Distinct Neural Control of Vocal Phases in Frog Calls

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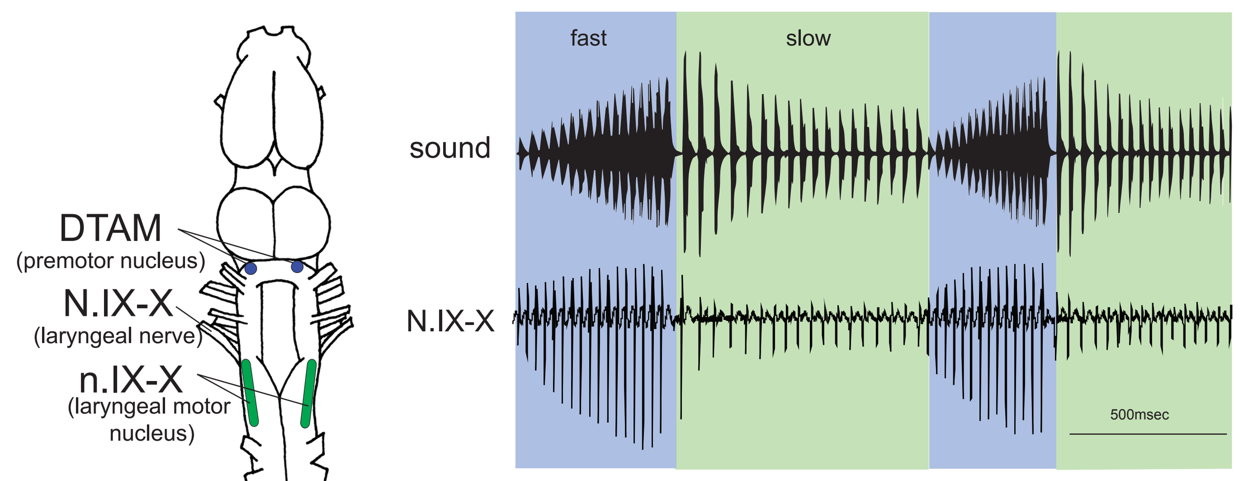
Introduction

The behavior of animals is comprised of a variety of motions. How are the motions underlying a functional behavior generated by the nervous system? We used the vocalizations of African clawed frogs (*Xenopus laevis*) to address this question. During the breeding season, a male *Xenopus* generates advertisement calls that consist of alternating fast and slow trill phases. Fast and slow vocal trill phases contain a series of clicks repeated at 70 and 30 Hz, respectively. 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) and a pair of laryngeal motor nuclei (n.IX-X) that are each interconnected. In this study, we examined how these nuclei contribute differently to the generation of fast and slow trill rhythms.

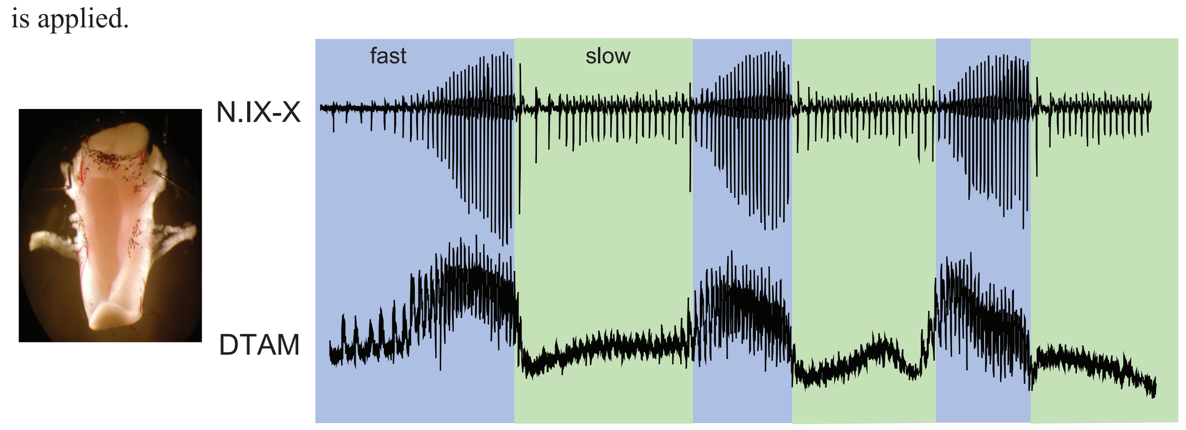
The Advertisement Call of *Xenopus laevis*



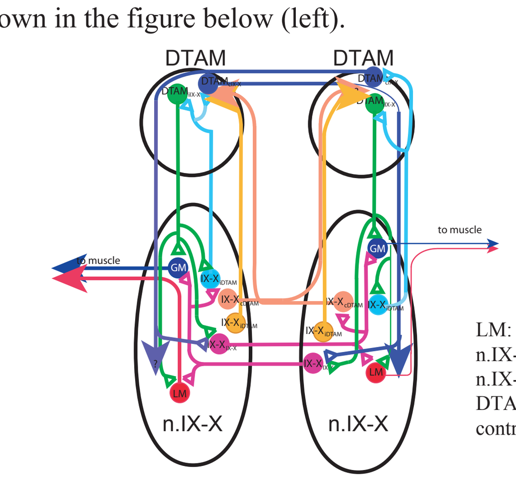
Xenopus advertisement calls consist of repetitive clicks that alternate between fast (70Hz) and slow (30Hz) rates (top trace, sound). The temporal organizations of the call are dictated entirely by motor nerve activity (bottom trace, N.IX-X). Motor command, in turn, is generated by the central pattern generator in the brainstem that consists of the dorsal tegmental area of medulla (DTAM, premotor nucleus, shown in red) and laryngeal motor nucleus (n.IX-X, shown in green).

