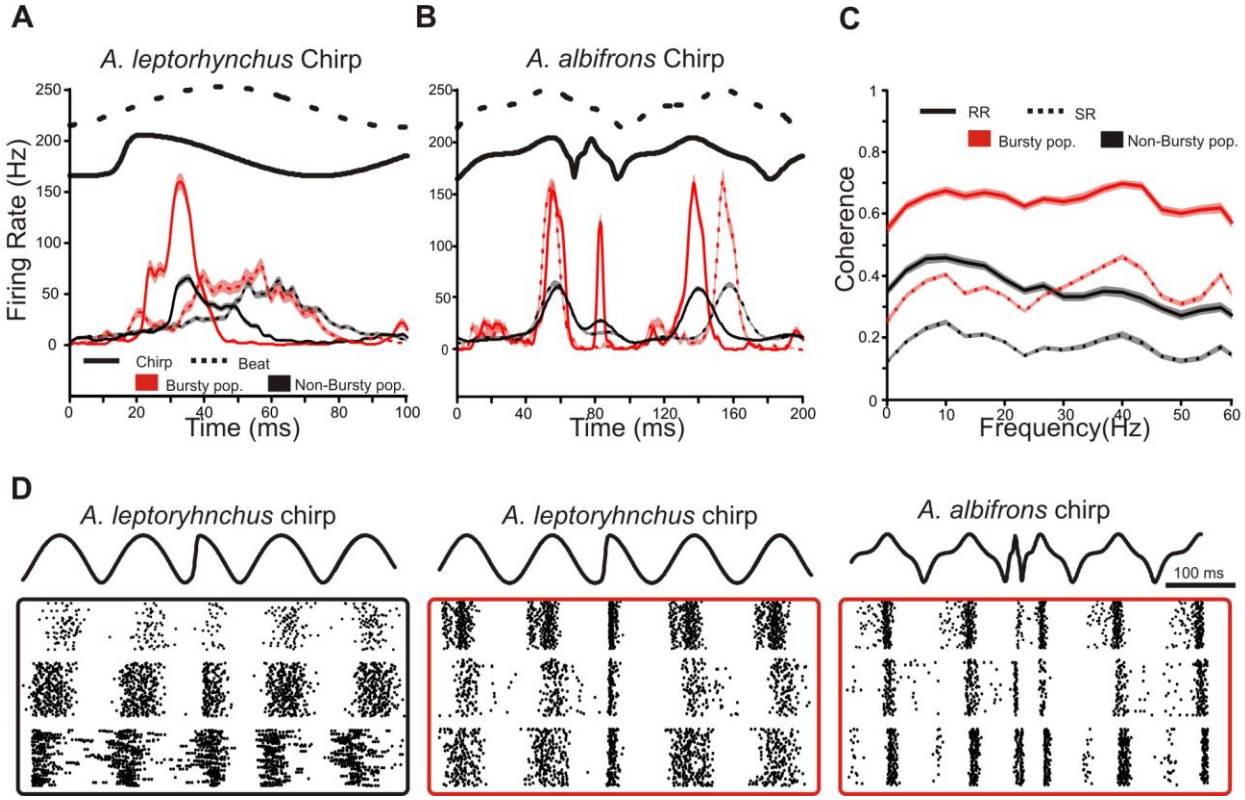


Figure 5: Coding of chirps by a small population of high firing neurons. A. The mean instantaneous firing rates of ON-cells over the time course of chirp and beat stimuli. The difference in peak firing rate indicates a small population (n=5, red) that bursts in response to *A. A. leptorhynchus* small chirps (Beat Responses, dashed lines: Peak FR 77.67 HZ +- 7.11; burst fraction 0.38 ± 0.02; Chirp responses, solid lines: Peak FR 159.57 Hz +- 8.68; burst fraction 0.76± 0.02, Wilcoxon ranked sum test (p<<.001). The majority of the ON-cell population (n=13, black) only showed a modest increase in bursting (Beat Response: peak FR 62.68 Hz +-1.27; burst fractions: 0.35±0.017; chirp response: peak FR 66.56 Hz+-4.21, burst fraction 0.44±0.02, Wicoxon ranked sum test (p=.002)). Shaded area represents standard error. B. Even the bursty population bursts less in response to *A. albifrons* chirps compared to the beat (beat response: peak FR 155.45 Hz +- 8.82, burst fractions 0.55± 0.03; chirp response: peak FR 117.75Hz +- 9.40, burst fraction 0.48± 0.03, Wilcoxon ranked sum test (p=.04)). C. The bursting population (red) tends to exhibit broad stimulus coherence compared to the non-bursting population (black). 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.).

