Population coding of natural stimuli in the nucleus praeeminentialis, a feedback center in the electrosensory system

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Table of Contents

Abstract

This master's thesis uses multi-unit recordings to analyze population-level neural activity in the nucleus praeeminentialis (nP), a key feedback area in the electrosensory system of weakly electric fish. The study has three main findings. First, noise correlations between neurons have minimal impact on the ability of a linear decoder to reconstruct stimuli from nP neural responses. Second, the research characterizes previously unrecorded neuron types in nP based on firing rates. Third, it reveals new aspects of sensory encoding, including nP multipolar cells' ability to encode envelopes, discriminate communication signals, and exhibit phase-invariant responses to those signals. Overall, the work elucidates feedback mechanisms for processing communication signals and environmental stimuli. It enhances understanding of the electrosensory system and highlights generalizable principles for sensory neuroscience.

Résumé

Cette thèse de maîtrise utilise des enregistrements multi-unitaires pour analyser l'activité neuronale au niveau de la population dans le noyau praeeminentialis (nP), une zone de rétroaction clé dans le système électrosensoriel des poissons faiblement électriques. L'étude présente trois constatations principales. Premièrement, les corrélations de bruit entre les neurones ont un impact minimal sur la capacité d'un décodeur linéaire à reconstruire les stimuli à partir des réponses neuronales du nP. Deuxièmement, la recherche caractérise des types de neurones auparavant non enregistrés dans le nP sur la base de leurs taux de décharge. Troisièmement, elle révèle de nouveaux aspects du codage sensoriel, y compris la capacité des cellules multipolaires du nP à coder les enveloppes, à discriminer les signaux de communication et à présenter des réponses invariantes en phase à ces signaux. Dans l'ensemble, le travail élucide les mécanismes de rétroaction pour le traitement des signaux de communication et des stimuli environnementaux. Il améliore la compréhension du système électrosensoriel et met en évidence des principes généralisables pour les neurosciences sensorielles.

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Author contributions

Maurice Chacron designed the experiments. Michael Metzen and Liam Easton collected the data. Liam Easton analyzed the data and wrote the manuscript.

List of figures

List of abbreviations

AM: amplitude modulation

CLS: centrolateral segment

CMS: centromedial segment

CSI: chirp selectivity index

EGp: eminentia granularis posterialis

ELL: electrosensory lateral line lobe

EOD: electric organ discharge

MS: medial segment

nP: nucleus praeeminentialis

PCA: principal component analysis

PSTH: peri-stimulus time histogram

STA: spike-triggered average

Introduction

The brain's ability to process sensory information is a fundamental aspect of how animals perceive and interact with the world. Sensory processing involves the reception, interpretation, and integration of sensory stimuli from various modalities including vision, hearing, touch, and others. The brain must accurately interpret these stimuli, filter out irrelevant information, and respond appropriately to environmental changes if the organism is to survive and reproduce. This complex task requires a delicate balance of stability (e.g., correcting for eye movements to maintain a stable visual field [1]) and adaptability (e.g., adjusting the sensitivity of auditory receptors to detect a stimulus of interest in the presence of noise [2]). Feedback mechanisms within the brain play a central role in ensuring both robustness against disturbances and flexibility in response to novel situations.

Feedback in living (and other adaptive) systems

Feedback refers to the ability of a system to adjust its behavior based on its previous state or output, thereby creating a loop of information flow. The concept of feedback is fundamental to understanding the complex dynamics of biological systems, where feedback mechanisms play a crucial role in maintaining the stability and robustness such biological systems against alterations in their parameters [3]. Feedback loops are ubiquitous in biology, governing processes from cellular regulation to ecosystem dynamics [4, 5]. Feedback is also integral to many technological systems. For instance, in an engineering context, feedback loops are used to control the temperature in a heating system or the speed of a car. In both contexts, feedback serves to adjust the system's behavior in order to minimize deviations from some preferred state, ensuring stability and adaptability in the face of changing conditions [6].

Feedback in sensory brain areas

In the context of the brain, feedback mechanisms are particularly prevalent and complex. The brain’s “wiring diagram” is characterized by a high degree of recurrent connections both within and between brain areas [7]. These connections are thought to play a crucial role in sensory processing by allowing for the integration of sensory input with prior knowledge that provides a context for that input [8-11]. Feedback connections from higher brain areas considerably outnumber feedforward connections even in early sensory areas and across sensory modalities,

indicating a universal principle of sensory processing [12-15], namely the modulation of sensory information based on the state of the organism and its environment.

For instance, in the visual system, feedback from higher visual areas to the early visual areas in primary visual cortex and the lateral geniculate nucleus of the thalamus can modulate the processing of visual information based on factors such as attention and expectation. This "top-down" feedback can enhance the processing of relevant visual stimuli and suppress the processing of irrelevant or distracting stimuli according to the behavioral context [1]. At the same time, information about eye movements from the superior colliculus, frontal eye fields and mediodorsal thalamus is fed back into the sensory pathway such that the effects of eye movements are subtracted from the sensory input, enabling brains to perceive the world as stable even when their sensors are in motion [16].

Similarly, in the auditory system, feedback loops from the auditory cortex to the cochlea can adjust the sensitivity of auditory processing based on the noise level in the environment. This feedback helps in protecting the auditory system from damage due to loud sounds [17], and aids in the detection of sounds in noisy environments by filtering out irrelevant frequencies based on selective attention to auditory stimuli [2]. In the somatosensory system, descending pathways can modulate the perception of touch [18] and pain [19]. For example, cortical feedback can modulate or inhibit the transmission of pain signals in the spinal cord, depending on emotion, attention, and many other factors [19].

More generally, feedback pathways are proposed to play a central role in predictive processing, an emerging framework for understanding brain function in which higher brain areas are thought to attempt to predict sensory inputs based on expectations derived from past experience; the error between these predictions and the actual input is then used to update the model of the world from which the expectations are drawn [20]. While this perspective on brain function has a long history [21], it has recently been supported by empirical findings and developed to include not just perception but also action. Thus, according to the predictive processing framework, the function of both perception and action alike is to reduce sensory prediction error resulting from an organism's interactions with the environment [8, 22]. The integration of bottom-up sensory

information and top-down expectations has furthermore been suggested to be necessary for conscious experience [11, 23, 24], but this is outside the scope of this thesis.

For present purposes, it is sufficient to state that feedback mechanisms play a vital role in sensory processing by modulating the reception and interpretation of sensory stimuli based on various factors. They can enhance the perception of relevant stimuli, suppress irrelevant stimuli, and adjust sensory sensitivity based on the environmental or behavioral conditions. Understanding these feedback mechanisms can provide insights into the functioning of sensory systems and can have implications for the treatment of sensory disorders. The study of feedback pathways in mammalian sensory systems is nonetheless a difficult task due both to the complex organization of the cortex (composed of many feedback loops) and to the "circular causality" inherent in feedback loops, in which the output is always affecting the input at the same time as the input is affecting the system [25].

Feedback in the electrosensory system

The electrosensory system of weakly electric fish presents a relatively simple model for the study of sensory processing in comparison to the mammalian cortex, with which it nevertheless shares many structural and functional similarities such as receptive fields with ON/OFF centers and surround inhibition, the presence of multiple topographic maps, and the importance of feedback from higher brain areas [26]. In particular, it includes a simple and accessible feedback circuit that makes it ideal for studying feedback mechanisms in sensory processing. The central node of this feedback circuit is the nucleus praeeminentialis (nP), a hindbrain structure that is hypertrophied in electric fish and plays a crucial role in processing electrosensory information. The nP receives input both from higher brain areas and from first order electrosensory neurons and sends feedback projections back onto these first order neurons, modulating their responses to stimuli.

Statement of problem

Half a century of experiments has revealed numerous roles for this feedback pathway in the electrosensory system, from the adaptive cancellation of redundant input to the enhancement of specific stimuli. However, our understanding of how nP neurons transform electrosensory inputs

in the course of implementing these and other feedback functions critical to the processing of electrosensory stimuli remains limited. Recordings from nP to date have focused entirely on responses of two well-characterized cell types—whereas at least ten cell types are known to exist in nP—to certain classes of stimuli. Notably, how nP neurons respond to electrocommunication stimuli is unknown. Moreover, all recordings in nP to date have employed single-unit electrodes, whereas it is generally recognized that population-level effects of the kind observable only by simultaneous recordings of many neurons are critical to understanding how brains process information.

In this thesis, I conduct multi-unit recordings of nP neurons in awake, behaving *Apteronotus leptorhynchus*, a South American weakly electric fish, in order to analyze the population-level responses of nP neurons to a range of natural stimuli. Through this approach, I hope to shed light on the collective activities of nP neuronal populations and begin to decipher the computations by which these neurons implement known (and perhaps novel) functions of feedback in the electrosensory system. By bridging this knowledge gap, this work contributes to a more comprehensive understanding of the important role of feedback in sensory processing and in shaping how organisms perceive the world.

Background information

Electroreception

Electroreception refers to the ability of some animals to detect external electrical gradients via specialized electroreceptors and associated neural circuitry. Such gradients are ubiquitous in aquatic environments and can be of both biological and nonbiological origin [27]. Despite being the most recently described sense modality [28, 29], electroreception is ancient; it is present in most non-teleost fish and considered to be an ancestral vertebrate trait [30]. Electroreceptors disappeared in most terrestrial vertebrates, perhaps unsurprisingly since atmospheric air is electrically insulating, and also disappeared for unknown reasons in most teleost fishes. *A. leptorhynchus* belongs to the gymnotiform family of neotropical weakly electric fishes, which, along with the mormyriform family of African fishes, is one of two lineages of teleost fish

featuring the reappearance of electroreceptors that not only detect external gradients, an ability sometimes referred to as *passive* electroreception, but also respond to self-generated electric fields resulting from the discharge of specialized electrogenic tissue known as the electric organ, an ability referred to as *active* electroreception [31]. In "wave-type" fish such as *A. leptorhynchus*, the electric organ discharge (EOD) is continuous and quasi-sinusoidal (Fig. 1A, right, green). Modulations in the EOD caused by objects with different conductances than the surrounding water, such as the small crustaceans that form the diet of these fish, conspecifics, rocks, and tree roots appear as "electric images" on the fish's skin, and from these images the fish can determine object distance, size, shape, and electrical properties [32].

Active electroreception thus allows gymnotiforms to communicate, navigate, forage, and orient themselves at night and in dark, turbid waters, and contributes to their considerable ecological success in neotropical aquatic ecosystems [33-35]. From the perspective of experimental neuroscience, the relatively simple spatiotemporal characteristics of natural electric stimuli, which can be easily mimicked in the laboratory, together with a relatively simple neuroanatomy, has led to the use of the electrosensory system as a model for understanding sensory processing. I describe several classes of natural stimuli that will form part of my experimental protocol below.

Natural stimuli

i. Beats

By emitting a continuous electric field, wave-type fish like *A. leptorhynchus* provide a continuous stimulus to other nearby electric fish. Whenever two wave-type fish come close to each other, their EODs overlap and result in a periodic amplitude modulation, often called a "beat". The frequency of the beat is equal to the difference between the EOD frequencies of the two fish. The frequency of a fish's EOD can communicate species identity, sex, and social status [36-38]. Since EOD frequency in *A. leptorhynchus* is sexually dimorphic, ranging from ~600–800 Hz in females and ~800–1000 Hz in males [36], a low-frequency beat generally corresponds to same-sex interactions while a higher-frequency beat corresponds to opposite-sex interactions.

ii. Envelopes

The amplitude of the beat forms a second-order stimulus called the "envelope" (Fig. 1A, right). Envelope contrast varies with the relative distance and orientation of the two fish [39, 40]; this is sometimes referred to as a "movement envelope". Movement envelopes tend to contain lower temporal frequencies (<1 Hz) than the beats themselves [41]. *A. leptorhynchus* respond behaviorally to very weak envelopes; in a natural setting such stimuli would correspond to the relative motion of a far-off conspecific [42]. A second type of "social" envelope arises from the interactions of the EODs of three or more fish [43]. In natural settings, social and movement envelopes will often occur in conjunction [44, 45]. The present study focuses on movement envelopes (hereafter simply referred to as envelopes). Envelope processing in the electrosensory system has been extensively investigated [42, 46-56] (reviewed in [41]). It is important to note that due to their distinct frequency contents, extracting an envelope from its carrier signal requires nonlinear processing [42]. As a practical matter, separate stimuli are used for studying neural responses to beats and envelopes (see Methods).

iii. Chirps

Although the EOD is relatively stable over long periods [57], short-term frequency modulations often occur in the context of social interactions. The fastest (typically <25 ms) frequency modulations are called "chirps" [58, 59]. Chirps are commonly emitted by males and come in two main varieties: big chirps, with EOD frequency increases of several hundred Hz, and the much more common small chirps, with EOD frequency increases of <100 Hz. In laboratory settings, small chirps are usually elicited by beats with frequencies <30 Hz (corresponding to other males) and are assumed to play a role in aggression [37, 60, 61], whereas big chirps are elicited by beat with frequencies >50 Hz and are assumed to be associated with courtship [62-64]. Recent field studies have shown that small chirps can also be elicited by higher beat frequencies during courtship [37]. Chirp encoding has been investigated at various stages of the electrosensory system [65-74] (reviewed in [75]).

In addition to chirps, a wide variety of frequency modulations over longer time scales have also been observed. The most well-studied is the jamming-avoidance response, in which a fish shifts its EOD frequency away from the interfering signal of a nearby fish with a similar EOD frequency [76]. Gymnotiforms have also been shown to modulate their EOD frequency to track

the time-course of low-frequency (<1 Hz) envelope stimuli [77], although the behavioral relevance of this, and many other of the wide variety of EOD frequency modulations, is still unclear. Here it is pertinent to note that unlike other genera of weakly electric fish whose electric organ is derived from muscle cells, the electric organ of *Apteronotus* species is derived from nerve cells [78-80] and its function is unaffected by the curare-like paralytics often used to immobilize these animals for experiments, which specifically act on muscle-type nicotinic acetylcholine receptors [81]. The fish thus continues to exhibit electrical behavior for the experiment's duration. Jamming avoidance, envelope-tracking and chirping behaviors are routinely observed in EOD recordings made during experimental protocols such as the one I propose.

