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SYMPOSIUM

Everything in Modulation: Neuromodulators as Keys to Understanding Communication Dynamics

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Synopsis Across the animal kingdom, the ability to produce communication signals appropriate to social encounters is essential, but how these behaviors are selected and adjusted in a context-dependent manner are poorly understood. This question can be addressed on many levels, including sensory processing by peripheral organs and the central nervous system, sensorimotor integration in decision-making brain regions, and motor circuit activation and modulation. Because neuromodulator systems act at each of these levels, they are a useful lens through which to explore the mechanisms underlying complex patterns of communication. It has been clear for decades that understanding the logic of input–output decision making by the nervous system requires far more than simply identifying the connections linking sensory organs to motor circuits; this is due in part to the fact that neuromodulators can promote distinct and temporally dynamic responses to similar signals. We focus on the vocal circuit dynamics of *Xenopus* frogs, and describe complementary examples from diverse vertebrate communication systems. While much remains to be discovered about how neuromodulators direct flexibility in communication behaviors, these examples illustrate that several neuromodulators can act upon the same circuit at multiple levels of control, and that the functional consequence of neuromodulation can depend on species-specific factors as well as dynamic organismal characteristics like internal state.

Introduction

Dynamics of animal communication: a role for neuromodulators

Animal communication is crucial for reproduction and survival across diverse taxa. Communication signals allow individuals to broadcast information about their identity and physiological state, and these signals can change in response to important environmental and social cues. For example, photoperiod is an important environmental trigger that promotes reproductive communication signals in European starlings on a circannual basis (Bernard and Ball 1997; Rouse et al. 2015), while in gray tree frogs, the presence of a female conspecific is an important social cue that leads the male to lower the dominant frequency of their calls (Reichert and Gerhardt 2013). Environmental or social cues can intersect with an organism's internal state to produce a specific behavioral response, for instance, gravid *Astatotilapia burtoni* females urinate more frequently in the presence of dominant males and brooding females than in isolation or in the presence of another gravid female (Field and Maruska 2017). How are these changes to communication signals rapidly executed by the brain? In this review, we explore how neuromodulators regulate the neuronal circuits that control context-dependent communication by describing several examples across species including frogs, birds, fish, and rodents.

Communication behaviors can vary over a wide range of timescales, from milliseconds to the lifespan of the animal. Processes such as circuit remodeling, changes in gene expression, and altered neuronal and synaptic function can produce behavioral flexibility. Some processes, such as building new neural circuits, are too slow to support rapid, context-dependent

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behavioral changes. One way that rapid behavioral changes can be mediated is via neuromodulators that induce physiological responses within sensory, sensorimotor, and/or motor circuits.

Neuromodulators are a diverse set of intercellular signaling molecules with conserved cellular mechanisms across species (e.g., Katz and Lillvis 2014; Kamhi et al. 2017). Neuromodulators trigger second messenger cascades that affect neural circuits in two broad ways: (1) changing the excitability or temporal pattern of neuron activity and (2) altering the strength of synaptic connections between neurons (Marder and Bucher 2001; Dickinson 2006). These signaling molecules are widely distributed and target many areas, from sensory circuits to motor circuits and everything in between, with the potential to orchestrate dynamic changes locally and globally. While many studies have investigated how neuromodulators alter neuron activity patterns, in this article, we focus on the broad effects of neuromodulators on rapid, context-specific circuit and behavioral dynamics.

Exploring the roles of neuromodulators in animal communication dynamics

Many studies investigating communication dynamics have focused on the roles of sensorimotor circuits—especially areas of the vertebrate social behavior network, such as the preoptic area (POA), the lateral septum, the ventromedial hypothalamus, the anterior hypothalamus, and the midbrain—as major sources and sites of action of behaviorally important neuromodulators (Goodson 2005; Goodson and Kingsbury 2013). In contrast, relatively few studies of communication dynamics have focused on the motor circuits, known as central pattern generators, which generate behavioral rhythms. Because many central pattern generators are multifunctional (Briggman and Kristan 2008), we argue that identifying the neuromodulatory mechanisms that promote flexibility of these circuits can, in turn, provide insight into the broader neural mechanisms of spatiotemporal dynamics in communication. We first focus on how neuromodulation of motor circuits contributes to communication dynamics. Next, we describe examples of social context-dependent behaviors and the roles of neuromodulators acting on sensory, sensorimotor, and cortical communication circuits. Finally, we describe the interactions between internal state and neuromodulation throughout all levels of behavioral circuits.

Xenopus vocal behaviors are a powerful system for revealing how neuronal circuit mechanisms are altered under varying conditions leading to dynamic patterns of communication. In this review, we focus on *Xenopus* vocal behaviors as a case study for investigating the relationship between neuromodulators and social interactions, and complement this work with examples from several well-studied vertebrate communication behaviors, including vocal and electric fish communication, birdsong, and mouse ultrasonic vocalizations. Together, these examples illustrate the diversity and complexity of neuromodulatory mechanisms across both species and levels of circuit organization.

Xenopus vocal dynamics

At the beginning of the South African winter, as temperatures drop and rain begins to fall, the calls of aquatic clawed frogs—Idwi in isiZulu (Phaka et al. 2019), also known by the Western scientific name, *Xenopus laevis*—resound throughout the region's ponds, lakes, and streams (Tobias et al. 2004). This scene of anuran reproduction appears decidedly more subdued than the charismatic multimodal chorusing of North American Gray treefrogs (*Hyla versicolor*) and Central American Túngara frogs (*Engystomops pustulosus*). However, below the water surface hidden from view, the rich repertoire of *X. laevis* vocalizations orchestrates a complex dance of courtship, reproduction, and agonistic behaviors.