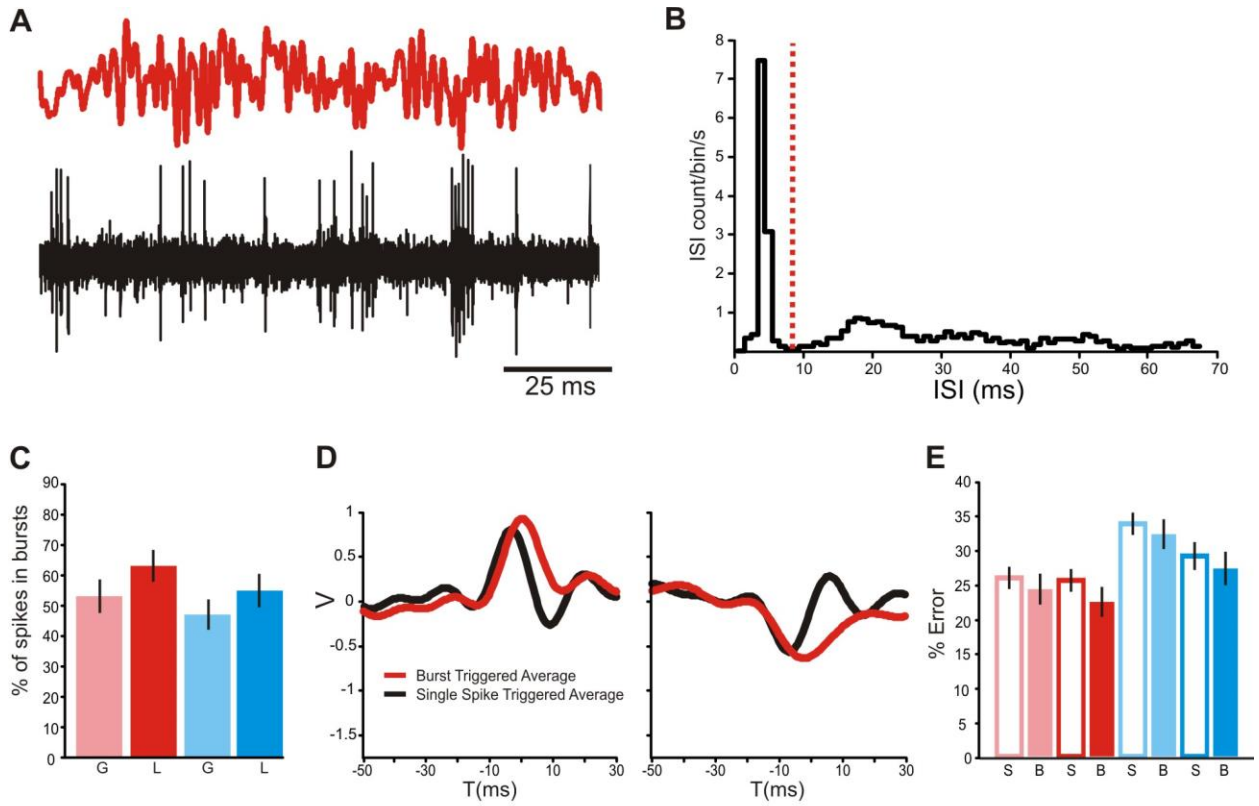


Figure 6: Coding of noise by bursts. A. Sample of noise stimulus (red) and representative spike train (black) from an OFF-cell. B. Example of ISI distribution used to determine burst threshold (dashed line). C. ON-cells (red) burst more than OFF-cells (blue) (ANOVA, p=.02), and local stimulation (L) produced more bursting than global (G) (ANOVA, p=.01). Error bars show standard error D. Mean burst triggered averages (red) and single spike triggered averages (black) from ON (right) and OFF-cells (left) show that bursts are triggered by wider (lower-frequency) stimulus features than single spikes. E. Bursts do not significantly reduce error in feature coding. In both ON and OFF cells in both stimulus configurations bursts (B) tend to have lower error rates than single spikes (S) but this trend is not significant (ANOVA, p=.10)