Neuroanatomy of the electrosensory system

Understanding how the above stimuli are processed at successive stages of the electrosensory system depends upon a detailed anatomical understanding of that system. Here I give an overview of the neuroanatomy of the electrosensory system, paying special attention to feedback pathways. Embedded within the skin of gymnotiform fish are two classes of electroreceptors: ampullary receptors, which respond to low-frequency external gradients [82], and tuberous receptors, which respond to frequencies in the range of the fish's EOD [83, 84]. Tuberous receptors can be further classified into P-type, which increase or decrease their firing rate in a probabilistic manner in response to increases or decreases in EOD amplitude, and T-type, which faithfully encode changes in EOD frequency. T-type receptors are far less numerous and are not considered further here.

Afferent fibres from electroreceptors project to the electrosensory lateral line lobe (ELL), a cerebellar-like structure of the hindbrain (Fig. 1B). Afferents from tuberous receptors trifurcate and project topographically to three parallel maps within ELL, the centromedial (CMS), centrolateral (CLS) and lateral (LS) segments, which are structurally identical but vary in frequency tuning, thus providing parallel information streams tied to different behavioral contexts [85]. CMS is associated with spatially localized, low-frequency (1–20 Hz) signals such as those caused by the fish's movements as it swims past/hunts a prey item [86, 87], while LS is associated with spatially diffuse, high-frequency (>50 Hz) electrocommunication signals, and

CLS responds to all behaviorally relevant frequencies. Ampullary receptors project to a fourth, medial (MS) segment.

Pyramidal cells within ELL are the main projection neurons associated with P-type receptor input. ON-type pyramidal cells have basal dendrites and are excited by P-type input, whereas OFF-type pyramidal cells have no basal dendrite and are inhibited by P-unit input. Pyramidal cells are further classified into superficial, intermediate and deep subtypes, and these differ significantly in ion channel composition [88-90], dendritic morphology [91] and plasticity [92]. Importantly, superficial cells (and, to a lesser degree, intermediate cells) are more plastic, have larger apical dendrites and receive considerably more feedback from higher brain areas, whereas deep cells, which are relatively linear encoders of AM stimuli [93], are the source of that feedback [94].

Efferent axons from ELL pyramidal cells project to the midbrain torus semicircularis. Deep pyramidal cells additionally provide input to nP, a large bilateral nucleus involved in feedback control of electroreception and the focus of this thesis. Torus in turn projects to higher brain areas including the optic tectum and motor areas involved in sending command signals to the electric organ; torus also send feedback projections to nP where it drives stellate cells [95, 96].

Output from nP feeds back onto ELL pyramidal cells in ELL either directly or indirectly via the eminentia granularis posterialis (EGp), forming the “direct” and “indirect” pathways, respectively. In the direct pathway, axons of nP stellate cells form a fibre tract and terminate in a topographic manner on the proximal spines of ELL pyramidal cell apical dendrites, as well as on interneurons [97, 98], forming excitatory connections [99]. Bipolar nP neurons also form part of the direct pathway but form spatially diffuse inhibitory connections within the ELL pyramidal cell layer [100]. In the indirect pathway, several other types of nP neurons including multipolar cells project indirectly to ELL via EGp, where electrosensory input is combined with proprioceptive information from the brainstem and transformed into granule cell activity [101]. EGp granule cells then feed back via typical cerebellar parallel fibres to ELL, where they terminate in a diffuse manner on the distal spines of pyramidal cell apical dendrites [97, 98].

Anatomical studies have classified neurons in nP into roughly ten morphological types in addition to three types of interneurons [102]. The firing properties of stellate and multipolar nP cells in response to moving electrolocation targets and global EOD amplitude modulations have been previously characterized. Multipolar cells have high spontaneous firing rates (30–100 Hz) and encode long-term changes in EOD amplitude with high resolution [103]. Stellate cells have very low spontaneous firing rates (<5 Hz) but respond well to moving electrolocation targets and low frequency AMs [96]. In contrast to multipolar cells that exhibit their strongest response (in terms of firing rate modulation) to ~64 Hz AM stimuli, stellates are inhibited by AM frequencies greater than ~16 Hz. The response properties of nP neurons other than stellate and multipolar cells have not been investigated to date.

Roles for feedback in the electrosensory system

Feedback plays a number of roles in the modulation, and in some cases generation, of responses in ELL pyramidal neurons, as reviewed in [104]. Predominantly, the indirect pathway handles functions such as gain control and cancellation of redundant stimuli, while the direct pathway, which is perhaps less well-understood, enhances or generates responses to specific stimuli.

i. Indirect pathway

Sensory systems face the challenging task of filtering relevant information from a rich and constantly changing environment. An important part of this task involves distinguishing between sensations that are caused by the organism's own actions, and hence uninformative, and those that are due to some potentially important change in the world. For example, gymnotiform fish often bend their body into an arc during active sensing behaviors. This results in large changes in EOD amplitude at different points on the body. While P-type afferents respond strongly to these amplitude changes, ELL pyramidal cells can cancel out these uninformative consequences of self-motion [105-107]. This ability relies on a "negative image" of the predicted stimulus received via the indirect feedback pathway, which is combined in ELL with the afferent signal, thereby cancelling the redundant stimulus and enhancing unexpected stimuli. Proprioceptive and electrosensory information, and possibly corollary discharge signals from motor commands, are combined in EGp and contribute to the generation of the negative image [92, 101-103, 105]. Plasticity at the feedback synapses allows the cancellation to adapt as the predicted input changes

[108, 109], and is likewise involved in gain control, whereby pyramidal cell responses remain relatively invariant with respect to changing stimulus amplitude [110-113].

The indirect feedback pathway is primarily activated by spatially diffuse stimuli [93, 94, 114-116]. In addition to attenuating responses of ELL pyramidal cells to low-frequency global stimuli, such as those caused by tail-bending or by a nearby conspecific [114, 117], indirect feedback enhances responses to localized stimuli, e.g., prey [118], and high-frequency communication signals [70, 93, 94, 114, 115, 119]. Indirect feedback input has also been shown to participate in the optimized coding of low-frequency envelopes by modulating ELL responses to match natural stimulus statistics [48].

ii. Direct pathway

Inhibitory input to ELL from nP bipolar cells was shown to generate oscillations in the gamma range (~30 Hz) in response to diffuse stimuli (e.g., conspecifics), but not in response to localized stimuli (e.g., prey) [120]. These oscillations can enhance the directionally selective responses of torus cells to moving objects [121]. Synchronization of neural activity by gamma oscillations is thought to play an important role in sensory processing across species [122], including in the selection between competing stimuli [123].

Stellate cell input to ELL is topographic and excitatory, and it has been proposed that stellate cells show the necessary characteristics of a "sensory searchlight" mechanism for highlighting important stimuli via positive feedback onto localized groups of ELL pyramidal cells [124], although specific evidence for this hypothesis is still lacking. Positive feedback from stellate cells in nP has been shown to drive responses in ELL pyramidal cells to certain stimuli, such as receding objects [125] and low-contrast envelopes [53]. Feedback from stellate cells also shares a role in the optimized coding of envelopes; whereas multipolar cells in the indirect pathway attenuate ELL responses to low-frequency envelopes to match natural statistics, stellate cells in the direct pathway are responsible for enhancing ELL responses to envelopes independently of envelope frequency [48]. Most recently, it was found that feedback from nP promotes heterogeneity in the responses of superficial ON cells in ELL to envelopes, with beneficial

effects on envelope coding [52]. Whether this effect of feedback involves the direct or indirect pathway, or both, is not yet known.

The direct feedback pathway can also participate in negative image formation [94, 106]. Since the direct pathway receives only electrosensory input, this shows that cancellation can be achieved using only information from the same modality without the need for proprioceptive input. Indeed, negative image responses can develop in response to repetitive electrosensory inputs alone [105-107].

Rationale, hypotheses, and specific aims

As described above, experiments have established a variety of roles for feedback in the electrosensory system, notably gain control, cancellation of redundant stimuli, and enhancing responses to moving objects, chirps, and envelopes. However, much remains to be understood about how population activity in nP underlies these functions. The response properties of nP neurons other than stellate and multipolar cells have not been investigated, and it is unknown how, or whether, nP neurons encode chirps. Moreover, all previous recordings in nP have been done with single-unit electrodes, resulting in a limited understanding of population-level response properties. The present study aims to fill these gaps. By recording from multiple nP neurons simultaneously, I hope to characterize how populations of nP neurons respond to a variety of naturalistic stimuli and investigate how these responses could underly established or novel roles of feedback in the electrosensory system. My findings will ideally guide further experiments with the broad aim of unravelling the ways in which feedback shapes sensory processing.

I hypothesize that multipolar cells will effectively encode the time course of global AM stimuli (‘beats’) at a range of frequencies. I expect stellate cells likewise to effectively encode the time course of AM stimuli below ~32 Hz, beyond which frequency they are inhibited [96].

I hypothesize that both stellate cells and multipolar cells will effectively encode envelope stimuli—as measured by the performance of a linear decoder (see below)—in agreement with

their respective roles in enhancing and sculpting envelope responses in ELL [48, 53]. Based on recent work showing that feedback from nP promotes heterogeneity in superficial ON (but not in OFF) pyramidal cell envelope responses in ELL [52], I further hypothesize that the envelope responses of stellate cells will be relatively heterogeneous. I make this hypothesis because previous work has shown that the indirect pathway affects both ON and OFF pyramidal cells in ELL [93, 94, 115].

How nP neurons will respond to chirp stimuli is more difficult to predict. While the indirect pathway is necessary for burst responses in ELL pyramidal cells to chirp stimuli, this occurs because the negative image input generated in response to the underlying beat enhances the chirp response, and not because of feedback deriving from the chirp stimulus itself [70]. Nevertheless, because multipolar cells respond strongly to AM stimuli in the frequency range at which chirps occur (i.e., >50 Hz) [103], I expect them to also respond strongly to chirps, although it is not clear how, or whether, this information would be used by downstream brain areas. Stellate cells, by contrast, are inhibited at these higher frequencies and likely will not show a strong chirp response [96].

The effect of correlated neural activity on signal encoding performance at the population level in nP is also hard to predict, as correlations can be detrimental [126, 127] or beneficial [128, 129]. Based on recent studies of population coding of chirps in ELL and torus, however, I hypothesize that correlations in trial-to-trial variability, i.e., noise correlations, will decrease the performance of a linear decoder by introducing redundancy [130, 131].

Overall, I expect considerable heterogeneity in responses, owing to the presence of ~10 morphologically distinct neuron types in nP [102]. As a further hypothesis, I suspect that many neurons will not respond to the presented stimuli in any obvious way, as has generally been observed in multi-unit electrode recordings across species [132, 133].

To test these hypotheses, I propose the following specific aims:

1. Record from populations of nP neurons in awake *A. leptorhynchus* in response to three classes of naturalistic stimuli that have been used in previous studies of the electrosensory system, namely beats, envelopes, and chirps.

2. Characterize the response properties of nP neurons to these stimuli using standard methods such as tuning curves and peri-stimulus time histograms.
3. Identify stellate and multipolar cells based on known response properties and use this information to establish which of the remaining neurons can be considered to reside in nP.
4. Look for groups of cells with similar response properties (including but not limited to stellate and multipolar cells)
5. Reconstruct AM and envelope stimuli from neural responses to investigate what information neural activity in nP contains about each stimulus.
6. Investigate the ability of nP neurons to discriminate between chirp stimuli with different attributes.
7. Analyze the effects of noise correlations on the ability of populations of nP neurons to encode information about the different stimuli.
8. Integrate the results of my analyses into our understanding of the roles of feedback within the electrosensory system.

Methods

Here I describe the methods for specific aims 1–7 above (the 8th aim will form the Discussion section). Following each description, I state the rationale for this choice of method, followed by its potential problems and their possible solutions.

1. Experimental protocol (animals, surgery, recording, and stimulation)

The neotropical weakly electric fish *Apteronotus leptorhynchus* (N = 4) was used in this study. Fish were purchased from tropical fish suppliers and were housed in groups (2–10) at controlled water temperatures (26-29°C) and conductivities (300–800 μS/cm) according to published guidelines [134]. All animal procedures were approved by McGill University's animal care committee.

Surgical procedures have been described previously [48, 53]. Briefly, the fish was first immobilized by intramuscular injection of 0.1–0.6 mg of tubocurarine (Sigma-Aldrich, St-Louis,

MO, USA). It was then transferred to an experimental tank (30 cm × 30 cm × 10 cm) containing water from its home tank and respired by a constant flow of oxygenated water through its mouth at a flow rate of 10 ml/min. The head of the fish was locally anaesthetized by applying lidocaine ointment (5%; AstraZeneca, Mississauga, ON, Canada). The skull was then partly exposed, and a small window opened over the nP recording site.

A Neuropixels probe (Imec inc., Leuven, Belgium) was inserted into the brain vertically with respect to the sagittal plane at transverse slice T4 of the *Apteronotus* brain atlas [135] and the tip moved 2000 μm into the brain as measured from the surface. After probe insertion the brain tissue was allowed to settle for thirty minutes before recording began.

A 100 s baseline period was recorded before stimulus presentation. Stimuli consisting of amplitude modulations (AMs) of the fish's own EOD were produced by triggering a function generator to emit 1 cycle of a sine wave for each zero crossing of the EOD, as done previously [63]. The frequency of the emitted sine wave was set slightly higher (40 Hz) than that of the animal's own EOD, which allowed the output of the function generator to be synchronized to the EOD discharge. The emitted sine wave was subsequently multiplied with the desired AM waveform (MT3 multiplier; Tucker Davis Technologies, Alachua, FL, USA), and the resulting signal was isolated from ground (A395 linear stimulus isolator; World Precision Instruments, Sarasota, FL, USA). The isolated signal was then delivered through a pair of chloridized silver wire electrodes placed 15 cm away from the animal on either side of the recording tank perpendicular to the fish's rostro-caudal axis (Fig. 1A). The resulting signal measured at the fish's skin was approximated using a dipole (1 mm distance between the two poles) positioned next to the fish 2 mm away. Our stimuli consisted of AMs (= 'beats') at frequencies ranging from 1 to 256 Hz, envelope stimuli, and chirps. The envelope stimuli consisted of a 5–15 Hz noisy AM carrier waveform (i.e., the first-order stimulus) whose amplitude (i.e., envelope) varied sinusoidally at frequencies ranging from 0.05 to 1 Hz. Small chirps with a 14ms duration and 60 Hz amplitude were presented at four different phases of an underlying 4 Hz beat (0°, 90°, 180° and 270°). Chirp amplitude (100 Hz, 140 Hz) and duration (5 ms, 24 ms) were also systematically varied and each such variation was presented at beat phases of 0 and 180. As such, a total of 12 chirp varieties were used (four variations each of phase, amplitude, and duration). Parameter ranges were chosen to match those in previous studies [69, 72, 73, 130, 131,

136]. The underlying beat frequency of 4 Hz was chosen as a typical frequency difference for the same-sex interactions in which small chirps are most frequently observed [62]. It should be noted, however, that recent field studies have found that small chirps can also be associated with high-frequency beats [37].