Xenopus vocal repertoires

The genus *Xenopus* includes over 25 species of African clawed frogs. Their vocalizations depend on both social context and internal state (e.g., sexual receptivity). Each call consists of trains of brief sound pulses, with the specific rate and temporal pattern of pulses defining each call. Full repertoires have been described for two species, *X. laevis* and *Xenopus borealis* (Fig. 1; Yager 1992; Tobias et al. 1998; Tobias et al. 2004). As in all described *Xenopus* species, *X. laevis* and *X. borealis* males produce advertisement calls in isolation or in the presence of conspecifics to advertise their sexual state to potential mates (Tobias et al. 2011). While the functions of these calls are similar, the temporal patterns are highly distinct: *X. laevis* advertisement calls consist of fast pulse trains (30–60 pulses per second), while *X. borealis* advertisement calls are much slower (1–3 sound pulses per second). Males of both species produce calls in several other social contexts: during agonistic encounters with conspecific males, when clasping a conspecific, and when interacting with (but not clasping) females. The complexity of vocal repertoires varies across species; for example, *X.*

laevis males produce three agonistic calls, while *X. borealis* males produce one. When clasping a conspecific, male *X. laevis* produce an "amplectant" call (Tobias et al. 2004), while *X. borealis* produce an "approach" call (Yager 1992). *Xenopus borealis* males switch regularly and rapidly between advertisement calling and approach calling as they interact with and clasp females. *Xenopus laevis* males produce a modified advertisement call, "answer" call, when they hear a receptive female (Tobias et al. 1998). Sexually unreceptive female *X. laevis* produce a "release" call when clasped by a male, and gravid females produce an advertisement call ("rapping") when they are unable to locate a calling male. In contrast, *X. borealis* females are only known to produce release calls when clasped by a conspecific while sexually unreceptive (Yager 1992). Regardless of species differences in vocal repertoires, individual frogs integrate a wide range of information about their own internal states with social sensory signals from nearby conspecifics to produce appropriate vocal responses.

The *Xenopus* vocal circuit

Many of the brain circuits underlying the processing and production of vocalizations in *Xenopus* have been identified (Fig. 2). Auditory information from the inner ear arrives in the hindbrain, and is processed in higher-order sensory nuclei including the midbrain torus and central thalamic nucleus. The central amygdala (CeA) and bed nucleus of the stria terminalis (BNST) appear to be sensorimotor integration centers; the CeA receives auditory information from the thalamus, and both project to hindbrain nuclei. Numerous studies have illuminated the connectivity of the vocal nuclei and identified hormone and neuromodulator systems that are present in the circuit (Kelley et al. 2020).

Xenopus presents an excellent system in which to probe hindbrain contributions to behavioral dynamics because the vocal motor circuit (a central pattern generator) has been well described and is experimentally accessible in a reduced preparation. The hindbrain vocal circuit consists of the premotor parabrachial nucleus (PBx) and the vocal motor nucleus (VMN; nucleus ambiguus [NA]). Vocal central pattern generator output can be recorded from the laryngeal nerve of the isolated *ex vivo* brain with suction electrodes; temporal patterns of these fictive vocalizations closely resemble *in vivo* vocalizations (Fig. 3; Yamaguchi and Kelley 2000; Rhodes et al. 2007). Extracellular and intracellular recordings in different nuclei allow for observation and perturbation of the motor circuit in action.

Behavioral dynamics from a motor perspective

Neuromodulation of central pattern generators

Much of what we know about the mechanisms and functions of neuromodulation has come from investigations of central pattern generators—motor circuits that autonomously generate rhythmic behaviors (Harris-Warrick 2011). Some of the best-studied examples include the crustacean stomatogastric ganglion controlling stomach movements (Marder and Bucher 2007), leech swimming and crawling circuits (Briggman and Kristan 2008; Puhl and Mesce 2008) and spinal locomotor circuits in vertebrates (Miles and Sillar 2011). There is ample evidence for complex neuromodulation of such circuits. For example, dozens of neuromodulatory substances are found in the stomatogastric ganglion, every neuron in the circuit is subject to modulation, and single neurons are regulated by multiple neuromodulators (Marder 2012). Co-transmission of multiple modulators is common, with distinct modulators often conferring opposing or additive effects on circuit output (Marder et al. 2005; Marder 2012; Nusbaum et al. 2017). While these complexities have been well described in a handful of circuits, detailed understanding of the multi-neuromodulatory mechanisms in many behavioral circuits remains to be unraveled (Nusbaum et al. 2017).

In this section, we review what is known about the neuromodulation of central pattern generators that drive communication behaviors in *Xenopus* as well as vocal and electric fish. While the role of neuromodulators has been appreciated for some time, recent studies are revealing that these circuits are also under complex neuromodulation like their invertebrate counterparts.

