Call initiation in African clawed frogs

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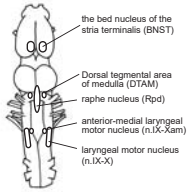
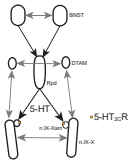
Introduction

Episodic behavior of animals are expressed only when appropriate. For example, escape behavior is expressed when an animal senses danger, and courtship behavior is expressed when the plasma levels of sex steroids are elevated and a potential mate is detected. How does the nervous system initiate episodic behavior? In our study, we use the vocalizations of African clawed frogs (*Xenopus laevis*) to address this question. During the breeding season, a male *Xenopus* generates advertisement calls. Previously, using a fictive preparation in vitro, we discovered that the advertisement call is generated by the central pattern generator (CPG) in the brainstem. The vocal CPG consists of a pair of premotor nuclei (DTAM, also known as parabrachial area) and a pair of laryngeal motor nuclei (n.IX-X) that are interconnected. Previously, we have shown that increasing the extracellular concentration of endogenous serotonin using a selective serotonin reuptake inhibitor elicits fictive advertisement calls by activating 5-HT2C receptors expressed by the anterior-medial n.IX-X[1]. The bed nucleus of the stria terminalis (BNST), a nucleus in the extended amygdala, directly projects to the dorsal raphe nucleus[2]. A previous study showed that electrical stimulation of BNST elicits fictive advertisement calls *in vitro*, and lesioning of BNST decreases calling behavior *in vivo* in male *X. laevis*[3]. Based on these results, we hypothesize that advertisement calls of male *X. laevis* are initiated by BNST activating raphe nucleus, which releases serotonin that binds to 5-HT2C receptors expressed by anterior-medial n.IX-X (nIX-Xam). To test this hypothesis, we obtained local field potential recordings (LFP) from n.IX-Xam.

The central vocal pathways of *Xenopus laevis*



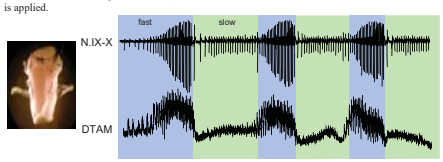
Hypothesized mechanism of call initiation



Black arrows indicate hypothesized pathways for vocal initiation. Gray arrows indicate anatomical connections between nuclei.

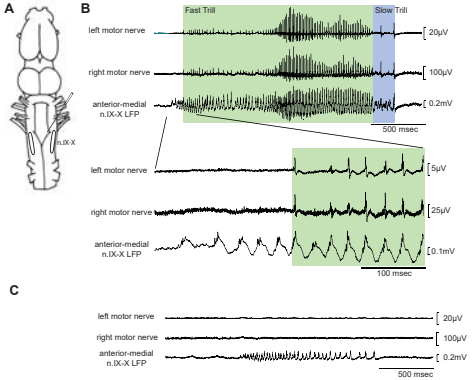
The Central Pattern Generator (CPG)

Fictive vocalizations (top trace, below) can be elicited from an isolated brainstem (left) *in vitro* when serotonin is applied.

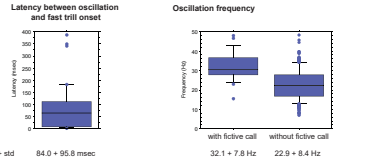


Question: How are vocalizations initiated in the brains of male *Xenopus laevis*?

Anterior-medial n.IX-X generates oscillatory activity that precedes the onset of fictive fast trills.

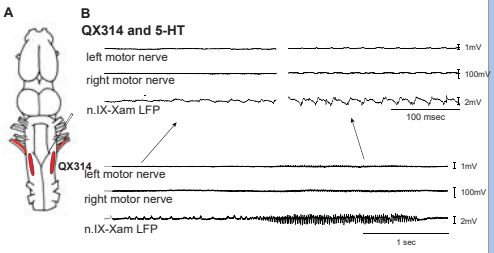


Oscillations in anterior-medial n.IX-X are not always accompaneid by laryngeal motor activity.



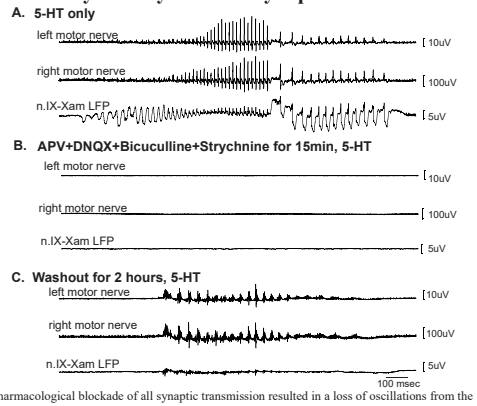
When local field potential (LFP) was obtained from the anterior-medial n.IX-X (n.IX-Xam) of a brain (A) in response to 5-HT, oscillatory activity (B, top traces) was recorded that largely coincided with the fictive advertisement calls. Although the oscillation was phase-locked to motor CAPs during fictive calls, the onset of the oscillation typically preceded the onset of the fictive advertisement calls (B, bottom traces) by ~80msec on average (D left). Oscillatory activity that preceded the fictive calls had a mean frequency of ~30Hz (D right). Furthermore, the oscillatory activity was sometimes recorded in the absence of the fictive advertisement calls (C) with initial oscillatory frequency similar to those that accompany fictive calls (D, right), although the amplitude is smaller. We hypothesize that the initial part of these oscillation in the n.IX-Xam represents neural activity that initiates advertisement calls in male *X. laevis* brains.