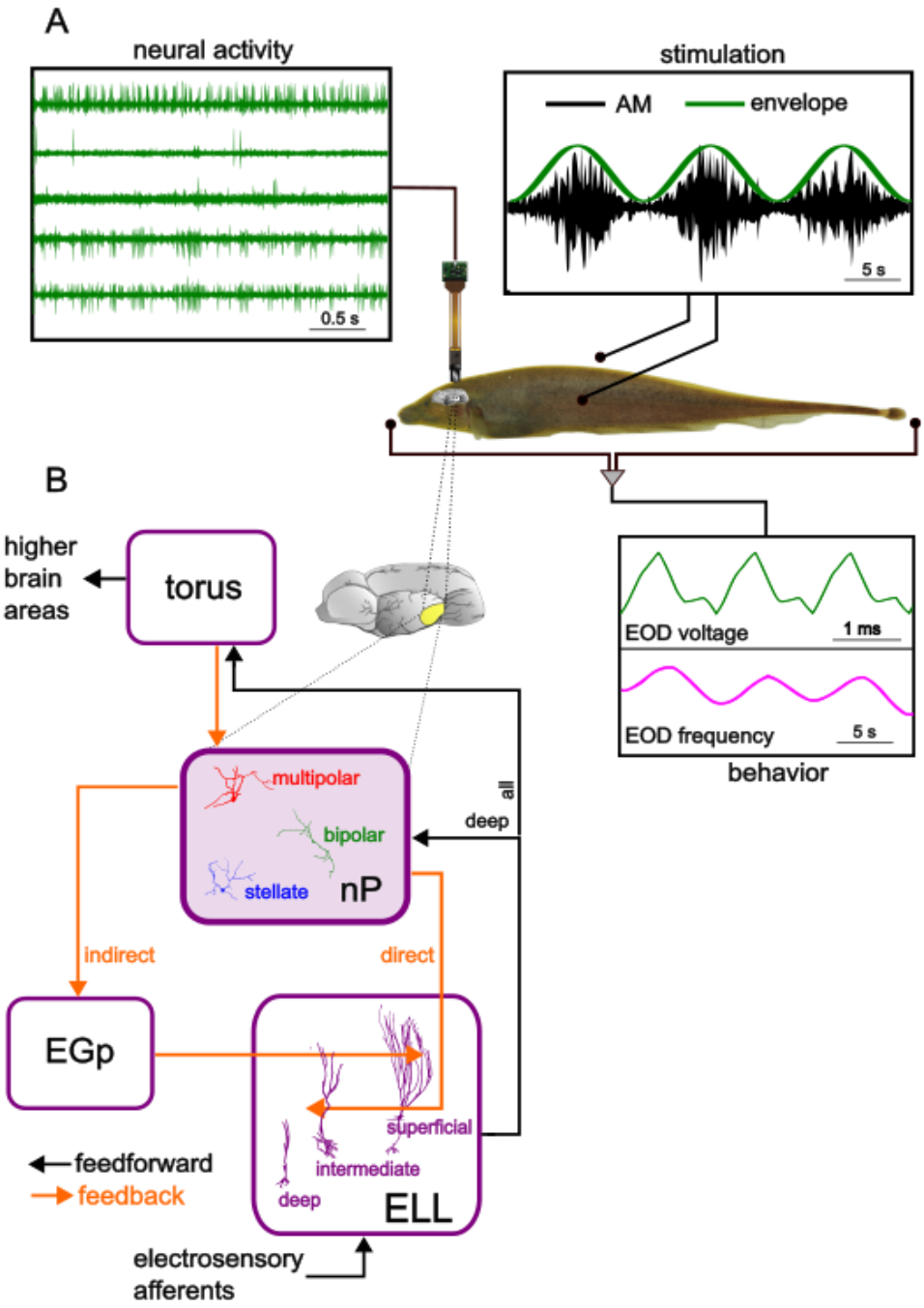

Figure 1. *(A) Schematic showing the experimental setup for multi-unit recordings made from awake* Apteronotus leptorhynchus *using Neuropixels probes. Raw voltage traces are shown for five example channels (top left). The stimuli consisted of amplitude modulations (AMs) of the fish's electric organ discharge (EOD); shown is an example envelope stimulus consisting of an AM carrier wave (black) whose amplitude modulation in turn forms the envelope (green). A pair of electrodes perpendicular to the fish's rostro-caudal axis were used to deliver the stimuli. A separate pair of electrodes located near the snout and the tail monitored the animal's EOD (bottom right, green). Behavioral responses consisted of changes in the EOD frequency; shown is an example response to the envelope stimulus (bottom right, pink). (B) Simplified diagram showing relevant brain areas in the electrosensory system. EOD AMs are encoded by electroreceptor afferents that project directly to ON-type pyramidal cells and indirectly via local inhibitory interneurons to OFF-type pyramidal cells in the electrosensory lateral line lobe (ELL), the first stage of sensory processing in the central nervous system (only ON cells are shown). Deep pyramidal cells project from ELL to the nucleus praeeminentialis (nP), which provides feedback to superficial and intermediate pyramidal cells in ELL via a direct and indirect pathway. Stellate and bipolar cells in nP feed back directly to ELL (direct pathway). Multipolar and several other cell types in nP (not shown) feed back indirectly to ELL via the eminentia granularis posterialis (EGp). All ELL pyramidal cells project to the torus semicircularis, which, besides also sending feedback to nP, sends information to higher brain areas that control the electric organ and give rise to behavior.*

At the end of the experiment, the fish was euthanized by MS-222 overdose (Sigma-Aldrich, 1 g/ L, gills) followed by decapitation as per approved protocol 5285 and according to the guidelines of the Canadian Council on Animal Care.

In addition to the recordings made using the Neuropixels probe, data from additional recordings in nP made using single-unit electrodes were used in the analysis. These recordings were achieved using metal-filled micropipettes as described previously [53] and used identical 1–128 Hz AM stimuli as used in the Neuropixels recordings.

Using spikeGLX (Janelia Research Campus, Howard Hughes Medical Institute), all recordings were digitized at 30 kHz and stored on a hard drive for offline analysis. Spike2 (Cambridge Electronic Design Ltd., Cambridge, UK) was used to manually sort spikes and extract spike times from the recordings. Well-isolated cells, that were stable across the recording session, were identified using Spike2's spike waveform template matching algorithm in combination with principal component analysis (PCA), with clusters merged or split as needed. The sorted neural response activity was then imported into MATLAB (MathWorks Inc., Natick, MA USA) where

spike times were converted to binary sequences sampled at 2 kHz. Custom code was used to analyse the data as described below.

Rationale (protocol)

The experimental paradigm just described is well established and the basis of a great deal of our knowledge of the neurophysiology of the electrosensory system. More generally, highly controlled experiments where the stimulus is precisely defined and the animal is immobilized allow for precise quantification of the neural and behavioural responses to that stimulus and are thus attractive for the study of neural coding [137]. Moreover, the present protocol benefits from the use of naturalistic stimuli with behavioral relevance to the animal.

Potential problems (protocol)

It can be argued that the standard experimental paradigm wherein controlled stimuli are presented to an immobilized animal implicitly relies on a conception of the brain as an input/output device that receives inputs, performs computations on these inputs, and then uses the results to guide behavior in a perception-action loop. This traditional conception is challenged by recent views influenced by control theory and predictive processing, according to which it is more accurate to say that brains initiate actions in order to control their sensory input, with action and perception forming parts of a unified whole [138-140]. From such a perspective—which may better account for the massively recurrent nature of the brain—it may be questioned whether measuring neural responses to controlled stimuli in an immobilized animal will give us a full understanding of sensory processing. Sensory systems certainly did not evolve in isolation from motor systems, and indeed recent large-scale neural recordings have discovered a surprisingly prevalent degree of [141, 142]-related neural activity in sensory brain areas [142].

Furthermore, brains have evolved to guide behavior in a rich, changing environment with many interacting variables. The assumption that neural computations can be de-contextualized, studied in isolation, and then reassembled into an accurate picture of brain function has been argued against [143], along with calls for more ecologically valid experimental design with complex stimuli and freely moving animals [144, 145]. The corollary of such complex experiments is the

difficulty of interpreting neural activity in terms of simple variables. Controlled experiments with simple stimuli like those used here have led, with much hard and careful work by many practitioners, to detailed descriptions of how electrosensory neurons encode stimuli. The technology to record neural activity in freely-moving fish has only recently become available [146]. It will be interesting to combine this technology with more ecologically valid experimental regimes in future experiments.

Finally, this protocol focuses on global electrosensory stimuli. These are spatially diffuse stimuli that impinge upon the entire body surface of the fish and correspond to the kinds of signals originating from other nearby electric fish. It is important to note that in natural settings, salient local (e.g., prey) and global stimuli can occur simultaneously. Indeed, feedback from nP is critical to the differential coding in ELL pyramidal cells of stimuli with differing spatial extents as well as different frequencies [94, 114, 115, 120]. Future studies of nP should include localized stimuli to investigate the important role played by nP in this differential coding.

Rationale (Neuropixels)

Neuropixels probes enable the recording of large populations of neurons and their relative locations with high temporal resolution [147], abilities which present significant advantages over single neuron recordings. It is generally accepted that population-level activity is important to understanding how brains compute, be it in terms of the distributed nature of brain computations [148-151] or the effects of correlated activity on the information capacity of groups of neurons [152, 153]. In the electrosensory system, ELL pyramidal cells display baseline correlations which give rise to noise correlations during stimulation [154-156]. The ability to simultaneously record from tens or hundreds of neurons allows for the effects of these correlations to be measured and allows for a more comprehensive understanding of the complex dynamics and interactions within neural networks, while also requiring fewer recording sessions and thus fewer animals to be sacrificed.

Potential problems (Neuropixels)

The potential challenge with such large-scale recordings is one that currently confronts neuroscience as a whole, namely the need for sophisticated data analysis techniques and perhaps

even new theoretical frameworks to interpret the large volumes of data generated. Bigger data will not necessarily lead to a more meaningful understanding of brain function with current methods of analysis [157]. A recent review highlighted several surprising insights and challenges to emerge from large-scale recordings [158]. For example, most sensory neurons in large-scale recordings do not respond in any obvious way to experimental stimuli [133, 159]. The same review highlights the importance of understanding behavior and the use of dimensionality reduction techniques for moving beyond purely descriptive accounts of large-scale neural data toward some deeper level of understanding.

2. Characterizing neural responses

Previous work has shown that, like ELL pyramidal cells, nP stellate and multipolar cells can be classified as ON or OFF according to whether they respond to increases or decreases in AM stimuli respectively [96, 103]. I thus first classified the nP cells according to their responses to a low-pass filtered 0-120 Hz noise amplitude modulation as done previously [131]. To do so, I calculated the spike triggered average (STA) by averaging stimulus segments during 1 s window centered at the action potential times of each neuron. I calculated the slope of the STA during an 8 ms window centered 10 ms before the action potential occurred to account for spike transmission delay [96, 103]. Neurons for which the slope was positive were classified as ON cells, whereas those for which the slope was negative were classified as OFF cells.

I computed baseline firing rates from 100 s of recording in the absence of stimuli. To quantify neural responses to AM and envelope stimuli I used linear systems identification techniques to compute gain and phase as done previously [47, 49]. Firing rate modulation was determined by averaging over the cycles of the stimulus and fitting a sinewave to the resultant cycle histogram. Gain is then defined as the amplitude of the firing rate modulation. Phase is defined as the average phase at which the filtered firing rate reaches its maximum relative to the peak of the stimulus waveform over each cycle divided by a period of 2π. To determine the degree of a neuron's phase locking to the AM and envelope stimuli, I computed vector strength as follows:

$$\text{vector strength} = \frac{1}{\mathrm{N}}\sqrt{\left(\sum_i cos\theta_i\right)^2 + \left(\sum_i sin\theta_i\right)^2},$$

where N is the number of spikes during 1 cycle of the stimulus and vector strength ranges from 0 (random spiking) to 1 (perfect phase locking) [160].

The decoding methods I used require time-varying firing rates as inputs; these were obtained by low-pass filtering the binary spike trains with a second-order Butterworth filter with a cutoff of 1.5*f*, where *f* is the stimulus frequency.

Rationale

These basic methods of quantifying and visualizing neural responses to AM and envelope stimuli have been used in numerous previous studies. They thus allow for comparison of the responses of nP neurons with those of neurons in other electrosensory areas and serve as a starting point for further analysis.

Potential problems

Although it is common and convenient to average across trials when computing/visualizing neural response properties like gain, phase, and firing rate, it should be kept in mind that brains do not average across trials when responding to events in the world. Moreover, responses can change across trials owing to, e.g., neural adaptation [161], habituation [162], and changing internal states of the animal such as arousal or fatigue [163]. It is therefore good practice to inspect the raw spike trains/raster plots for nonstationarities prior to averaging.

3. Identifying nP stellate and multipolar cells from response properties

I defined stellate cells as those cells with a baseline firing rate <5 Hz and a lowpass response to AM stimuli and multipolar cells as those cells with a baseline firing rate >30 Hz and whose tuning curves (i.e., gain as a function of stimulus frequency) peaked at AM stimuli>32 Hz [96, 103]. Cells falling within the vertical range with limits defined by the most ventral and dorsal stellate/multipolar cell in each recording were considered nP neurons; cells outside this range were excluded from analysis as potentially belonging to other brain areas.

Rationale

I follow Huang et al. [48] in identifying stellate and multipolar cells based on their baseline firing rate and AM response properties. Using these known nP cells as delimiters allowed me to confidently assume that other recorded neurons occurring within these limits were nP neurons.