Neuromodulation and behavioral dynamics in the *Xenopus ex vivo* brain

Serotonin is the best-characterized neuromodulator within the *Xenopus* vocal motor circuit. A tract-tracing study showed a robust connection between the *X. laevis* vocal hindbrain nuclei and the dorsal raphe (the primary source of serotonin in the brain; Brahic and Kelley 2003), and led to the hypothesis that serotonin modulates vocal circuit function. Rhodes et al. (2007) tested this hypothesis in the *X. laevis ex vivo* brain and found that bath application of serotonin most commonly elicits fictive

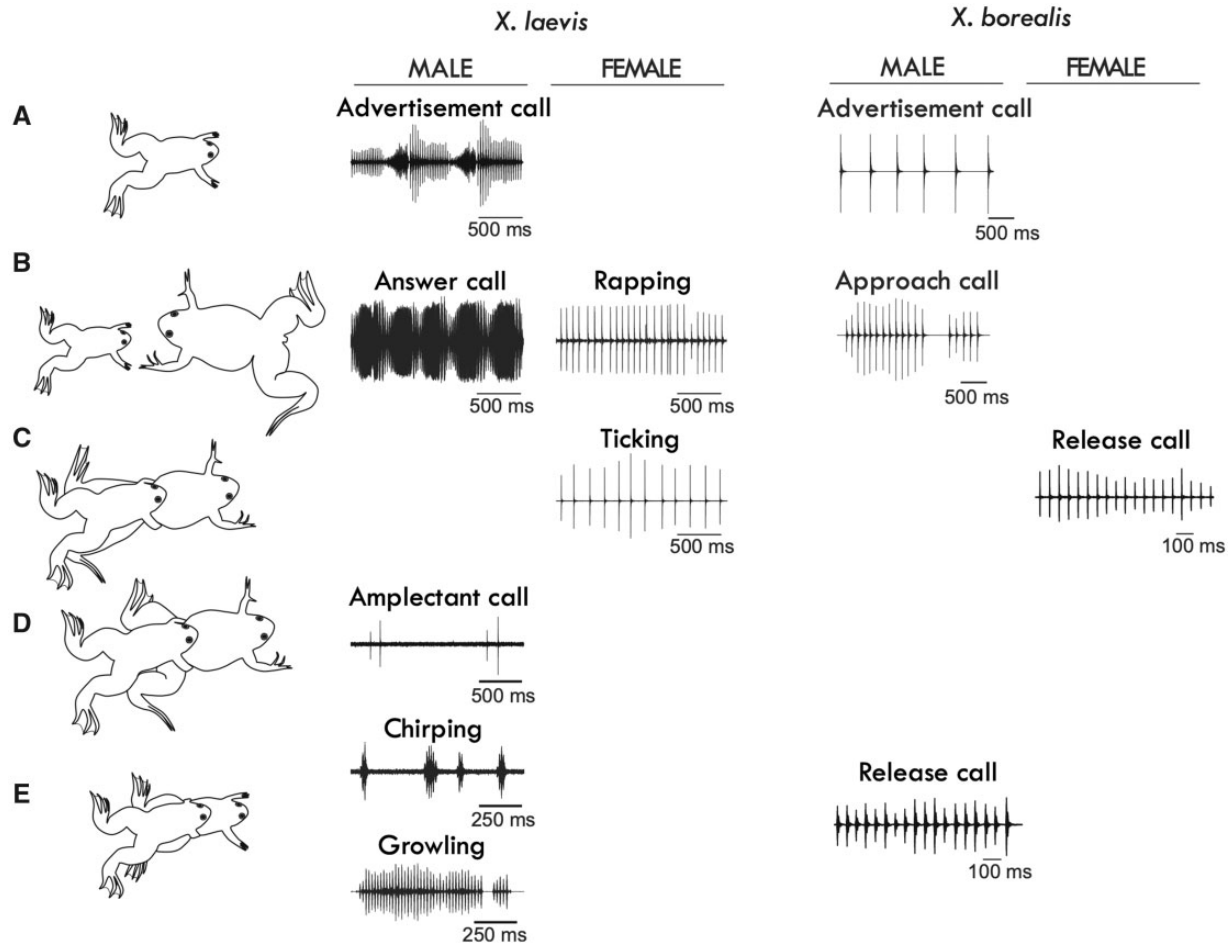

Fig. 1. Vocal repertoires of two species of African clawed frog, *X. laevis* and *X. borealis*. Vocalizations occur in specific social contexts. (**A**) Both *X. laevis* and *X. borealis* males produce advertisement calls to attract mates. (**B**) Female *X. laevis* have a fertility call (rapping), which elicit answer calling from males. *Xenopus borealis* males emit an approach call when attempting to clasp a female. (**C**) Unreceptive female *X. laevis* and *X. borealis* produce release calls (known as "ticking" in *X. laevis*) when clasped by a male. (**D**) *X. laevis* males produce an amplectant call during a prolonged clasp. (**E**) Agonistic calls produced by male *X. laevis* during physical interactions include chirping (produced by the clasper) and growling (produced by the frog being clasped). *X. borealis* males also produce an agonistic release call when clasped.

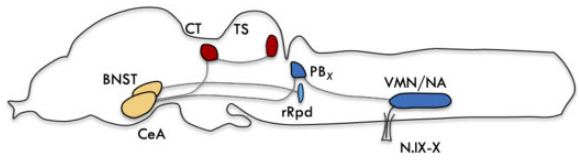

Fig. 2. Schematized anatomy of the *Xenopus* vocal circuit. Sagittal view; rostral is to the left; dorsal is up. Vocalizations are encoded by the auditory system (red), including the torus semicircularis, which projects to the CT. Sensorimotor areas (yellow) such as the CeA and BNST link auditory circuits to vocal circuits (blue). The rostral division of the dorsal raphe nucleus (rostral division of the dorsal raphe nucleus [rRpd]; light blue) is the major source of serotonin within the vocal circuit. The vocal motor circuit is a central pattern generator that includes the premotor parabrachial nucleus (PBx) and the vocal motor nucles (VMN homolog of the mammalian nucleus ambiguus, NA: Albersheim-Carter et al. 2016); the vocal pattern produced by the circuit exits the brain to the activate the larynx via the fourth rootlet of cranial nerve (N.) IX-X.

advertisement calls in male brains and fictive release calls in female brains. Serotonin also elicits fictive advertisement calling in at least four additional species (Leininger and Kelley 2013; Barkan et al. 2017), suggesting that serotonin-mediated induction of advertisement calling is conserved across the genus.