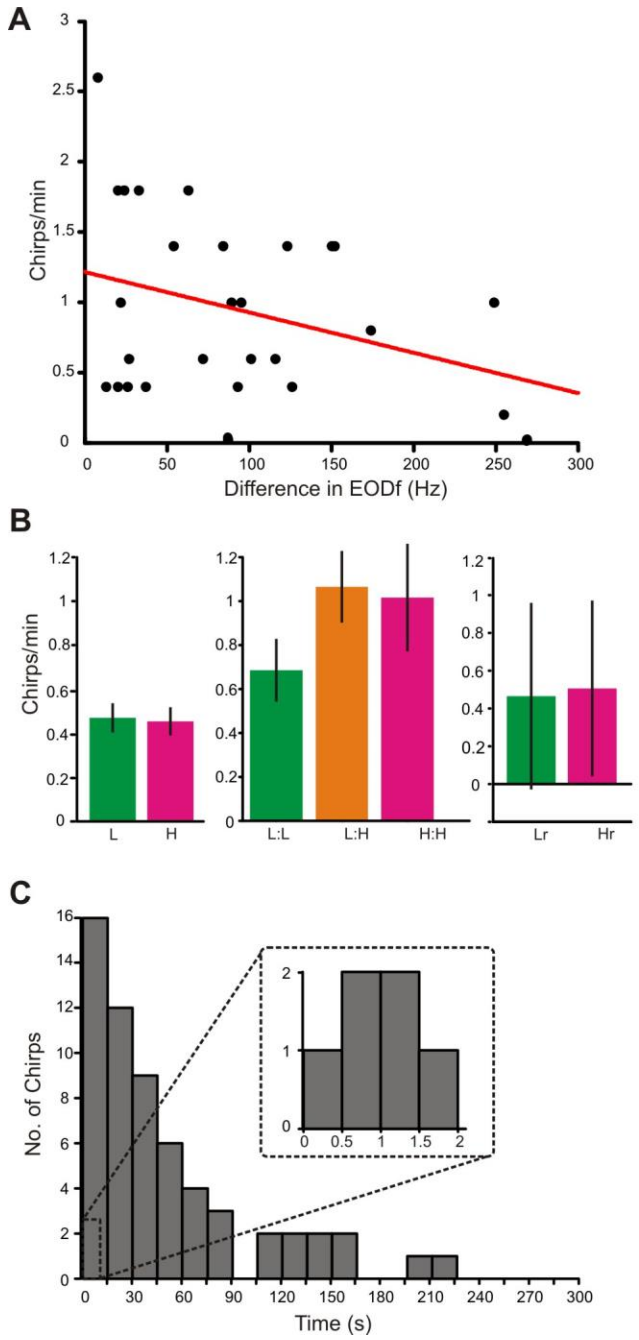


Figure 7: Chirping behavior in freely swimming pairs. A. The number of chirps produced during interaction is correlated with difference in EODf (r^2=.1093) B. Chirping does not differ by absolute EODf, relative EODf, or pairing. Mean chirp rate for low frequency (<1100Hz, L) and high frequency fish (>1100, H) was similar (Student's t test, p=.86). Mean), mean chirps per trial based on EODf of pairing (low frequency: low frequency, L:L; low: high, L:H; high: high, H:H) (ANOVA, p=.47), and by relative frequency within the pairing (Lower frequency individual, Lr; Higher frequency individual, Hr) (Student's t test, p=.55) were all extremely low and similar in all groupings. Error bars indicate standard error. C. Interchirp intervals for all recorded chirps

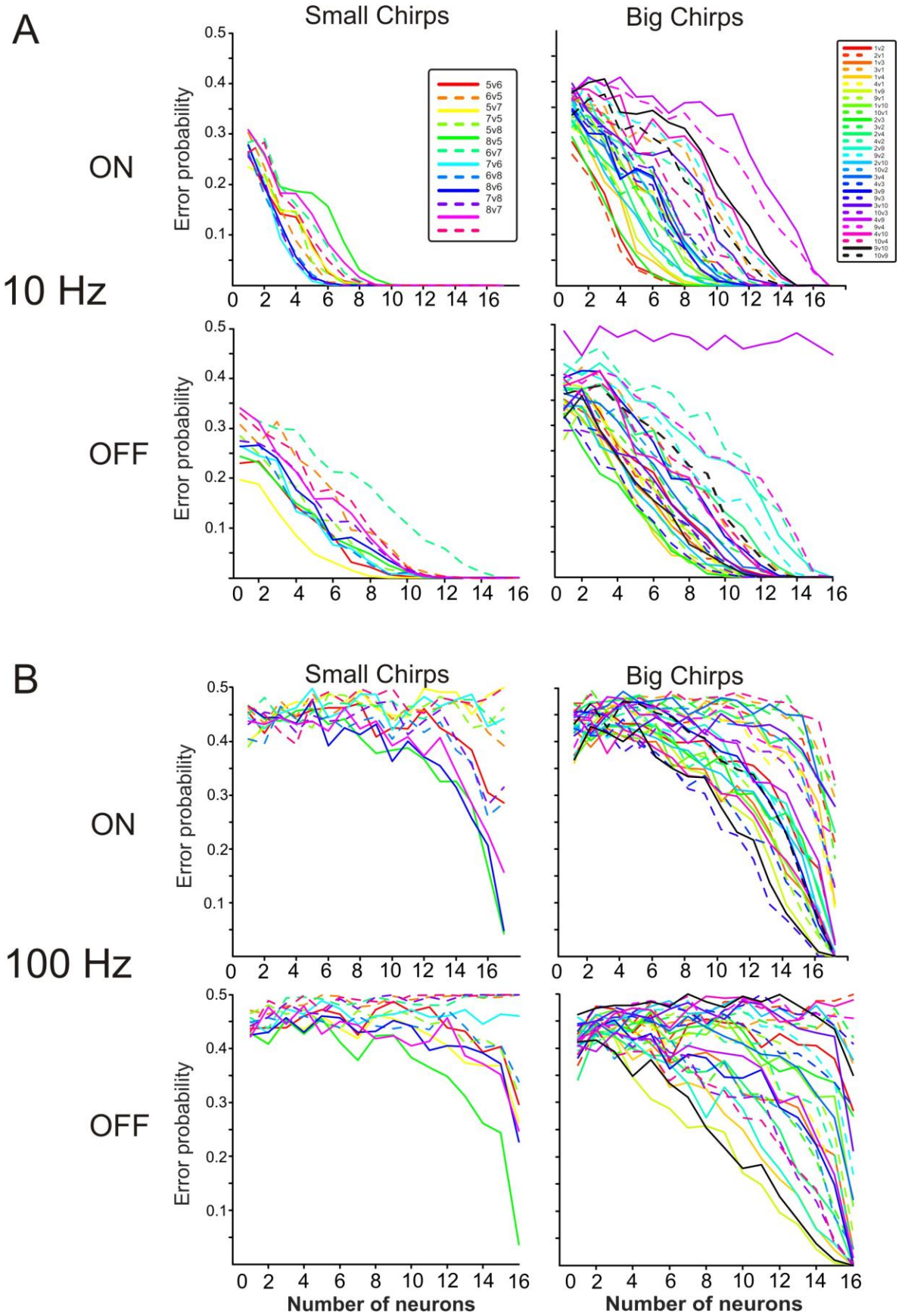
show no echoing or frequent chirp exchanges. Inset: Enlarged section show that very few chirps occur within 2s of each other.