Potential problems

It is possible that some nP neurons occurring above or below the uppermost or lowermost stellate/multipolar neuron were excluded from analysis. However, this seems preferable to the alternative of mistakenly including neurons from adjacent brain areas (e.g., torus). As these other areas are not known to have cells with response properties similar to those of stellate or multipolar cells, it is unlikely that I included cells from adjacent areas.

4. Clustering on neural responses

As the tuning properties of nP neurons other than stellate and multipolar cells are unknown, I investigated whether the responses of nP neurons fall into discernible groups using a variety of dimensionality reduction and clustering techniques, of which PCA and hierarchical clustering based on Ward's distance [164] proved to be the most informative. As inputs, I used both baseline firing rates and trial-averaged measures of neural responses (i.e., firing rates, gain, phase and vector strength) for each neuron in response to the range of AM stimuli. Due to the large differences in firing rates between cell types and to avoid outliers, I normalized responses prior to PCA using the inverse hyperbolic sine transformation. This acts similarly to a log transformation for normality, but can accommodate null values, e.g., firing rates equal to zero, which are prevalent in my data [165].

Rationale

Dimensionality reduction can reveal underlying structures in high-dimensional spike train data and help to identify distinct neural response patterns [166].

Potential problems

Finding clusters in data is not an exact science, and the clustering algorithm and number of clusters chosen depends on context [167]. My purpose here is heuristic: the number and

separation of clusters should give some idea of the heterogeneity of responses within nP and some clues to the varieties of computations being carried out. Since the AM response properties of stellate and multipolar cells are already known, I was particularly interested in whether the responses of remaining neurons would form distinct clusters. This information can potentially then be related to the various known functions of feedback in the electrosensory system.

Alternatively, it would be possible to unambiguously characterize the response properties of the different morphological cell types by marking the cells being recorded from with fluorescent dye, and examining tissue slices to identify the morphological type of the marked cell with fluorescent microscopy, but this is beyond the scope of this project.

5a. AM and envelope stimulus reconstruction via linear decoder

To investigate how much information neural activity in nP contains about AM and envelope stimuli, I reconstructed the stimuli from neural responses using a linear decoder. Specifically, I obtain the optimal weights for a linear reconstruction of the stimulus using least-squares regression [168] as follows:

$$\boldsymbol{w} = \frac{\mathbf{X}^{\mathrm{T}}\mathbf{y}}{\mathbf{X}^{\mathrm{T}}\mathbf{X}},$$

where **X** is matrix of neural responses in the form of time-varying firing rates, **y** is the actual stimulus, and $\boldsymbol{w}$ is the vector of weights resulting in the minimum mean squared error between the actual and reconstructed stimulus (Fig. 3A). Neurons with no responses during a given stimulus were not considered.

The reconstructed stimulus is then the sum of weighted responses of *N* neurons:

$$\text{Reconstruction} = \sum_{i=1}^{N} w_i \, \mathbf{x}_i.$$

Decoder performance was measured as follows:

$$\text{Performance} = 1 - \frac{\sqrt{\text{MSE}}}{\text{std}(\text{stimulus})},$$

where MSE is the mean squared error between the reconstructed and original stimulus. Performance thus varies between 0 and 1, with 1 indicating perfect reconstruction of the stimulus.

To further investigate differences in envelope responses between cell types, I quantified the heterogeneity of responses for a given cell type by computing the correlation coefficients between all possible pairings of filtered firing rates in response to envelope stimuli.

Rationale

Stimulus reconstruction by neural decoding is a widely-used technique to determine the amount of information a brain region contains about a given stimulus, and potentially give clues about how information about the stimulus is encoded [169, 170]. High decoding performance indicates that information about the stimulus is present in the neural responses but does not necessarily reveal how, or whether, that information is actually used by downstream brain areas [171].

Potential problems

Linear decoders assume a linear relationship between stimulus and neural activity. Although actual decoding strategies in the brain are, in general, nonlinear [172], linear models are often used in a neural decoding context due to their ease of interpretation [171]. To gain a clearer picture of the actual neural computations being carried out in a given brain area, however, it is useful to compare multiple decoding models, including artificial neural network models that can learn arbitrary, nonlinear mappings between stimulus and neural response [173].

5b. Comparing linear decoder and nonlinear decoders

I trained neural decoders using various common machine learning algorithms to compare their performance at reconstructing AM and envelope stimuli from nP responses. Following Glaser et al. [173], these comprised simple linear regression (identical to the linear decoder described above), Wiener Cascade, Support Vector Regression, Extreme Gradient-Boosted regression, fully-connected feedforward neural network, vanilla Recurrent Neural Network, Gated Recurrent Unit network, and Long Short-Term Memory Network models. Descriptions of these models can be found in [173]; I also used the specific hyperparameters suggested by those authors for each

model. To evaluate each model's ability to generalize to unseen data, I used 10-fold cross-validation and then averaged the performance across the ten test sets. (This contrasts with the approach used with the linear decoder described above, which was trained on the entirety of the neural responses to each stimulus.) I compared the performance of the decoders on each stimulus variety and their aggregated mean performance across all AM and envelope stimuli using one-way ANOVA and post-hoc Tukey tests.

Rationale

Machine learning algorithms able to learn complex relationships between inputs and outputs can be used as performance benchmarks with which to compare the least-squares linear model for neural decoding. A large gap in performance between a neural network decoder and a linear decoder, for instance, may suggest that the linear model does not fully capture how the neural responses are encoding the stimulus. Cross-validation prevents the decoding models from overfitting to the training data and tests their ability to generalize to withheld data.

Potential problems

While useful as performance benchmarks, the complexity of neural network and other machine learning models makes their interpretation difficult [174]. Furthermore, training neural network models can involve a degree of trial-and-error to find the hyperparameters that result in optimal performance for a given task [173].

6. Chirp analysis: selectivity, discrimination, invariance

I used the chirp selectivity index (CSI) to quantify the response of each neuron to chirp stimuli as done previously [175]:

$$\mathrm{CSI} = \frac{\mathrm{R_{chirp}} - \mathrm{R_{beat}}}{\mathrm{R_{chirp}} + \mathrm{R_{beat}}},$$

where R_{chirp} is the maximum firing rate obtained in a peristimulus time histogram (PSTH) during a time window of 60 ms starting with chirp onset, and R_{beat} is the maximum firing rate obtained during one beat cycle before chirp onset.

The CSI ranges between –1 and 1, representing perfect selectivity for the beat at –1 and the chirp at 1. To measure the selectivity of a neuron to multiple chirp stimuli, the average CSI was used, as follows:

$$\mathrm{CSI_{avg}} = \frac{1}{\mathrm{N}} \sum_{i=1}^{N} \mathrm{CSI}_i \,,$$

where N is the number of chirp stimuli tested and $\mathrm{CSI_i}$ is the CSI to chirp stimulus *i*.

To investigate whether nP neurons can discriminate between chirp waveforms that differed in their amplitude, duration and phase, I use a chirp classifier as previously described in [130]. For each chirp variety, the sum of neural responses (the "population response") to a random trial was used as the template for that variety. Thereafter, each population response was assigned to the chirp variety whose template it most resembled based on the van Rossum distance metric [176] with timescale τ = 6 ms. In the resulting confusion matrix (Fig. 7A and C), the element (i, j) gives the probability that a population response was assigned to chirp variety j given that it was actually generated by chirp variety i. The diagonal elements of this matrix are the probabilities that a response was assigned to the correct chirp variety, whereas off-diagonal elements correspond to misclassifications. Discrimination performance is computed by averaging over the diagonal elements. The chance level for discrimination performance was 0.083 (i.e., 1 of 12) because I used a total of 12 different chirp stimuli.

Studies have shown that midbrain neurons (in torus) can respond invariantly to chirps occurring on different phases of the underlying beat, and moreover that such phase invariance progressively increases across successive stages of electrosensory processing [73, 177]. Whether feedback plays a role in this process is an open question. I thus quantified the phase invariance of nP responses to chirps. The invariance score was defined as:

$$\mathrm{Invariance} = \; 1 - \frac{\sum_{\substack{i \\ j}} \left[\frac{\mathrm{D}\left(\mathrm{FR}_i(t), \mathrm{FR}_j(t)\right)}{\mathrm{D}\left(\mathrm{S}_i(t), \mathrm{S}_j(t)\right)} \right]}{\left(\mathrm{N_{chirps}}\right)\left(\mathrm{N_{chirps}} - 1\right)},$$

where N_{chirps} is the number of chirp stimulus waveforms, and $D(x,y)$ is a distance metric between x and y that was computed as in [177]:

$$D(x,y) = \frac{\sqrt{\langle (x - \langle x \rangle - y + \langle y \rangle)^2 \rangle}}{\max\left[\frac{\max(x) - \min(x)}{\sqrt{2}}, \frac{\max(y) - \min(y)}{\sqrt{2}}\right]},$$

where <. . .> denotes an average over a time window of 60 ms after chirp onset, $FR_i(t)$ is the PSTH response of a given cell to chirp stimulus waveform $S_i(t)$, and max(. . .), min(. . .) denote the maximum and minimum values, respectively. All responses were normalized prior to computing the distance metric.

Rationale

The CSI, invariance, and classifier approaches have been used in previous studies on chirp encoding in other electrosensory areas and will thus provide a useful point of comparison for the role of nP in the processing of these stimuli.

Potential problems

While previous studies have shown that weakly electric fish perceive chirps with different attributes [178], the behavioral relevance of different chirps remains rather unclear as emphasized by a recent study [179]. Without further studies on the ethology of these electrocommunication signals, any measurement of neural responses to such stimuli will likely be merely descriptive and difficult to relate to a behavioral function.

7. Examining the effect of noise correlations on decoding performance

To quantify the effects of noise correlations on population coding in nP, the performance of both the various AM and envelope decoders and the chirp classifier were evaluated on both the unaltered neural responses and neural responses that were randomly shuffled with respect to trial order, as done previously [130, 131].

Rationale

Understanding how populations of neurons encode information is complicated by the fact that neural activities are correlated [153]. Of particular interest are the effects of correlations between the trial-to-trial variability of neural activities, i.e., noise correlations. While noise correlations can limit information transmission by introducing redundancy [180, 181], they can also introduce synergy and be beneficial to coding [182-184].

Comparing the performance of a neural decoder with trial order intact to the performance of the same decoder after randomizing neural responses with respect to trial order (effectively removing noise correlations) provides a convenient quantification of the effect of noise correlations.

Potential problems

The actual effect of correlations depends both on the structure of those correlations [126] and on the type of decoder used [185]. Furthermore, it is important to note that what we consider 'signal' and 'noise' in neural responses results from what the experimenter considers to be the stimulus event, but this cannot be assumed to equate to the actual input to the organism [138, 140, 186]. Indeed, several recent studies have made it clear that what may appear to be task-irrelevant neural activity (so-called "noise") is not simply due to the stochastic nature of neurons, but can result from brain-wide activity related to ongoing behavioral or cognitive processes [141, 142]. How such activity interacts with the processing of sensory stimuli is not well understood and may require a holistic approach linking brain-wide activity to behavior [186].

Results

I recorded a total of 36 nP neurons in over 3 Neuropixels recording sessions (n = 8, 5, and 23, respectively) in N = 2 fish. Two of the recording sessions occurred during a single surgery. Based on EOD frequency (900 Hz and 700 Hz) and chirp emission rate (1.6 chirps/s and 0.04 chirps/s during 100 s of 0–120 Hz noise stimulation), the 2 fish consisted of one male and one female. For my analysis of responses to 1–128 Hz AM stimuli, I also incorporated 20 additional single-unit nP neuron recordings from N=2 additional fish, both with EOD frequencies <800 Hz (680

Hz and 770 Hz) and hence presumed to be female. Thus, when considering AM responses, I used n=56 total neurons, whereas for envelope and chirp responses only the Neuropixels recordings were available.

Based on baseline firing rates and AM tuning curves, I identified 22 stellate cells and 9 multipolar cells (Fig. 2A and B). The response properties of the remaining 25 cells did not match our definition criteria for either stellate or multipolar cells and are presumed to belong to one or more of the ~8 other neuron types in nP [102].

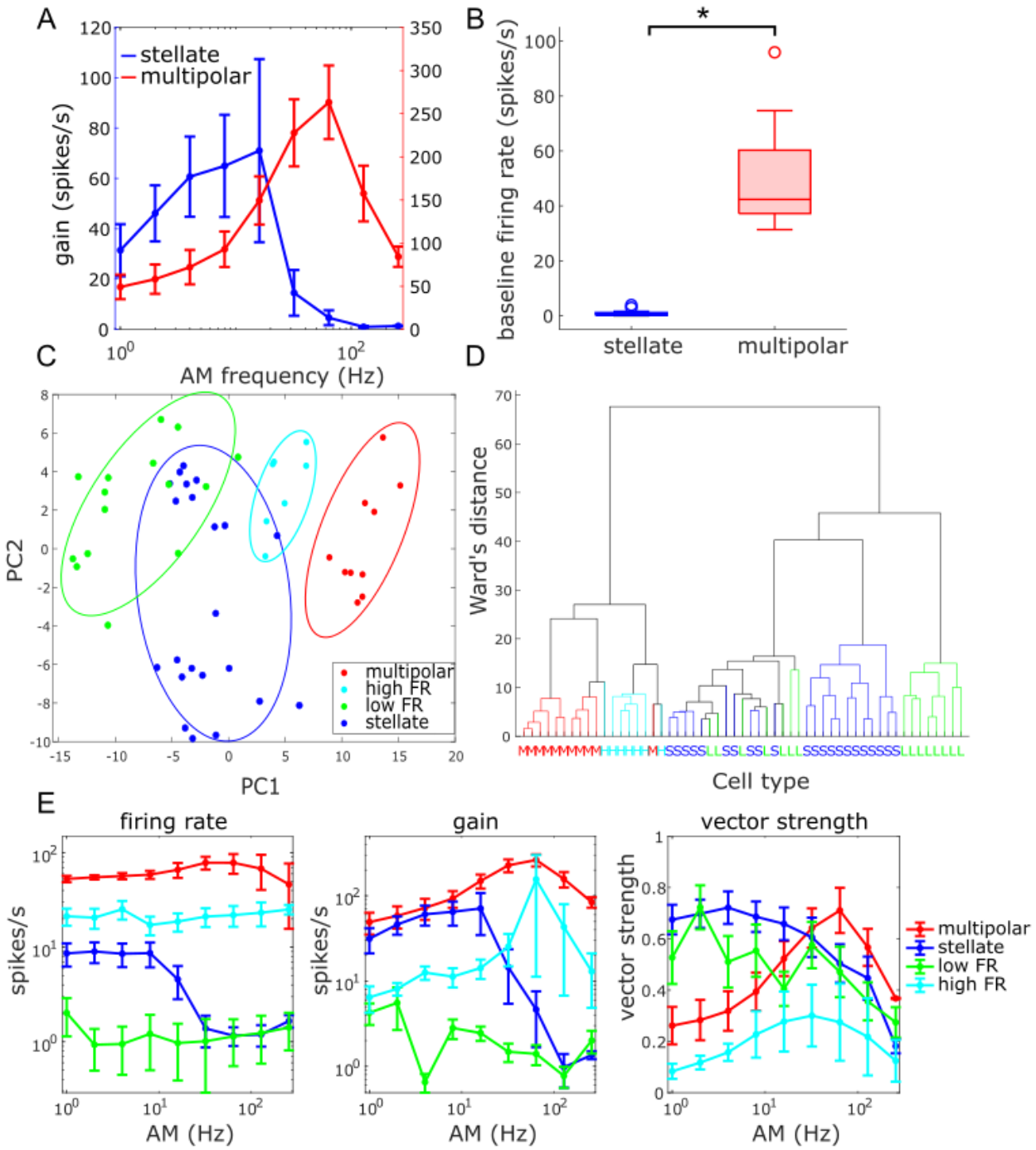

Figure 2. *AM responses. (A) AM frequency tuning curve for nP stellate (blue) and multipolar (red) cells. Stellate gain (defined as amplitude of firing rate modulation; see "Methods") rapidly drops off at frequencies >32 Hz, whereas multipolar cell gain increases at higher frequencies >32 Hz, consistent with previous studies [96, 103]. (B) Boxplot of baseline (i.e., in the absence of stimulation) firing rates of stellate (blue) and multipolar (red) cells. In agreement with previous studies [96, 103], baseline firing rates are significantly higher in multipolar cells than stellates ($\chi 2 = 18.6$, $p=1.64*10^{-5}$, Kruskal-Wallis ANOVA), as denoted by the (*) symbol. (C) Results of*