While social context mediates behavioral switching *in vivo*, behavioral dynamics can still persist in the isolated brain preparation, which inherently lacks social context. Fictive vocal patterns resembling calls made when males are clasping (amplectant call) or being clasped (release call) are also occasionally observed in *X. laevis* brains, but are not reliably elicited by serotonin (Rhodes et al. 2007; Zornik and Kelley 2008). *In vivo, X. borealis* males dynamically interact with and clasp conspecifics, and in the process alternate between advertisement and approach calls (Yager 1992). In response to serotonin, isolated *X.*

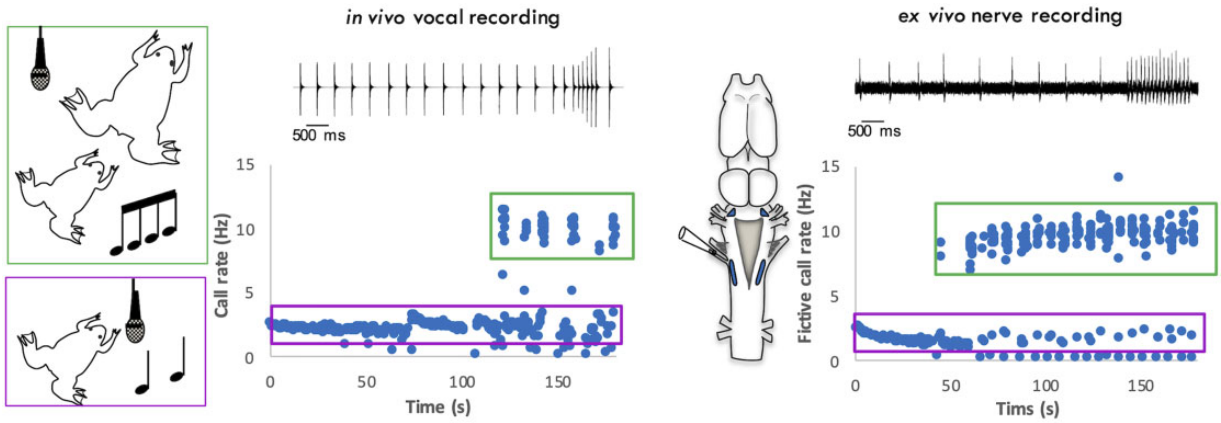

Fig. 3. Pattern switching in *X. borealis* occurs both *in vivo* and *ex vivo*. Left panels: Social context, sound oscillograms, and call rates over time, showing call temporal dynamics. Vocal pattern depends on social context; approach calls (upper boxes, call rate 5–15 Hz) occur during attempted clasps of conspecifics, and advertisement calls (lower boxes, call rate 1–3 Hz) occur when the male is not contacting a conspecific, and can occur in isolation. Within a period of calling, call rate changes dynamically and switches between advertisement call and approach call, as illustrated by sound oscillogram (top trace) and by tracking call rate over the course of minutes (lower plot). Right panels: Dynamic temporal properties persist in the *ex vivo* brain, a reduced preparation, which by definition lacks social context. Fictive motor output can be recorded with a suction electrode placed on the vocal motor nerve. Top trace: Nerve recordings from the *ex vivo* brain show impulses of activity that change dynamically over time. Lower plot: Within a period of fictive calling, fictive call rate changes dynamically over time, switching between fictive advertisement (lower box) and approach calling (upper box).

borealis brains reliably produce both fictive advertisement calls (which contain 1–3 sound pulses per second) and approach calls (consisting of 5–15 pulses/s). Patterns of nerve output frequently switch between these two calls over the course of seconds to minutes (Fig. 3; Leininger and Kelley 2013). It is unknown how fictive behavioral switching occurs in *ex vivo* brains. Future experiments can test the role of additional neuromodulators in contributing to these fictive vocal dynamics. By first identifying the neuromodulatory basis of vocal pattern switching *ex vivo*, we can ultimately test whether these same neuromodulatory signals drive behavioral switching *in vivo*.

Complex neuromodulation of *Xenopus* vocal circuits

Understanding the neuromodulatory mechanisms controlling behavioral switching through actions on receptor subtypes (and receptor distributions in the brain) is an open area of inquiry. Neuroanatomical, pharmacological, and transcriptomic approaches are beginning to uncover the role of neuromodulators in vocal production.