Table 1

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

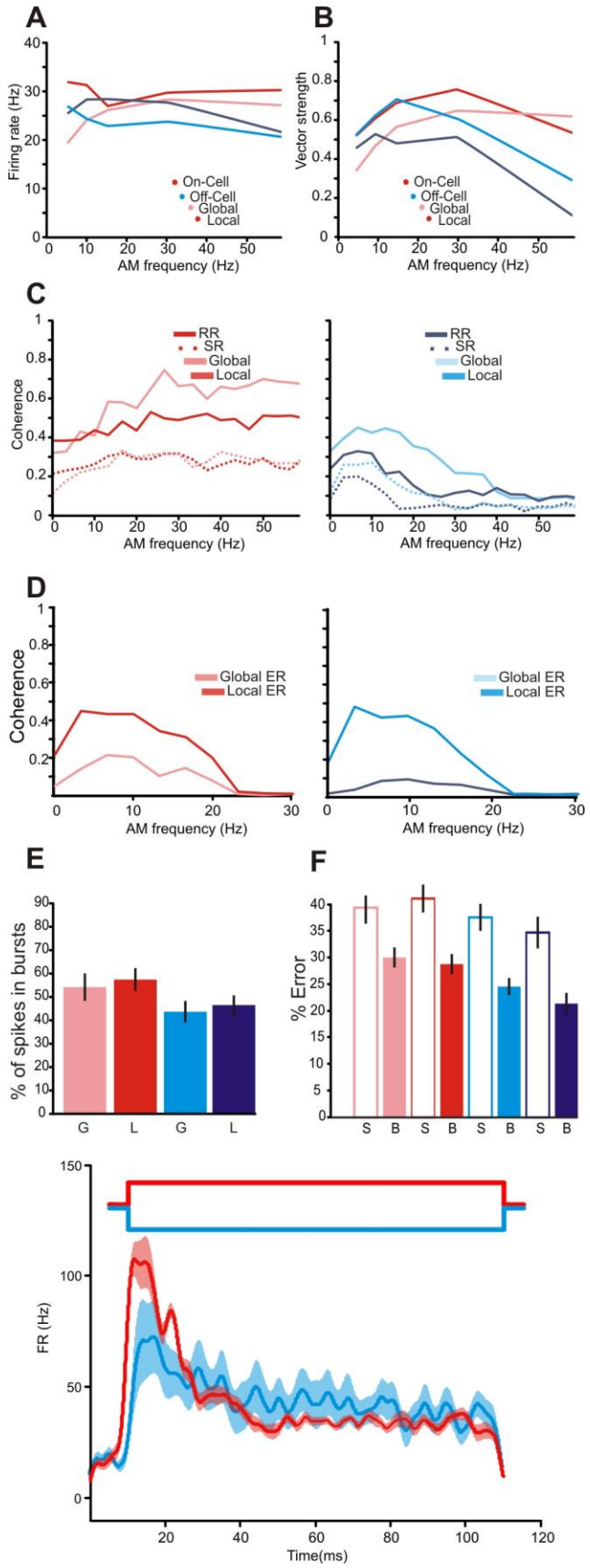
Frequency and duration properties for all chirps used