principal component analysis on AM responses. Clusters are assigned based on the known properties of stellate and multipolar cells and division of the remaining cells into "high firing-rate" and "low firing-rate" types. (D) Hierarchical clustering of neural responses properties to AM stimuli based on Ward's distance [164] (M=multipolar, H=high firing-rate, S=stellate, L=low firing-rate). (E) AM response measures by cell type: (left) mean firing rate, (middle) mean gain, (right) mean vector strength (see "Methods" for explanations). Error bars indicate standard error.

nP cells form clusters based on firing rate.

Fig. 2C shows principal component scores for the first two principal components of neural responses to AM stimuli, which together explain 64.7% of the variance in those responses. The first principal component (PC1) accounted for 48.8% of the variance and was predominantly weighted by firing rate and gain, while the second (PC2) was more influenced by the phase of neural responses. Fig. 2D shows the result of hierarchical clustering based on Ward's distance. Based upon the results of these two analyses I chose to provisionally divide the unknown cell types into "high firing rate" (cyan in both figures) and "low firing-rate" (green in both figures) classes for subsequent analyses. In effect, this amounted to separating the unknown cells into those with a baseline firing rate >10 Hz (n=6) and those with a baseline firing rate <10 Hz (n=15). It should be noted, however, that there is some overlap in the response properties of these groups—particularly the stellate and low-firing rate groups—as evidenced by both the PCA and hierarchical clustering analyses. Average measures of AM responses (firing rate, gain and vector strength) used in the PCA and hierarchical clustering analyses are shown in Fig. 2E for each of the four cell types.

AM linear decoder performance varies with cell type and stimulus frequency.

Fig. 3B and C show the performance of a linear decoder as a function of population size for example low (2 Hz) and high frequency (64 Hz) AM stimuli using the pooled responses from the Neuropixels recordings. In each case the three panels show performance with each cell type considered separately (left) and for the population as a whole with cell types added to the population in different orders (middle, right; note that the maximum performance is identical). Reconstructions of the AM stimulus waveform at different population sizes are shown above/below the performance plots. At 2 Hz (Fig. 3B), the decoder quickly reaches a relatively high performance (>0.8 at a population size of 20) at reconstructing the stimulus, irrespective of

the order in which different cell types are considered. As expected, [187, 188] decoder performance generally increases with population size. In contrast, at 64 Hz, only multipolar cells encode the stimulus to any appreciable degree (Fig. 3C).

Shuffling neural responses with respect to trial order revealed that noise correlations have no significant effect on decoder performance (Fig. 3F). Since noise correlations do not appear to play a significant role in AM decoding, I then added responses from the single-unit recordings to the decoder. The increased population size resulted in significantly better decoding performance (Fig. 3D and F).

Both stellate and multipolar cells perform relatively well at encoding AM frequencies ≤16 Hz, but their performance decreases rapidly at frequencies >16 Hz and >64 Hz respectively (Fig. 3E), consistent with the preferred AM frequencies of these cell types [96, 103]. Low-firing rate cells show a similarly steep decline for frequencies >2 Hz. The performance of high firing-rate cells, by contrast, decreases in a linear fashion with stimulus frequency until reaching negligible levels beyond 32 Hz.

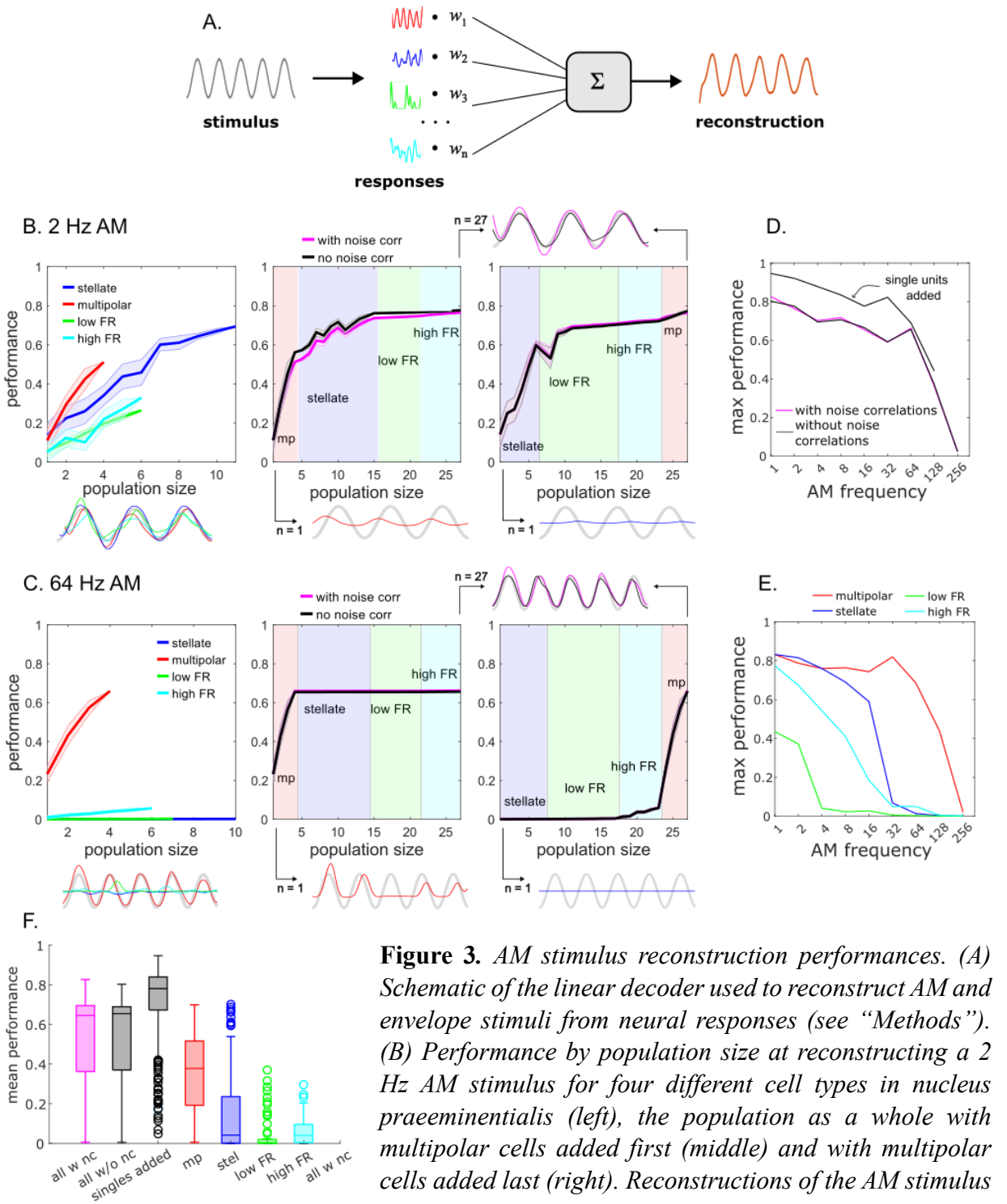

Figure 3. *AM stimulus reconstruction performances. (A) Schematic of the linear decoder used to reconstruct AM and envelope stimuli from neural responses (see "Methods"). (B) Performance by population size at reconstructing a 2 Hz AM stimulus for four different cell types in nucleus praeeminentialis (left), the population as a whole with multipolar cells added first (middle) and with multipolar cells added last (right). Reconstructions of the AM stimulus waveform at different population sizes are shown above/below the performance plots. (C) As before, but with a 64 Hz AM stimulus. (D) Maximum performance for population with noise correlations intact, with noise correlations removed by shuffling responses with respect to trial order, and with the addition of single unit recordings (see text for explanation), across all AM frequencies. (E) Maximum performance by cell type across all AM frequencies, including single-unit recordings (n=11 multipolar, n=20 stellate, n=15 low firing-rate, n=6 high-firing*

rate). (F) Boxplot comparing mean performance of different groups at reconstructing envelope stimuli of frequencies ranging from 1–256 Hz. Performances were significantly different among groups and among frequencies (two-way ANOVA, p <<<0.001). All pairs of neuron classes differed significantly according to Tukey's honestly significant difference procedure, with the exception of the 'all w nc' (all neurons with noise correlations) / 'all wo nc' (all neurons without noise correlations) pair and the 'low FR' / 'high FR' pair.

Envelope linear decoder performance varies with cell type.

Results of envelope stimulus reconstruction by a linear decoder (Fig. 4A and B) show that, as with low-frequency AM stimuli, performance rises quickly with increasing population size before levelling off. As with the AM decoder, shuffling neural responses with respect to trial order revealed no significant effect of noise correlations on decoder performance (Fig. 4C). Performance increases considerably more rapidly with multipolar cells than any other cell type. Indeed, relatively high performance is achieved with only a few multipolar cells (Fig. 4A and B, left panels), indicating that these cells tend to linearly encode the time course of envelopes with high resolution. A decoder using only stellate cells can reach an equivalent level of performance but requires more neurons to do so, i.e., their mean performance is less (Fig. 4C). Low firing-rate cells exhibit similar envelope responses and decoder performance to stellate cells. High firing-rate cells, by contrast, do not seem to linearly encode envelopes whatsoever (Fig. 4C).

While the lower overall firing rates of single stellate cells may render them less suitable for encoding the detailed time course of envelope stimuli compared to multipolar cells, the population as whole performs nevertheless performs better than any single cell type (Fig. 4C). Interestingly, the envelope responses of stellate cells and low firing-rate cells are more heterogeneous than those of multipolar cells (Fig. 4D and E), which could contribute to the increase in overall performance contributed by the former. Indeed, multipolar cell responses tended to be in phase with the envelope, while the phase of stellate responses was more varied (Fig. 5A, top and bottom). Multipolar cells also showed a notable high pass response with respect to envelope frequency (Fig. 5D), consistent with a previous study [48].