Serotonin receptor subtypes and distributions

Pharmacological approaches have helped illuminate how serotonin stimulates fictive calling *ex vivo*. Agonists for serotonin receptor subtype 2C (5-HT_{2C}) activate the *X. laevis* vocal central pattern generator, suggesting that 5-HT_{2C} receptor activation is sufficient for vocal initiation (Yu and Yamaguchi 2009). Application of selective serotonin reuptake inhibitors to isolated *Xenopus* brains initiates fictive advertisement calling and this effect is abolished by a 5-HT_{2C} receptor antagonist, suggesting that endogenous serotonin binding to 5-HT_{2C} receptors initiates vocal activity (Yu and Yamaguchi 2010). Immunohistochemistry experiments located 5-HT_{2C}-like receptors in the raphe and VMN (Yu and Yamaguchi 2010). Interestingly, 5-HT_{2C} receptors do not appear to be expressed in PBx, despite apparent projections from the raphe. Therefore, if serotonin acts directly on PBx neurons, it may do so through a different receptor subtype. In terms of behavioral dynamics, addressing the expression patterns of various serotonin receptors across vocal nuclei may provide clues about mechanisms of vocal switching, while studies across species may reveal species-specific functions of serotonin.

Next-generation methods for probing complex vocal neuromodulation

More recently, calcium imaging of cultured *Xenopus* vocal neurons has allowed high-throughput characterization of individual neurons' responses to multiple neuromodulators (Inagaki et al. 2020). This approach has revealed that neurons in the premotor vocal nucleus PBx can respond to a range of neuromodulators in combination, including Substance P, acetylcholine, serotonin, and adenosine triphosphate (ATP); (Inagaki et al. 2020).

Finally, transcriptomic approaches can be used to identify transcripts related to neuromodulator synthesis, degradation, and receptor expression within subregions of the vocal circuit. Differentially expressed genes across sex, hormonal treatment, or species may identify which neuromodulators in the vocal central pattern generator underlie behavioral dynamics.

Complex neuromodulation of motor circuits across vertebrates

Research in *Xenopus* described above supports two hypotheses: (1) the temporal dynamics of the vocal central pattern generator is regulated by several neuromodulators and (2) because vocal repertoires vary across *Xenopus* species, it may be possible, but thus far untested, that species differences in neuromodulation contribute to behavioral differences. Here we describe examples of neuromodulation of pattern-generating circuits in vocal and electric fish, which indicate that complex regulation of motor circuits may be universal across vertebrates.

As we hypothesize in *Xenopus*, vocal production in fish is also under complex neuromodulation. The Batrachoididae, commonly known as toadfish, vocalize via contractions of intrinsic swimbladder muscles (Bass 2008). Evidence of catecholaminergic signaling has been revealed throughout the vocal central pattern generator of two species: plainfin midshipman (*Porichthys notatus*) and the gulf toadfish (*Opsanus tau*; Forlano et al. 2014; Goebrecht et al. 2014; Rosner et al. 2018), suggesting a conserved role for catecholamines (e.g., dopamine and norepinephrine). Rosner et al. (2018) expanded the search for additional neuromodulators. Their results revealed that all hindbrain vocal motor nuclei appear to receive serotonergic inputs, while cholinergic neurons and putative synaptic terminals were observed throughout the *O. tau* vocal motor circuits, as also previously described in midshipman (Brantley and Bass 1988). Therefore, strong anatomical evidence indicates that the toadfish vocal central pattern generator is controlled by multiple neuromodulators; however, the effects of these signaling molecules have not yet been tested in physiological or behavioral experiments.

Species-specific modulation of communication in electric fish

Weakly electric fish generate electric organ discharges (EODs) for navigation, hunting, and social communication. The rate and waveform of the EOD convey a variety of information to conspecific fish including the sender's identity, sex, age, and reproductive state (Caputi et al. 2005). The hindbrain pacemaker nucleus (a central pattern generator) generates the EOD temporal pattern and relays it to electromotor neurons that control the electric organ. Descending inputs to the pacemaker nucleus modulate the EOD rate in response to environmental and social cues. Electroreceptors are distributed across the body of these fish and detect changes in the electric field they generate in order to detect the presence of objects such as prey and conspecifics.

As in *X. laevis* and *X. borealis*, neuromodulation of the electric pacemaker nucleus is conserved across species, but the same modulator has slightly distinct effects. *Gymnotus omarorum* is a solitary species that is highly aggressive and territorial during all seasons. In contrast, *Brachyhypopomus gauderio* is a gregarious species and only displays aggression during the breeding season and only toward males. Systemic injection of the neuropeptide arginine vasotocin (AVT) in *B. gauderio* and *G. omarorum* increases diurnal EOD rate. In *B. gauderio*, the effect is persistent but in *G. omarorum* the effect is transient. AVT perfusion onto a reduced pacemaker nucleus preparation recapitulates these behavioral differences: in *B. gauderio*, the pacemaker nucleus spike rate increases and this increase persists, while in *G. omarorum*, the spike rate of the pacemaker nucleus only briefly increases (Perrone et al. 2010, 2014). Thus, the same neuromodulator acts on the motor pattern generator of both species but elicits different effects.

In this section, we described various ways in which central pattern generator neuromodulation may contribute to vertebrate communication dynamics. Frog and toadfish vocal circuits are influenced by multiple neuromodulatory systems, and many more likely remain undiscovered, suggesting that vertebrate communication motor circuits are under complex neuromodulatory control, similar to their invertebrate counterparts. Identifying the full suite of neuromodulators in a given circuit can then provide clues about which upstream sensory and sensorimotor circuits are responsible for orchestrating changes at the motor level, leading to a more comprehensive understanding across levels of behavioral control. Such "bottom-up" approaches can complement the more common "top-down" strategies investigating modulation of sensory and sensorimotor circuits, discussed in the next section.