SUPPLEMENTAL FIGURES:


A
Small Chirps
Big Chirps
ON
10 Hz
OFF
Error probability
5v6
6v5
5v7
7v5
5v8
8v5
6v7
7v6
6v8
8v6
7v8
8v7
B
ON
100 Hz
OFF
Number of neurons

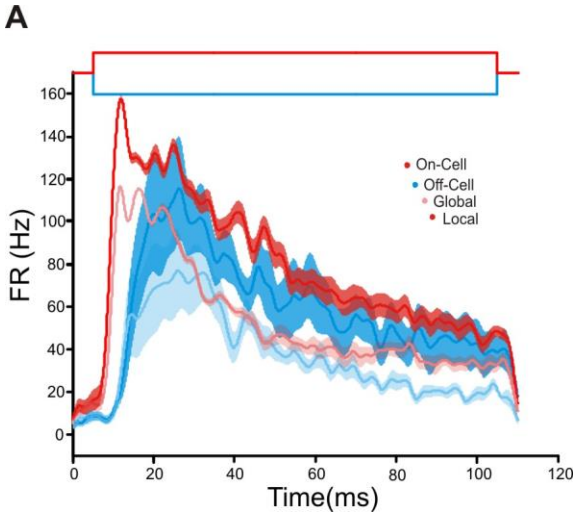
836

S1: 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.

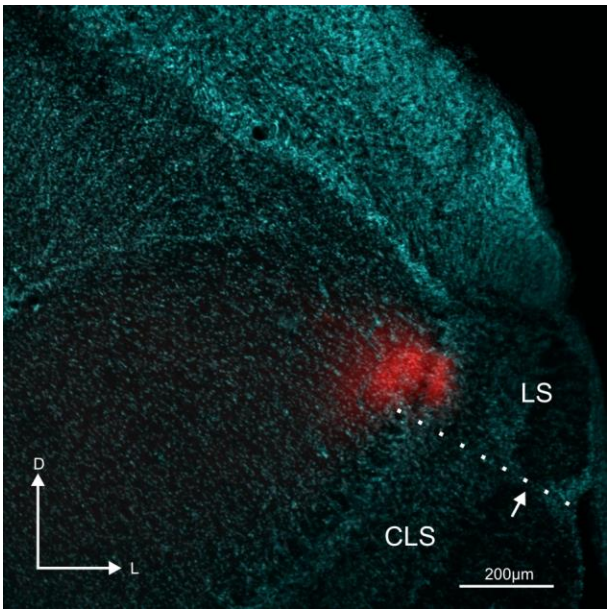

A
Firing rate (Hz)
On-Cell
Off-Cell
Global
Local
AM frequency (Hz)
B
Vector strength
On-Cell
Off-Cell
Global
Local
AM frequency (Hz)
C
Coherence
RR
SR
Global
Local
AM frequency (Hz)
RR
SR
Global
Local
AM frequency (Hz)
D
Coherence
Global ER
Local ER
AM frequency (Hz)
Global ER
Local ER
AM frequency (Hz)
E
% of spikes in bursts
G
L
G
L
F
% Error
S
B
S
B
S
B
S
B
FR (Hz)
Time(ms)

842

S2: *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 are 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. D. *A. leptorhynchus* adaptation to step stimulation (global stimulation shown).



S3: *A. albifrons* adaptation to step stimulation. Adaptation curves are not as sharp as those observed in *A. leptorhynchus*. We compared adaptation time constant, τ, by fitting exponent curves to the portion of the stimulus response after peak firing rate. For *A. albifrons* there was no difference in τ between ON and OFF cells (ANOVA p= 0.13) or between local and global stimulation (ANOVA p= 0.08).



S4: Histology showing location of recording within ELL. Red is Dextran Texas Red pressure injected at recording site, Cyan is Syto 59 nuclear marker. Arrow indicates the tract separating LS and CLS regions.