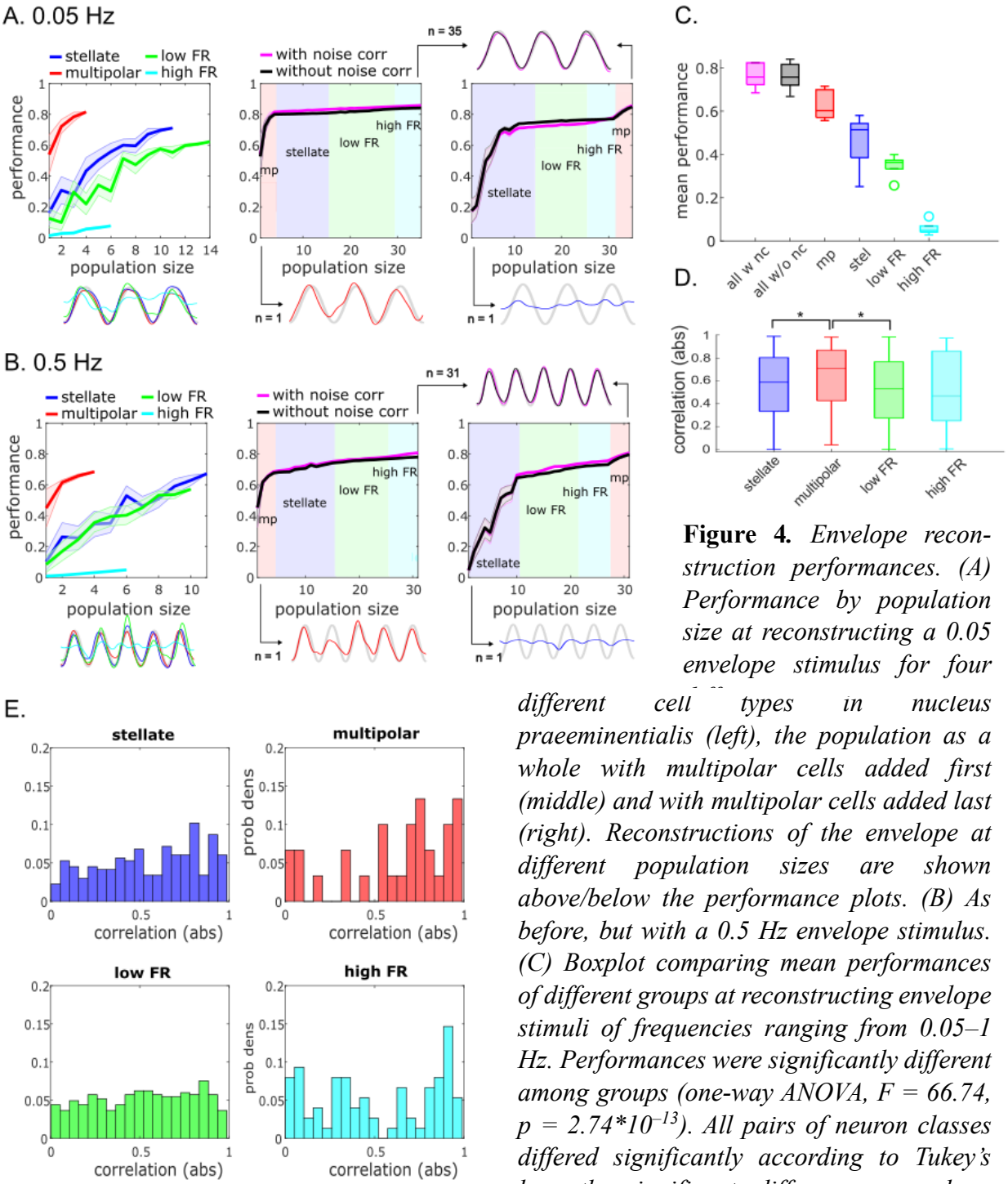

Figure 4. *Envelope reconstruction performances. (A) Performance by population size at reconstructing a 0.05 envelope stimulus for four different cell types in nucleus praeeminentialis (left), the population as a whole with multipolar cells added first (middle) and with multipolar cells added last (right). Reconstructions of the envelope at different population sizes are shown above/below the performance plots. (B) As before, but with a 0.5 Hz envelope stimulus. (C) Boxplot comparing mean performances of different groups at reconstructing envelope stimuli of frequencies ranging from 0.05–1 Hz. Performances were significantly different among groups (one-way ANOVA, $F = 66.74$, $p = 2.74*10^{-13}$). All pairs of neuron classes differed significantly according to Tukey's honestly significant difference procedure, with the exception of the 'all w nc' (all neurons with noise correlations) / 'all wo nc' (all neurons without noise correlations) pair. (D) Boxplot comparing heterogeneity in envelope responses among four cell types. The (*) symbol indicates significantly different distributions according to a Kolmogorov-Smirnov test with Bonferroni correction. (E) Histograms showing the probability densities for absolute correlation values computed between firing rates for all possible pairs of a given neuron type in response to envelope stimuli.*

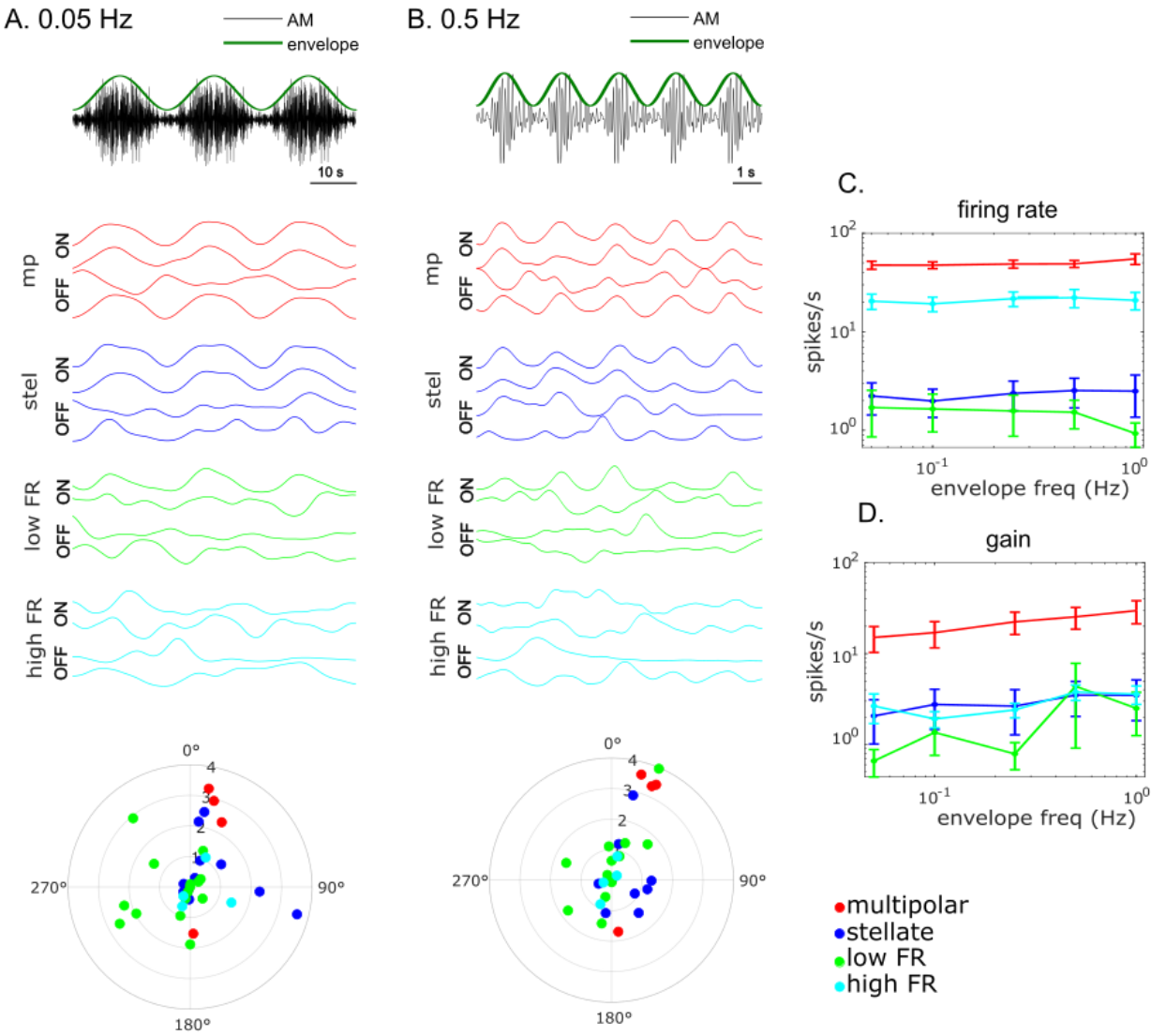

Figure 5. *Envelope responses. (A) Example responses of ON- and OFF-types of four types of nucleus praeeminentialis cells (multipolar, stellate, low firing-rate and high firing-rate cells) to a 0.05 Hz envelope stimulus. Shown are the carrier waveform whose amplitude modulation forms the envelope stimulus (top), filtered firing rates for two examples of each class of cells, and polar plots showing gain (radial coordinate, log scale) and phase (angular coordinate) for all cells in response to the stimulus. (B) As before, but with a 0.5 Hz envelope stimulus. (C) Mean firing rates of each cell type across five envelope frequencies. (D) Mean gain of each cell type across five envelope frequencies. Error bars indicate standard error.*

Linear and nonlinear decoders did not differ significantly in overall performance.

One-way ANOVA tests revealed no significant difference in aggregated mean performance across AM ($p = 0.333$, Fig. S1B) or envelope ($p = 0.798$, Fig. S2B) stimuli for the various decoders tested. Moreover, in no case was there a significant difference in performance between decoders trained on shuffled and unshuffled responses (Fig. S1A, Fig. S2A). However, there were significant differences between decoders at the level of specific stimuli. In particular, the Extreme Gradient-Boosted regression decoder was significantly better than any other model ($p < 0.001$) at decoding 128 Hz and 256 Hz AM stimuli (Fig. S1A). In addition, Extreme Gradient-Boosted regression achieved the highest performance in 24 out 28 total stimuli, with Long Short-Term Memory networks coming achieving top performance in the remaining 4.

Multipolar cells respond strongly to chirps.

Raster plots and filtered PSTHs showing responses to four different chirp stimuli for an example ON and OFF cell from each of the four cell types are shown in Fig. 6A. A comparison of CSI values (Fig. 6B) reveals no neurons in nP that respond only to chirps, such as have been observed in torus [177]; on the contrary, nP cells generally respond more strongly to the beat. Multipolar cells are the exception in having a mean CSI >0. Based on this and the readily apparent change in firing patterns visible in the raster plots, and consistent with my prediction, multipolar cells appear to respond strongly to chirps.

The strong chirp response in multipolar cells is reflected in their ability to discriminate between chirps with different amplitude, duration, and phase characteristics (Fig. 7C). Only a small number of multipolar cells are needed to achieve >0.7 discrimination performance (Fig. 7D). Stellate cells also appear to show increasing performance with bigger population sizes, but the increase is much more gradual. This is nevertheless somewhat surprising, since stellate cells are inhibited by high-frequency AM stimuli. The performance of low firing-rate cells, by contrast, remains barely above chance even at a population size of 15.

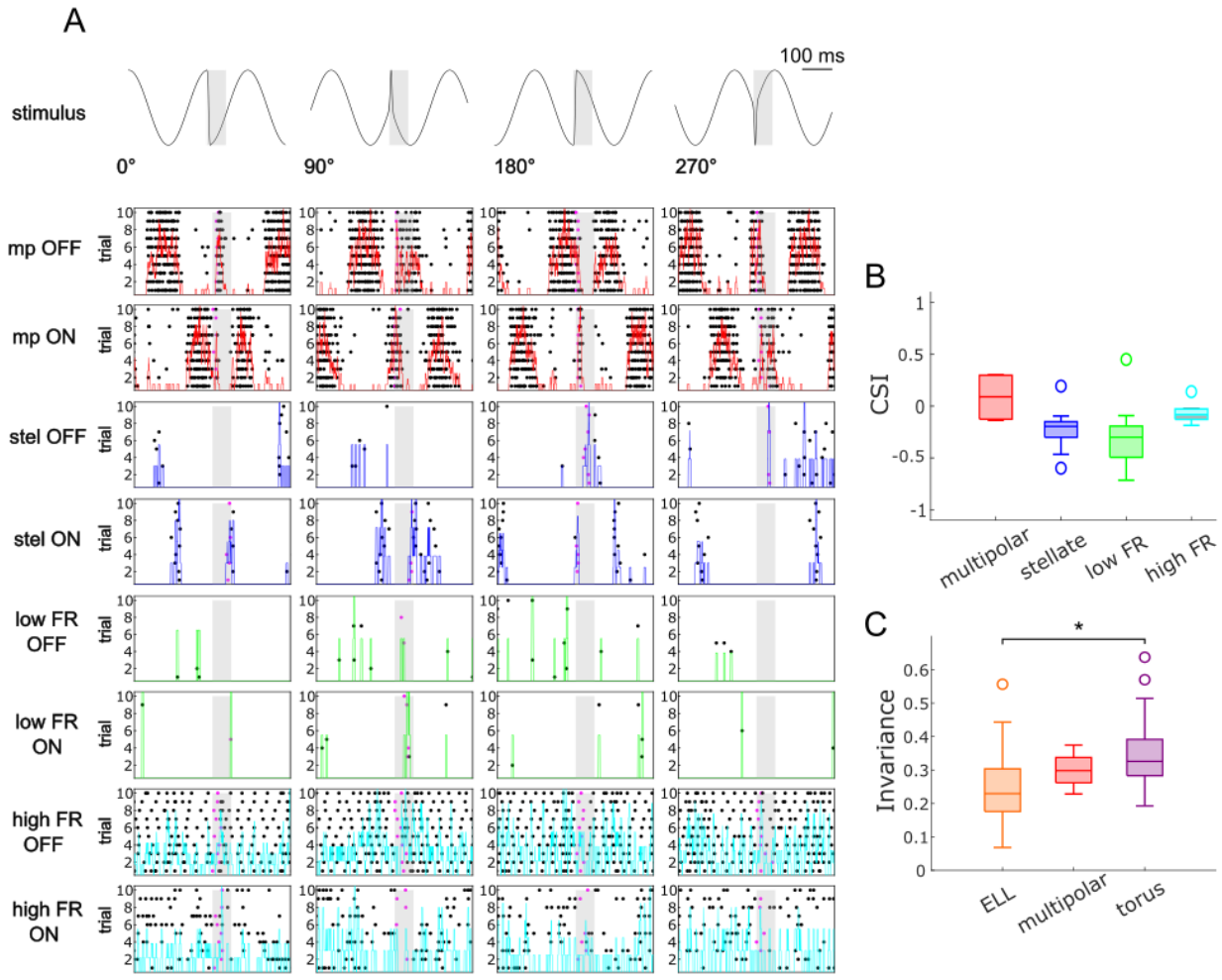

Figure 6. *Chirp responses. (A) Example responses of ON- and OFF-types of four types of nucleus praeeminentialis cells (multipolar, stellate, low firing-rate and high firing-rate cells) to four different chirp stimuli. Shown are the stimulus waveforms, raster plots showing responses to 10 presentations of each stimulus with the grey rectangle representing the chirp evaluation window (see "Methods"), and filtered peri-stimulus time histograms. (B) Chirp selectivity index four the four cell types. (C) Invariance of the responses of the four cell types to chirps occurring at different phases of the underlying beat. Invariance was significantly different between groups (one-way ANOVA, $F = 7.15$, $p = 0.002$). The (*) symbol indicates significant difference between groups according to Tukey's honestly significant difference procedure.*

I found that multipolar cells displayed an average phase invariance that was intermediate between that obtained for ELL pyramidal cells and torus neurons (Fig. 6C) using data from a previous study [73] (ELL: 0.24±0.02; multipolar: 0.30 ± 0.03; torus: 0.35±0.02). The difference between multipolar cells and ELL/torus cells did not reach the level of statistical significance,

perhaps due to the small number of multipolar cells recorded. Since other cell types in nP did not show notable responses to chirps on average, their invariance scores are not informative and thus not displayed.

Finally, as with the AM and envelope decoders, trial shuffling revealed no significant effect of noise correlations on the performance of the classifier (Fig. 7A and E). My hypothesis that noise correlations would cause decreased performance is thus not supported. This result is however in agreement with previous findings that the amount of noise correlation for spatially diffuse stimuli is relatively small [156], and exists mainly in neurons with overlapping receptive fields [154].