The role of social context in behavioral dynamics

While we can learn much about behavioral dynamics through the lens of hindbrain pattern generating circuits, we also want to understand what higher-order mechanisms in sensory and sensorimotor regions trigger those downstream changes. Here we discuss

what is known about social contexts and brain regions that regulate *Xenopus* vocal dynamics; we then give examples of how neuromodulation of sensory and sensorimotor circuits promotes behavioral flexibility across vertebrate taxa.

Context dependence of *Xenopus* vocalizations

Many *Xenopus* vocalizations are tightly connected to social context. For example, *X. laevis* males only produce amplectant calls when clasping another animal (Tobias et al. 2004). Similarly, across the genus, release calls occur specifically when a male or unreceptive female is being clasped by a conspecific (Tobias et al. 2014). Social context can also vary the temporal dynamics of a specific call; Yager (1992) observed that *X. borealis* male advertisement call intervals are more variable when the male is advertising in the presence of a conspecific versus when the male is advertising alone.

How is social context information integrated by the *Xenopus* brain in order to select an appropriate behavioral response? In the *Xenopus* forebrain, the CeA and the BNST project to PBx and the dorsal raphe nucleus, respectively, and these areas are important for regulating an individual's response to vocalizations (Hall et al. 2013). Electrical stimulation of the CeA or BNST elicits fictive calling in *ex vivo* brains. *In vivo*, lesioning the CeA or BNST disrupts socially appropriate vocal responses. Thus, these connections are likely involved in the process of behavioral initiation and switching.

The role of neuromodulators in vocal decision making is not well studied, but histological studies have identified candidates. As described above, the BNST projects to the serotonergic dorsal raphe nucleus. The BNST, in turn, is immunoreactive for acetylcholine (Marín et al. 1997), vasotocin, and mesotocin (González and Smeets 1992). Future physiological studies of the *ex vivo* brain can test whether and how these neuromodulators contribute to vocal selection.

Complex neuromodulation driving social context-dependent behavior is found across vertebrates

Neuronal circuits upstream of the central pattern generator are important for vocal dynamics in *Xenopus*, but how neuromodulators operate in these areas is open for inquiry. Here we describe a few recent studies in songbirds, electric fish, and mice that provide examples of how neuromodulators can influence communication dynamics through actions in a range of midbrain and forebrain nuclei.

Parallel actions of multiple neuromodulators in songbirds

In many songbird species, males produce distinct behaviors when their singing is "directed" toward a female versus when their songs are "undirected"—produced in isolation or facing away from other conspecifics (Sossinka and Böhner 1980). Many song characteristics are altered in the switch from undirected to female-directed behavior: temporal and spectral properties become less variable and song tempo and rate increase. Recent research has identified two neuromodulators, norepinephrine and acetylcholine, that target forebrain song nuclei and are implicated in the control of directed song.

Basal ganglia inputs to the forebrain motor region, the robust nucleus of the arcopallium (RA), provide a source for variability during undirected song, and norepinephrine weakens these synaptic inputs (Jarvis et al. 1998; Sizemore and Perkel 2008). In zebra finches, Sheldon et al. (2020) showed that stimulation of locus coeruleus (the major source of norepinephrine) modified undirected song toward a less variable, more directed-like song, while application of norepinephrine into RA only partially recapitulated these effects. Therefore, norepinephrine projections from the locus coeruleus onto additional nuclei likely also contribute to the switch from undirected to directed song.

Acetylcholine application excites neurons in nucleus HVC, a song nucleus that plays a critical role in song production, sensorimotor integration, and learning (Shea and Margoliash 2010). In Bengalese finches, Jaffe and Brainard (2020) showed that cholinergic signaling in HVC may promote the switch from undirected to directed song. Targeted application of acetylcholine into HVC decreased temporal variability and increased song tempo as observed in directed song. Consistent with this result, blocking muscarinic acetylcholine receptors in HVC reduced changes typically associated with directed song.

Acetylcholine and norepinephrine may function synergistically to promote a behavioral switch between two distinct context-dependent vocal behaviors. Because both of these neuromodulators have also been shown to modulate sensory processing (Cardin and Schmidt 2004; Ikeda et al. 2015; Lee et al. 2018), they may coordinate social context-specific circuit properties at each level of the song control system.

Serotonin modulation of sensory circuits in electric fish and rodents

Sensory circuits are an important target of neuromodulators. Serotonin is known to modulate sensory

systems across diverse taxa, including the electrosensory systems of weakly electric fish, and the auditory system in rodents. While the presence of serotonin may be conserved in general, the exact nature of how serotonin modulates a sensory system varies across species.

The electrosensory lateral line lobe (ELL: the primary hindbrain sensory nucleus in electric fish) receives synaptic input from the electroreceptors. When two fish are near each other, their electrical signals are "jammed," generating a sinusoidal modulation, or beat frequency, resulting in an interfering signal that can disrupt coding of environmental cues (Rose 2004). To compensate, the fish produce a jamming avoidance response that changes their EOD frequency away from the contaminating frequency.

The existence of serotonergic inputs (from the dorsal raphe) to theELL suggested a likely source of neuromodulation. Injection of serotonin in *A. leptorhynchus* and *A. albifrons* directly into the ELL increases the jamming avoidance response magnitude and also increases EOD modulation in response to envelope stimuli. Serotonin increases the excitability of ELL neurons in both species by increasing bursting (Marquez and Chacron 2020a, 2020b). These studies indicate that neuromodulation of a single sensory nucleus is sufficient to enhance behavioral responses to communication stimuli.