Laryngeal motoneuron activity is not critical for generating 5-HT-induced oscillations in the anterior-medial n.IX-X.



To determine if 5-HT induced oscillations in the anterior-medial n.IX-X depend on motoneuron activity, we applied QX-314 (an intracellular Na+ channel blocker) to the laryngeal motor nerves bilaterally and selectively blocked the activity of all the laryngeal motoneurons (A). Application of 5-HT still elicited oscillations from the anterior-medial n.IX-X (B) although the amplitude of the oscillations was smaller compared to those obtained from control brains.

Oscillatory activity relies on synaptic transmission.



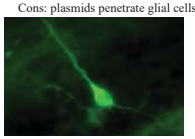
Pharmacological blockade of all synaptic transmission resulted in a loss of oscillations from the anterior-medial n.IX-X indicating that the activity is not mediated by pacemaker-like neurons responding directly to 5-HT.

Techniques: Transgene expression in *Xenopus* nervous system.

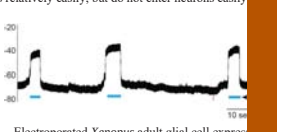
Optogenetic tools have revolutionized the field of neuroscience in recent years. Howev these tools have not been available for Xenopus laevis because it is not a genetic model To overcome this obstacle, we have been developing a reliable technique to express tra at high levels in Xenopus central vocal pathways. Specifically, our goal is to express optogenetic actuators (i.e., channelrhodopsin, halorhodopsin) and sensors (i.e., GCamP voltage-sensitive fluorescent proteins) in the Xenopus vocal neurons using either target electroporation or viral injection techniques.

1. Electroporation of plasmids into the Xenopus nervous system

Pros: plasmid can be easily obtained, fast gene expression
Cons: plasmids penetrate glial cells relatively easily, but do not enter neurons easily



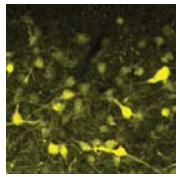
Electroporated *Xenopus* adult neuron expressing EGFP.



Electroporated *Xenopus* adult glial cell expres that depolarize in response to blue light.

2. Recombinant adeno-associated virus (rAAV) injection ino the Xen nervous system

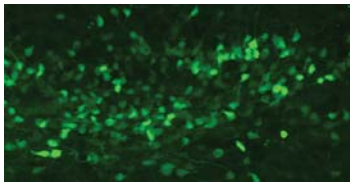
Pros: rAAV with a variety of transgenes can be readily purchased
Cons: low success rates in Xenopus adult brains (less than 10%), take a long time to (>3 weeks)



Xenopus adult neurons expressing reporter gene (EYFP) after injection of pAAV-hS hChR2(H134R)-EYFP.

3. Vesicular stomatitis virus injection into the Xenous nervous system

Pros: fast expression (6 hours at room temperature), trans-synaptic virus with presu direction-specificity (anterograde or retrograde) .
Cons: BSL2, appear to have high toxicity to neurons.



Xenopus adult neurons expressing reporter gene (EGFP) after injectio of VSV-G VSV

Conclusions

We conclude that there is a population of neurons medial to the anterior n.IX-X that likely play a role in call initiation in response to 5-HT in the *Xenopus* brainstem. Their oscillatory activity does not depend on the laryngeal motoneuron activity; the oscillations are elicited in response to 5-HT even in the absence of laryngeal motoneuron activity, or when the motoneurons are selectively silenced. In addition, these neurons are unlikely to show intrinsic oscillatory activity directly in resonse to 5-HT because pharmacological blockade of synaptic transmission blocks the 5-HT-induced oscillation. The anterior-medial n.IX-X expresses 5-HT$_{2C}$ receptors that are known to initiate fictive vocalizations *in vitro*. Thus, we suggest that activation of 5-HT$_{2C}$ receptors by the neurons in IX-Xam results in oscillatory activity that initiates the fictive vocalizations in ~80msec. We plan to use otogenetic tools (shown on the right column of this poster) to dissect the call initiation pathways further in the future.

References

[1] Yu and Yamaguchi (2010) Endogenous serotonin acts on 5-HT2C-like receptors in key vocal areas of the brain stem to initiate vocalizations in Xenopus laevis. J. Neurophysiol 103: 648-658.
[2] Moreno et al. (2012) Characterization of the bed nucleus of the stria terminalis in the forebrain of anuran amphibians. J Comp Neurol. 2012 Feb 1;520(2):330-63.
[3] Hall IC, Ballagh IH, Kelley DB (2013) The Xenopus amygdala mediates socially appropriate vocal communication signals. J Neurosci. 33:14534-48.

Funding: NSF 1146501, NSF IOS-1146501, 1557945, Startup funds from the University of Utah, Department of Biology and USTAR.