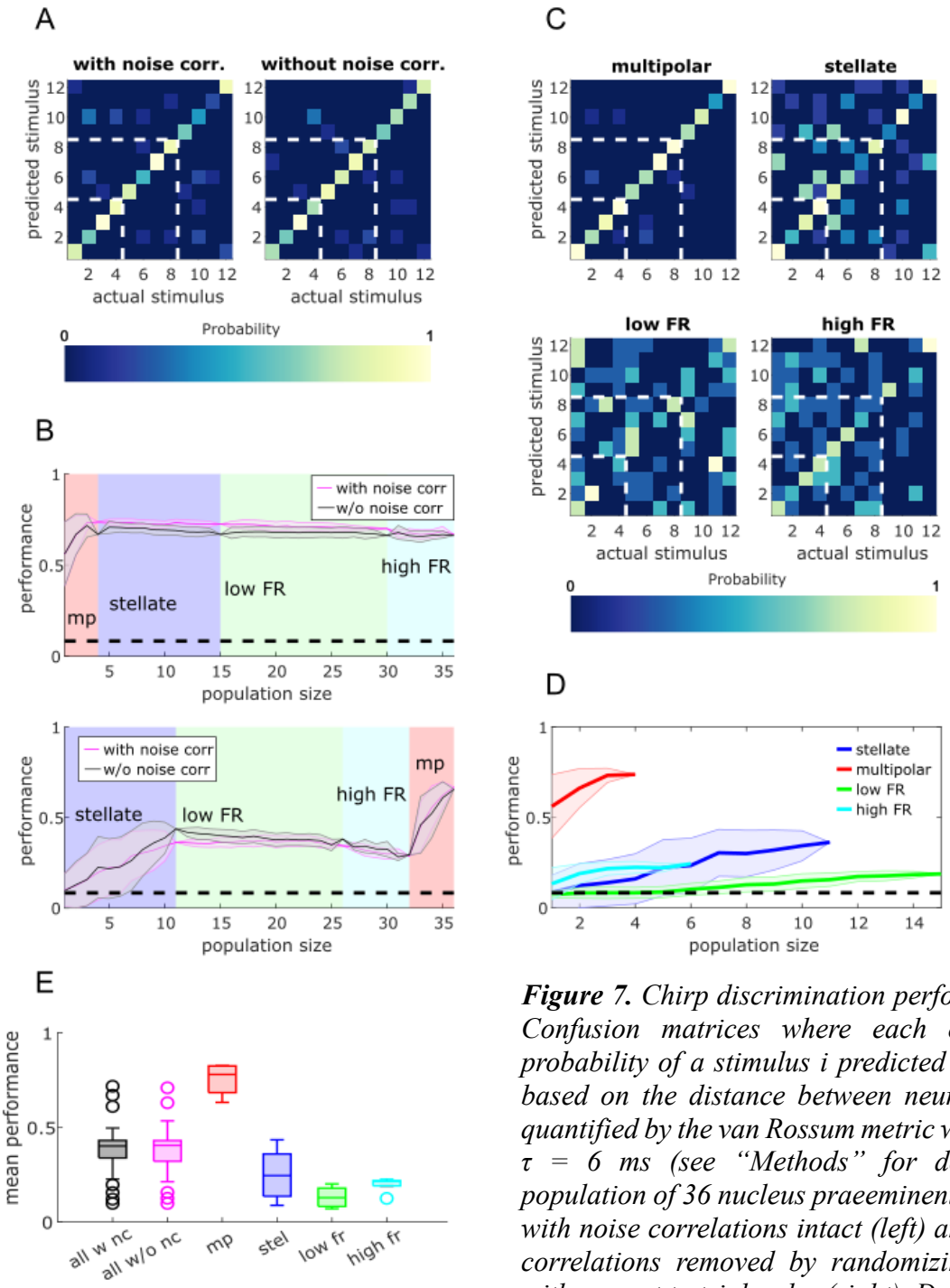

Figure 7. *Chirp discrimination performances. (A) Confusion matrices where each entry is the probability of a stimulus i predicted as stimulus j based on the distance between neural responses quantified by the van Rossum metric with timescale* τ *= 6 ms (see "Methods" for details) for a population of 36 nucleus praeeminentialis neurons with noise correlations intact (left) and with noise correlations removed by randomizing responses with respect to trial order (right). Dashed lines indicate boundaries between different stimuli classes (see text for stimuli descriptions). (B) Discrimination performance by population size, with cells added by type, and with multipolar cells added first (top) or last (bottom). Dashed line indicates chance level. (C) Confusion matrices (with noise correlations intact) for n=4 multipolar cells, n=11 stellate cells, n=15 low firing-rate cells and n=6 high firing-rate cells. (D) Discrimination performance by population size for individual cell types. (E) Boxplot comparing mean performance of different*

groups at discriminating among chirp varieties. Performances were significantly different among groups (one-way ANOVA, $F = 25.049$, $p = 2.15*10^{-16}$). Multipolar cell performance was significantly different ($p < 0.001$) than that of all other groups according to Tukey's honestly significant difference procedure.

Discussion

Summary of results

This study advances our understanding of neural population activity in feedback circuits as it relates to natural stimuli processing within the electrosensory system. Specifically, I examined the impact of noise correlations on the performance of linear decoders for different stimulus classes, characterized previously unrecorded neurons in nP, and investigated the role of multipolar and stellate cells in encoding AMs, envelopes, and chirps. The findings indicate that noise correlations have a minimal effect on decoding performance, aligning with previous work on spatially diffuse stimuli in the electrosensory system. Furthermore, the study distinguishes at least two classes of neurons in nP apart from the previously studied stellate and multipolar cells, characterized by their unique firing rates and general unresponsiveness to stimuli. Finally, the research uncovers new facets of electrosensory encoding, including the capability of multipolar cells to accurately encode low-frequency envelopes and discriminate between different types of chirps, along with a possible role in phase-invariant chirp responses. Stellate cells displayed more varied responses, contributing to a nuanced understanding of how these cell types might complement each other in tasks such as localizing conspecific signals in noisy environments. Overall, this study broadens our understanding of the feedback mechanisms involved in processing electrosensory stimuli and opens avenues for further research.

Effects of noise correlations on decoding performance

Using multi-unit electrodes, I was able to measure the correlated activities of neural populations and quantify the effects of co-fluctuations in trial-to-trial variability on the performance of a linear decoder. For all three classes of stimuli (AMs, envelopes, and chirps), I saw no significant difference in performance in any case, suggesting that the amount of noise correlation may be small in nP responses to spatially diffuse stimuli. Previous studies have found that the amount of noise correlation for spatially diffuse stimuli in ELL is likewise relatively small [156], and exists

mainly in neurons with overlapping receptive fields [154]. More broadly, my results contribute to the ongoing question of the importance of correlated variability in neural computations [153]. Noise correlations have variously been found to have beneficial [128, 129, 182, 189], detrimental [126, 127, 181], and in the present case no significant effect on signal-encoding performance at the level of populations of neurons. However, it is important to keep in mind the limitations of the current approach in understanding the true relevance of such correlations to sensory processing as previously discussed (see Methods), viz. the dependence of correlation effects on the type of the decoder used in analysis [126, 129-131], the fact that correlations are plastic [190-193], and the fact that "signal" and "noise" are concepts defined by the experimenter [186].

Characterizing previously unrecorded neurons in nP

Another aim of the present study was to characterize the responses of nP neurons other than the previously characterized multipolar and stellate cells. I divided these unknown cells into low firing-rate and high firing-rate varieties. Some of the low firing-rate cells behave similarly to stellates (and may in fact be stellates), while others fired very sparsely. Some high firing-rate cells did not seem to respond to any stimuli but exhibited continuous slow (~0.1 Hz) oscillations throughout the recording session. These could be considered "dark neurons," i.e., sensory neurons that do not respond to stimuli in any obvious manner. Such neurons have been found to be surprisingly prevalent since the advent of large-scale neural recordings [132]. Since nP receives input from higher brain areas via torus, the activity of these neurons could potentially be related to any number of ongoing brain-wide behavioral or cognitive processes not directly related to the experimental stimuli, as has been observed in other recent large-scale recordings [141, 142]

Linear vs. nonlinear decoders of AM and envelope stimuli

Decoding a continuous stimulus from neural responses is an example of a regression problem, a class of problems in which machine learning techniques have exhibited considerable success [194]. Indeed, the abilities of artificial neural networks to learn complex structure in data have led to their widespread use as computational models of the brain, despite concerns about the inscrutable complexity of such models [195]. Nonetheless, I found that the performance of a simple linear decoder at reconstructing AM and envelope stimuli from nP responses was broadly

comparable to that of more complex nonlinear models. This suggests that the relationship between these stimuli and responses in nP is largely linear and justifies the more fine-grained analysis of the linear decoding models that follows.

AM encoding in multipolar and stellate cells

The ability of both multipolar cells and stellate cells to accurately encode low frequency AM stimuli, as revealed by the performance of a linear decoder, is not entirely surprising, as these cells are known to respond to such stimuli (with multipolar cells responding most strongly to higher frequencies and stellate cells ceasing to respond beyond ~32 Hz; see Fig. 1A). This accurate encoding is necessary for the generation of the negative image used in the cancellation of spatially diffuse low frequency stimuli caused by self-motion or nearby conspecifics. The ability to cancel redundant AM stimuli is of paramount importance for these fish, which are largely nocturnal and rely heavily on the electric sense for navigation and foraging [196]. Since the EODs of wave-type fish are generated continuously, the electroreceptors of a given fish are liable to receive a continuous barrage of noise caused either by the fish's own movements or by interference ('beats') from the EODs of other nearby fish. The solution arrived at by gymnotiform weakly electric fishes is the exquisite adaptive cancellation mechanism previously described (see Background Information) in which both the direct and indirect feedback pathways contribute to the generation of negative image that arrives at the apical dendrites of ELL superficial cells and results in the subtraction of predictable components of sensory input. A strikingly similar solution to the same problem was arrived at independently in African mormyriform weakly electric fishes [197]—in these fishes, however, electrosensory and proprioceptive input are combined with corollary discharge signals from the electric-organ command centre [198-200], while the existence of an analogous corollary discharge signal in gymnotiforms is so far only speculative [201].

Envelope encoding in multipolar and stellate cells

The ability of multipolar cells to encode the time course of low frequency (<1 Hz) envelopes with high accuracy is a novel result, and somewhat unexpected since these cells are known to respond preferentially to high frequency (~ 64 Hz) AMs. Previous studies have shown that envelope extraction already takes place via feedforward mechanisms in ELL and thus may

simply be inherited in nP multipolar cells [54]. Nevertheless, it has also been shown that both the indirect and direct feedback pathways from nP play roles in sculpting ELL pyramidal cell responses to envelopes [48, 53]. *A. leptorhynchus* respond with EOD frequency modulations to very weak envelopes [53, 77] which suggests that these stimuli (representing the movements of other fish) likely have strong behavioral relevance, a notion further supported by observations that these fish are adept at detecting and localizing conspecifics [37, 40, 202]. How the electrosensory system is able to localize conspecific signals despite the diffuse nature of these signals and the presence of noise remains unknown [203].

My results further refine our understanding of the role of feedback in envelope coding, with multipolar cells appearing to encode the time course of the envelope with high resolution, and stellate cells showing more heterogeneous responses. The heterogeneity in stellate responses lends support to the idea that the direct feedback pathway is responsible for promoting heterogeneity in superficial ON cell responses to envelopes in ELL with presumed beneficial effects on population coding performance, as described in a recent study [52]. Interestingly however, another recent study found that deep cells (which receive less feedback) outperform superficial cells in their ability to discriminate the location of conspecific-type stimuli [204]. Both of these studies, like the present one, are limited by their use of immobilized animals. This highly unnatural scenario fails to account for the fact that action and perception are intimately connected, as evidenced in the well-known "active sensing" behaviors of weakly electric fish [205, 206] and many other animals [207, 208]. For example, a fish could move such that the contrast of the envelope signal resulting from the EOD of a conspecific undergoes slight differential changes on different parts of its body and use this information to triangulate the source of the signal. In this scenario, I conjecture that multipolar cells and stellate cells could perform complimentary roles, with multipolar cells providing a high temporal resolution encoding of the envelope (perhaps inherited from deep pyramidal cells in ELL), and stellate cells providing excitatory feedback to particular ELL pyramidal cells to enhance the spatial resolution of the information gleaned by active sensing, consistent with the proposed role of stellate cells as sensory searchlight [95] and their known role in motion encoding [125]. Information from the two pathways (i.e., direct and indirect) could perhaps then be integrated in superficial pyramidal cells and contribute to the ability of these fish to locate conspecifics in noisy environments [37].

Multipolar cells can discriminate between chirp varieties

My results clearly show that multipolar cells respond strongly to chirps and can discriminate between chirps with different duration and amplitude. This is a novel finding of this project, as it was previously unknown how or whether nP neurons respond to electrocommunication stimuli. Interpreting this finding within the broader context of the electrosensory system at present requires some speculation. Multipolar cells may simply inherit their response to chirps from ELL pyramidal cells; indeed, classifier performance by population size for multipolar cells (Fig. 7D) appears to be very similar to that found for ELL ON cells in a previous study [131]. But whether this information from multipolar cells is used in downstream brain areas is uncertain. While the indirect pathway is necessary for burst responses in ELL pyramidal cells to chirp stimuli, this is because the negative image input generated in response to the underlying beat depolarizes the apical dendrites of ELL pyramidal cells and increases their proclivity to burst in response to chirps—it is not, apparently, due to feedback from the chirp itself [70]. Because indirect feedback input reaches the apical dendrites of ELL pyramidal cells ~20 ms or more after stimulus onset, feedback from the chirp stimulus itself cannot influence the generation of the second spike in the burst response, which occurs on average ~16 ms after the chirp [69-71]. Instead, any influence of the chirp via feedback would occur after the generation of several spikes in the bursting chirp response in ELL. However, ELL pyramidal cells have been shown to exhibit two distinct firing patterns in response to chirps: a rapid, feature-invariant burst response thought to be associated with the rapid detection of a chirp, and a graded heterogeneous response thought to contain sufficient information to support the evaluation and discrimination of different varieties of chirp waveforms [65]. Information on chirp characteristics from multipolar cells could perhaps contribute to this second pathway. Similar parallel pathways for chirp detection vs. discrimination exist downstream in torus [177], and may correspond to different behavioral needs, e.g., the need for rapid detection of chirps in agonistic contexts or for accurate evaluation of chirp characteristics during courtship [61, 65, 68, 178]. This question could likely be addressed experimentally using a discrimination task [65] and a feedback block.