Like in electric fish, serotonin also enhances sensory processing in rodents. Mice have well-studied context-dependent vocal communication behaviors, and connections between sensory, sensorimotor, and neuromodulatory centers have been described. Serotonin concentrations increase in the auditory processing midbrain region, the inferior colliculus, during a variety of social contexts, and this is thought to increase the salience of relevant signals. Arginine vasopressin (AVP) and serotonin work together in a feedback loop in order for the social behavioral network (SBN) to modulate sensory systems via the dorsal raphe, thus aiding selection of context-appropriate motor responses (Petersen and Hurley 2017). In a model proposed by Petersen and Hurley (2017) the SBN encodes contextual cues from multiple sensory inputs, and activates the dorsal raphe via AVP. In turn, the dorsal raphe nucleus projects to the inferior colliculus, altering the animal's sensory sensitivity to auditory stimuli. It is then possible that the inferior colliculus feeds back on the SBN. In this way, multiple neuromodulators can be involved in feedback loops that can tune a system to change sensitivity to a given signal.

A key takeaway from the studies described above is that neuromodulators are known to act virtually at every level of behavioral control, from sensory inputs to motor output, resulting in production of appropriate communication signals. Next, we explore ways in which changes in internal state can alter neuromodulatory signals and how circuits respond to those inputs.

Intersection of internal state and neuromodulation

While environmental and social cues are key determinants of behavioral output, decision-making processes also depend on an organism's internal state. Internal states are multidimensional, integrating diverse physiological conditions such as energy balance, hormonal state, and place in social hierarchy (Kanwal et al. 2021, submitted for publication). An animal's internal state can aid in generating appropriate behaviors within environmental and social contexts. Here we describe a few examples in which behaviors are altered by internal state, and explore how neuromodulation may contribute to variability across a range of timescales.

Xenopus vocal communication is regulated by internal state

Sexual receptivity in females

During courtship, calls produced by *X. laevis* females depend on their reproductive state. Females produce receptive (rapping) calls when they are ready to lay their eggs but cannot physically locate a male (Tobias et al. 1998), or unreceptive (ticking) calls when they are clasped by a male while unreceptive (Russell 1955). Females lay eggs during the winter breeding season, which is likely mediated by elevated levels of gonadotropin (Lutz et al. 2001). In the lab, injection of gonadotropin leads a female to transition to a sexually receptive state and begin laying eggs and producing receptive calls (Tobias et al. 1998). How does gonadotropin influence vocal decision making? Gonadotropin receptors are present in the *Xenopus* brain, including areas upstream of the vocal central pattern generator, such as the CeA and thePOA, providing a molecular link between reproductive condition and vocal behavior (Yang et al. 2007). Furthermore, electrically stimulating the CeA in isolated female brains produces fictive receptive calls, in contrast to serotonin application, which produces fictive unreceptive calls (Ballagh 2014). Collectively, this suggests that gonadotropins may influence the output of the vocal circuit through the CeA.

Social dominance and auditory processing

Social status is another internal state that influences vocal choices. *Xenopus laevis* males form vocal dominance hierarchies in which only one or a few males in a pond call at a given time while the rest remain silent; in the lab, vocal playbacks are sufficient to induce vocal suppression (Tobias et al. 2010). A frog must integrate its own internal state—dominant or subordinate social status—with sensory information about the sender's identity to decide whether or how to respond to a call. The exact locations and mechanisms of auditory decision-making, and the role neuromodulators play in gating or tuning salient vocal signals, remain unstudied physiologically, though candidate brain regions have been identified, including central thalamus (CT) and CeA (Fig. 2; Hall et al. 2013). How these sensorimotor centers are modulated by social dominance status remains to be investigated.

Interactions between internal state and neuromodulation across vertebrate communication

Additional states, such as seasonal and circadian rhythms also determine when *Xenopus* frogs call (Tobias et al. 2004), though their underlying mechanisms remain poorly understood. Furthermore, parental care behaviors, though not present in *Xenopus*, are common in many vertebrate species. Below we explore additional examples of neuromodulation associated with a given state in a range of behaviors and species.

Neuromodulation of nocturnal vocal fish behavior

Midshipman males typically call at night. This circadian rhythm appears to be endogenous because this behavioral pattern persists when fish are placed in constant darkness. However, placing fish in constant light abolishes this rhythm. Suppression of vocalization by constant light is melatonin dependent; a melatonin analog implant rescued singing under constant light conditions (Feng and Bass 2016). Researchers found that melatonin receptors were expressed throughout sensory, sensorimotor, and motor nuclei, including areas of the hindbrain central pattern generator, suggesting that melatonin may be able to act globally across relevant vocal control regions to regulate call timing.

Seasonal gating of sensory inputs

On a longer timescale, seasonality affects reproductive behaviors in many species. Plainfin midshipman males call during the summer breeding season to attract mates. Inner ear hair cells in females become more sensitive to male calls during the breeding season and this change is mediated by dopamine. Forebrain efferent supplies the inner ear with dopaminergic inputs (Perelmuter et al. 2019). Both the amount of dopaminergic innervation and expression of dopamine receptors decrease in the breeding season, indicating that seasonal regulation of this neuromodulatory system may be used to enhance auditory sensitivity (Perelmuter et al. 2019). In addition to the inner ear, forebrain catecholaminergic neurons send projections to many vocal regions of the forebrain, midbrain, and hindbrain (Forlano et al. 2015). An intriguing possibility is that global changes in dopaminergic signaling promote increased activity across vocal circuits during the breeding season.