Multipolar cells display phase invariance in chirp responses

As opposed to chirp amplitude and duration, characteristics which are under the control of the emitting fish, the phase of the underlying beat at which the chirp occurs is random and thus is not

thought to carry semantic information or have behavioral relevance [177]. However, chirps occurring at different beat phases result in rather dissimilar waveforms (Fig. 6A, top) that nevertheless must all be recognized as chirps in the nervous system of the receiving fish. In other words, neurons must respond invariantly to chirp features despite differences caused by the underlying beat phase. Indeed, the phase invariance of neural responses to chirps generally increases at successive stages of electrosensory processing, and behavioral responses are nearly completely phase invariant [73]. Invariance in neural responses has traditionally been thought of as a feedforward process [209-211]. Such models have tended to ignore feedback loops, which are, as previously discussed, ubiquitous in sensory areas. Recent studies have, however, begun to uncover interesting roles for feedback in invariant object recognition [212, 213]. Intriguingly, I found that multipolar cells displayed an average phase invariance that was intermediate between that obtained previously for ELL pyramidal cells and torus neurons. Although the difference did not reach the level of statistical significance, likely due to the small number of multipolar cells recorded, the potential role of feedback in creating phase invariant responses to chirps may be worthy of further study.

Future work

My results on chirp encoding in nP suggest two potential studies that could further investigate the role of feedback in chirp discrimination and phase-invariant responses to chirps. These studies would be relatively straightforward to conduct using a protocol like that of the present study and comparing measures of discrimination and invariance before and after blocking the feedback pathway to ELL, e.g., with lidocaine.

Further exploration in this area could also benefit from more ethological studies on electrocommunication in these fishes in their natural habitat. Such research would provide a foundational understanding of what various chirps signify to the fish and could lead to deeper insights into sensory processing. Indeed, to move beyond merely descriptive, correlational accounts of brain activity neuroscience likely needs to focus on understanding behavior (which is, after all, what brains are for) in all its richness [144]. Along these lines, there is currently a good deal of excitement about applying machine learning techniques in combination with

behavioral data to decode animal communication, although it remains an open question whether this approach will yield fruitful results [214].

Another logical next step for future research involves recording population activity with Neuropixels probes (or similar) in EGp, which forms part of the indirect feedback pathway but remains relatively unexplored. This area is where proprioceptive information is combined with electrosensory information to generate the negative image used to cancel self-stimulation due to movement and is thus likely crucial for active sensing.

Finally, it is important to emphasize this protocol focuses on global electrosensory stimuli. These are spatially diffuse stimuli that impinge upon the entire body surface of the fish and correspond to the kinds of signals originating from other nearby electric fish. It is important to note that in natural settings, salient local (e.g., prey) and global stimuli can occur simultaneously. Indeed, feedback from nP is critical to the differential coding in ELL pyramidal cells of stimuli with differing spatial extents as well as different frequencies [94, 114, 115, 120]. Future studies of nP should include localized stimuli to investigate the important role played by nP in this differential coding.

Implications for other systems

Despite its seemingly exotic nature, the electrosensory system faces many of the same challenges as do other senses. As noted previously (see Background Information), the ELL is a cerebellum-like structure, the architecture of which is well-suited to its role as an adaptive filter able to cancel the effects of self-stimulation [12]. This function of the cerebellum has long been known [215, 216], but its contribution to a wide range of behaviors in vertebrates is recently becoming appreciated [217, 218]. In the primate cerebellum, for example, an adaptive cancellation signal suppresses expected sensory consequences of active movement [219], while the importance of cerebellar function in human brains is argued for by its comprising 70% of the brain's total neurons [220]. A detailed understanding of the feedback pathways underlying neural learning in ELL could thus provide insights for the treatment of cerebellar-related disorders. Furthermore, the downweighting of repetitive or expected stimuli and enhancing of novel ones is a hallmark of the predictive processing framework, and the adaptive cancellation mechanism in weakly electric fish could serve as a model for testing specific predictions of this theory, such as role of

precision-weighted prediction errors in sensory perception [22], with implications extending to potential applications in neuroprosthetics and machine learning algorithms that mimic biological sensory filtering.

Envelope coding in the electrosensory system likewise has parallels with the encoding of second-order stimuli in other sensory systems, notably contrast modulation or luminance envelopes in the visual system [221] and motion envelopes in the vestibular system [222]. Finally, the use of alternate coding strategies for discrimination and detection tasks seen in the context of electrocommunication has parallels in the visual [223] and insect auditory [224] systems. It is likely that principles of feedback uncovered in the electrosensory system will also manifest in these other systems.

Final remarks

By conducting multi-unit recordings of nP neurons in awake *A. leptorhynchus* to explore their population-level responses to a range of natural stimuli, this study resulted in several novel findings with respect to feedback mechanisms in electrosensory processing. I characterized the responses of previously unrecorded neurons in nP, analyzed the role of noise correlations in the ability of nP neurons to accurately encode stimuli, and elucidated the roles of different neuron types in encoding various electrosensory inputs. The results extend our understanding of feedback mechanisms in sensory processing, revealing minimal effects of noise correlations on decoding performance, and highlighting the nuanced roles of multipolar and stellate cells in encoding beats, envelopes, and chirps. The work uncovers novel aspects of electrosensory encoding, such as the ability of multipolar cells to linearly encode motion envelopes, to discriminate between different chirps, and the potential role of feedback in phase-invariant chirp responses. The behavioral relevance of these abilities, i.e., how they are used by these remarkable fish to locate and communicate with conspecifics using electroreception, should be addressed in future behavioral studies. The insights gleaned from the current study enhance our understanding of the electrosensory system but also hold implications for broader sensory neuroscience, potentially informing the study of other neural systems with similar feedback mechanisms.

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Supplementary figures

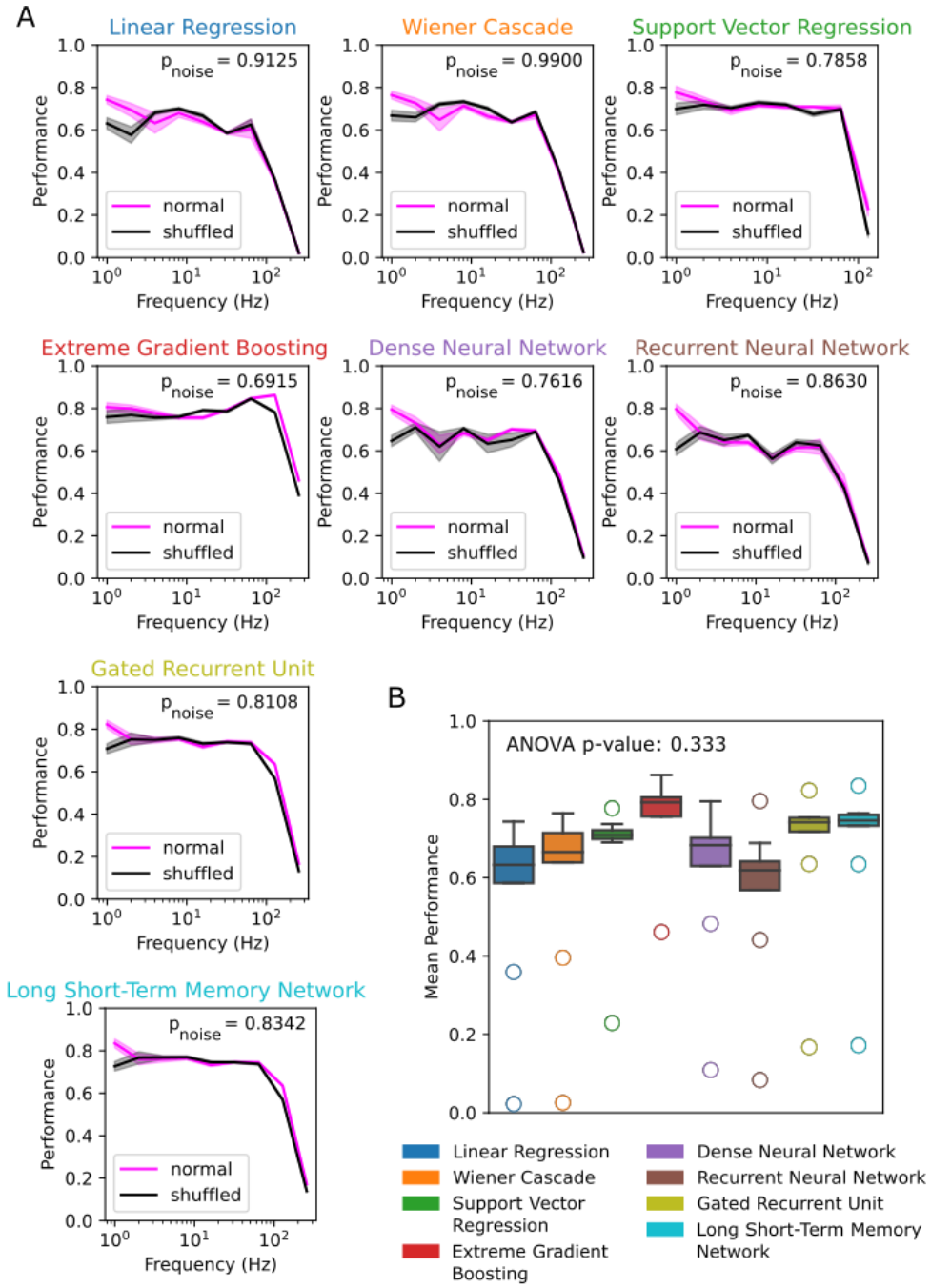

Figure S1. *Results of training various decoders to reconstruct AM stimuli from nucleus praeeminentialis neuron responses. (A) Mean (SE) performance of various decoders with noise correlations intact (magenta) and with noise correlations removed by shuffling responses with respect to trial order (black) across all AM frequencies, along with results of a t-test for significant difference in mean performance. (B) Boxplot comparing mean aggregated performance of different decoders at reconstructing AM stimuli of frequencies ranging from 1–256 Hz. Performances were not significantly different (one-way ANOVA, p=0.333).*

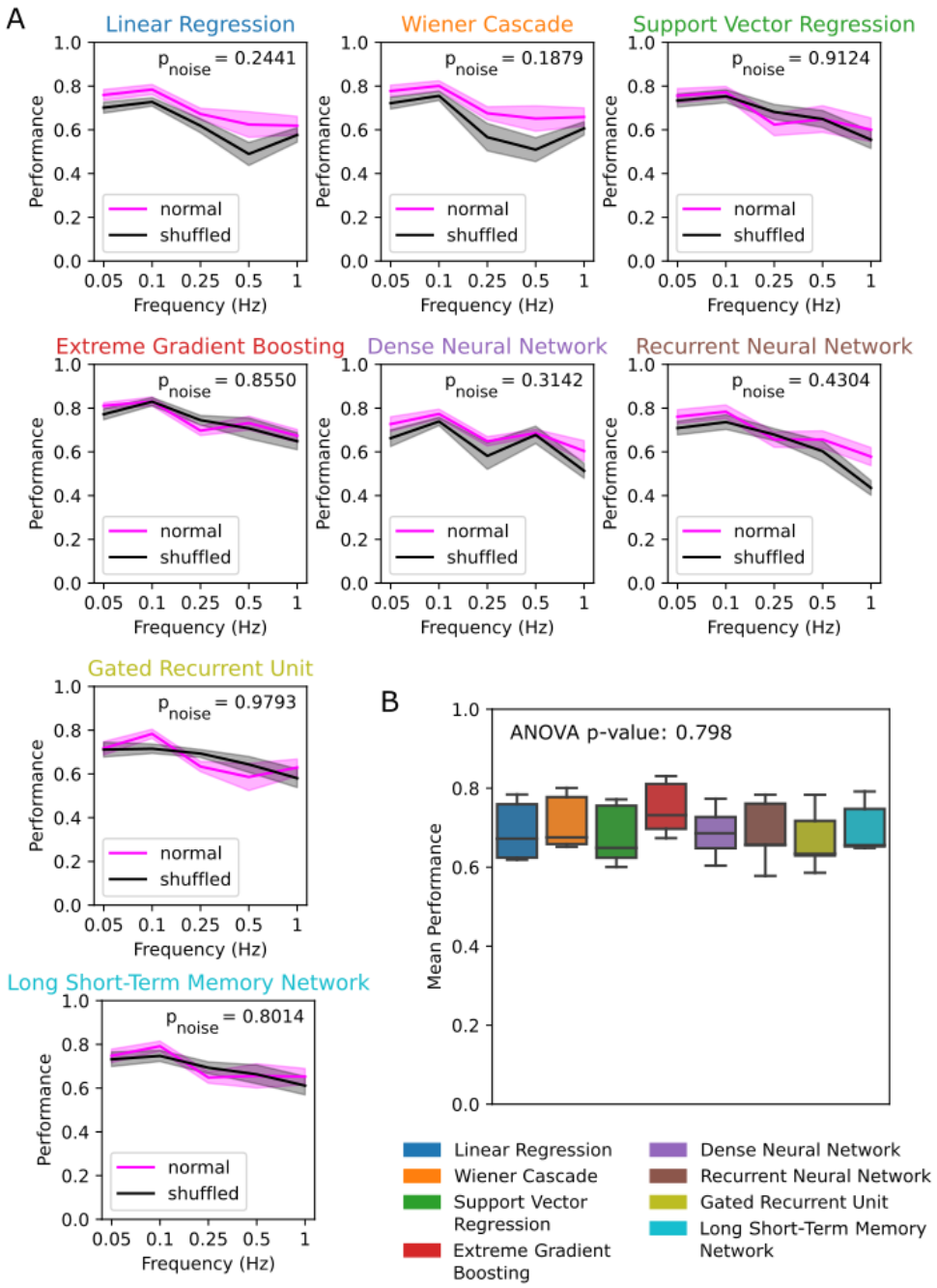

Figure S2. *Results of training various decoders to reconstruct envelope stimuli from nucleus praeeminentialis neuron responses. (A) Mean (SE) performance of various decoders with noise correlations intact (magenta) and with noise correlations removed by shuffling responses with respect to trial order (black) across all envelope frequencies, along with results of a t-test for significant difference in mean performance. (B) Boxplot comparing mean aggregated performance of different decoders at reconstructing envelope stimuli of frequencies ranging from 0.05–1 Hz. Performances were not significantly different (one-way ANOVA, p=0.798).*