Parental status

While parental care is not present in *Xenopus*, it is common across taxa, including some species of frogs. For example, activity of neurons containing the neuropeptide mesotocin (homolog of the mammalian oxytocin) is altered during nursing behaviors (i.e., egg provisioning) in two species of poison frogs (e.g., Fischer et al. 2019a, 2019b). To our knowledge, however, effects of neuromodulators on parental-related communication behaviors have not been described in frogs. In rodents, some forms of parental care are also mediated by oxytocin, including certain communication behaviors. For example, mouse pups produce ultrasonic vocalizations when they are outside their nest to allow their mother to locate them. Experienced mothers respond to ultrasonic vocalizations and retrieve their pups, while female mice who have never been mothers fail to respond to the distress calls. However, when nonmothers are treated with exogenous oxytocin, they begin to respond to and retrieve the calling pups (Pedersen et al. 1982; Marlin et al. 2015). This response is mediated by activation of oxytocin receptors in the auditory cortex, which enhances neuronal and behavioral responses to pup calls (Marlin et al. 2015). Oxytocin is produced in the supraoptic nucleus and paraventricular nucleus of the hypothalamus and regulates the social behavior network (Goodson 2005). While its role in social behaviors is nearly ubiquitous, the expression of oxytocin receptors varies widely across species; thus exploring differences in oxytocin modulation across brain regions may reveal mechanisms of divergent social behaviors (Boender and Young 2020).

Modulation depends on the social status of electric fish

In *Xenopus* neuromodulators that contribute to vocal dominance remain unidentified, but roles of neuromodulators in social hierarchies have been studied in other species. For example, in one species of weakly electric fish, *G. omarorum*, when two males interact for several minutes, a stereotyped dominant–subordinate behavioral pattern emerges: subordinate males (1) interrupt their EOD in order to remain undetected by the dominant male, (2) produce chirps (brief modulation of their EOD), and (3) decrease their EOD rate after the conflict; dominant males, on the other hand, aggressively defend their territory. Interestingly, the hypothalamic neuropeptide AVT affects dominant males differently than subordinate males. AVT exposure increases all three submissive electric behaviors (described above) in subordinate males. In contrast, AVT treatment has no effect on electric communication in dominant males, but blocking V1a AVT receptors does lead to a significant decrease in attacking behavior (Perrone and Silva 2018). In a reduced preparation, AVT applied to the pacemaker nucleus of subordinate males induces chirp-like signals (Comas et al. 2019). These findings support the ability of AVT to act directly on the central pattern generator and influence behavior in an internal state-specific manner.

Conclusions

While it is well established that neuromodulation plays a role in regulating behaviors, much research in vertebrates tends to focus on either a single neuromodulatory system and/or a small number of brain regions. In this review, we described research into neuronal mechanisms that collectively highlight the complex nature of neuromodulation-dependent behavioral regulation and support three broad conclusions: (1) many circuits have been shown to be under the control of several neuromodulatory substances; (2) A single neuromodulator tends to act at multiple levels of behavioral control, including motor circuits; and (3) the function of each neurochemical may vary considerably both within species (dependent on internal state differences) and across species.

Given the complexity of neuromodulation observed in small invertebrate circuits, we should not be surprised by complexity in neuromodulation of vertebrate communication systems. Examples described in this article should serve as reminders that a discovery about the actions of a single neuromodulator in a single brain region should not signal the end of the search for additional neuromodulatory inputs. Likewise, the lack of an effect of a particular substance on a circuit or behavior may only reflect the response during a single internal state, while the substance may have dramatic effects in other physiological contexts.

Our hope in writing this review is that it inspires readers who study behavioral dynamics to reconsider the role of neuromodulators in shaping brain activity, and to cast a wide net. While "one brain region, one neuromodulator" studies are pragmatic starting points in exploring behavioral mechanisms, new high-throughput approaches are making a broader search more manageable. For example, transcriptomics enables an unbiased means of discovering new signaling pathways that may have previously evaded detection using traditional approaches such as drug injection. New techniques that pair monitoring of neuronal activity with functional characterization can reveal previously unidentified regulatory mechanisms. For example, patch-seq (Cadwell et al. 2016)—in which electrophysiological analysis of a single neuron is followed by RNA sequencing of that same cell—could reveal the presence of transcripts for receptors of neuromodulators that had not already been known to influence a certain behavior. In cases where single-cell physiology is impractical, sequencing of transcripts being translated in active neurons (e.g., PhosphoTRAP; Knight et al. 2012) has been a successful approach for discovering signaling pathways associated with behavioral dynamics (e.g., Fischer et al. 2019a, 2019b; Baran and Streelman 2020) and could be adopted for work on communication systems. Finally, while systemic application of agonists and antagonists can tell us about global impacts of neuromodulators, a detailed understanding of a modulator's role requires spatially and temporally precise perturbations (Nusbaum et al. 2017). Expanded use of optogenetics beyond traditional genetic model organisms will enable more precise inquiries into the roles of select neuromodulatory neurons under a wider range of experimental conditions (Hisey et al. 2018). Altogether, we believe a broad search for neuromodulators across circuits and species is likely to reveal a level of complexity beyond that described in this article, and to provide novel insights into the common logic of neuromodulation within communication systems.

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Conflict of interest

The authors declare no competing financial interests.

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