The Central Pattern Generator (CPG)

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



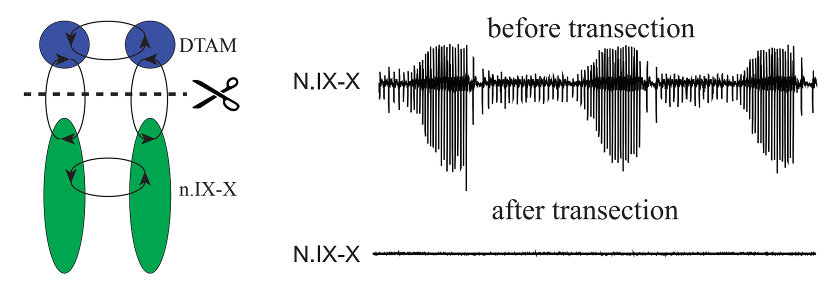
The central pattern generator that generates vocalizations consist of a pair of dorsal tegmental area of medulla (DTAM, premotor nucleus) and a pair of laryngeal motor nuclei (n.IX-X). There are extensive reciprocal connections, including bilateral, descending, and ascending projections, among the DTAMs and n.IX-Xs as shown in the figure below (left).



LM: laryngeal motoneurons, GM: general visceral motoneurons, IX-XIX-X: n.IX-X projecting n.IX-X neuron, IX-XiDTAM: ipsilateral DTAM rojecting n.IX-X neuron,IX-XcDTAM: contralateral DTAM projecting n.IX-X neuron, DTAMiIX-X: ipsilateral n.IX-X projecting DTAM neuron, DTAMcDTAM: contralateral n.IX-X projecting DTAM neuron.

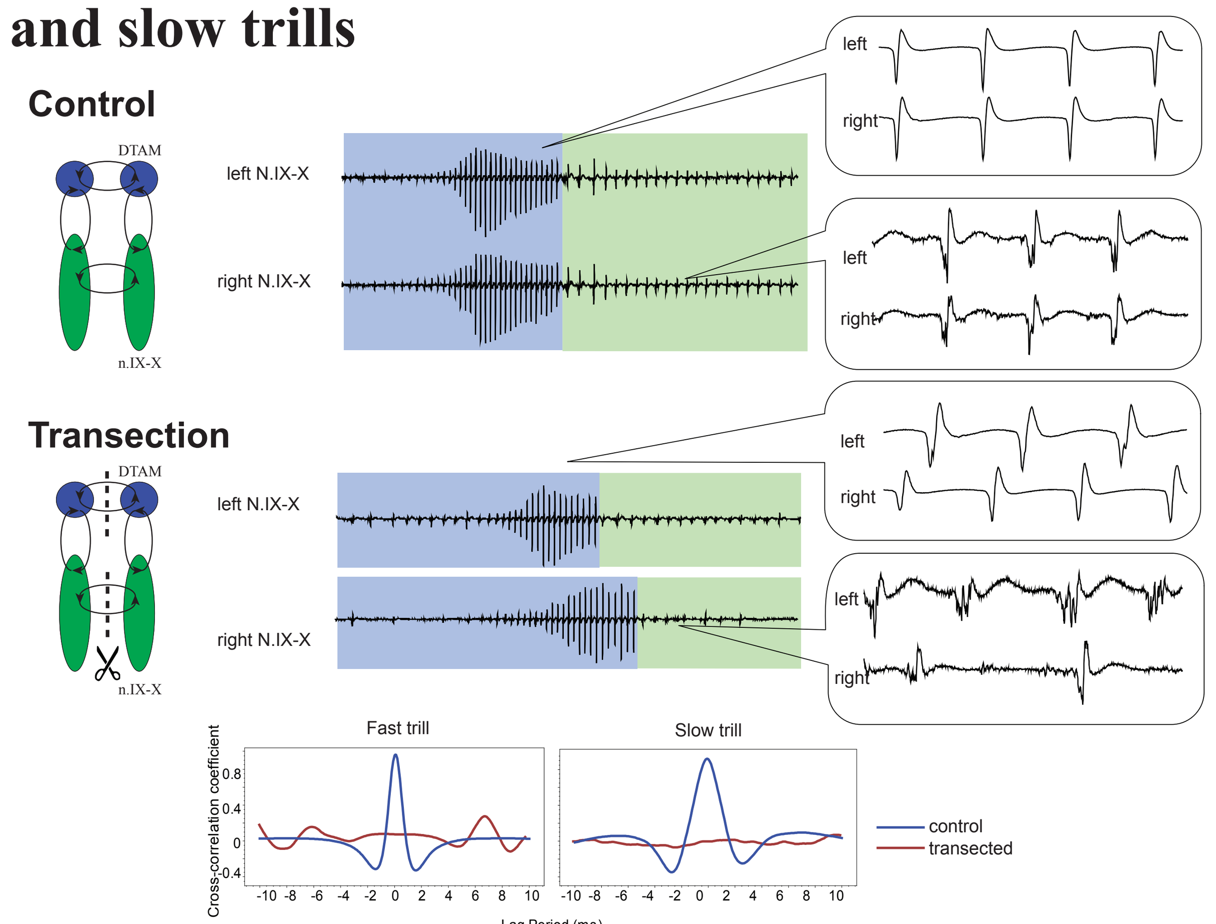
Question: How are two distinct vocal rhythms initiated and generated by the central pattern generator?

The connections between DTAM and n.IX-X are critical for the activation of both fast and slow trills



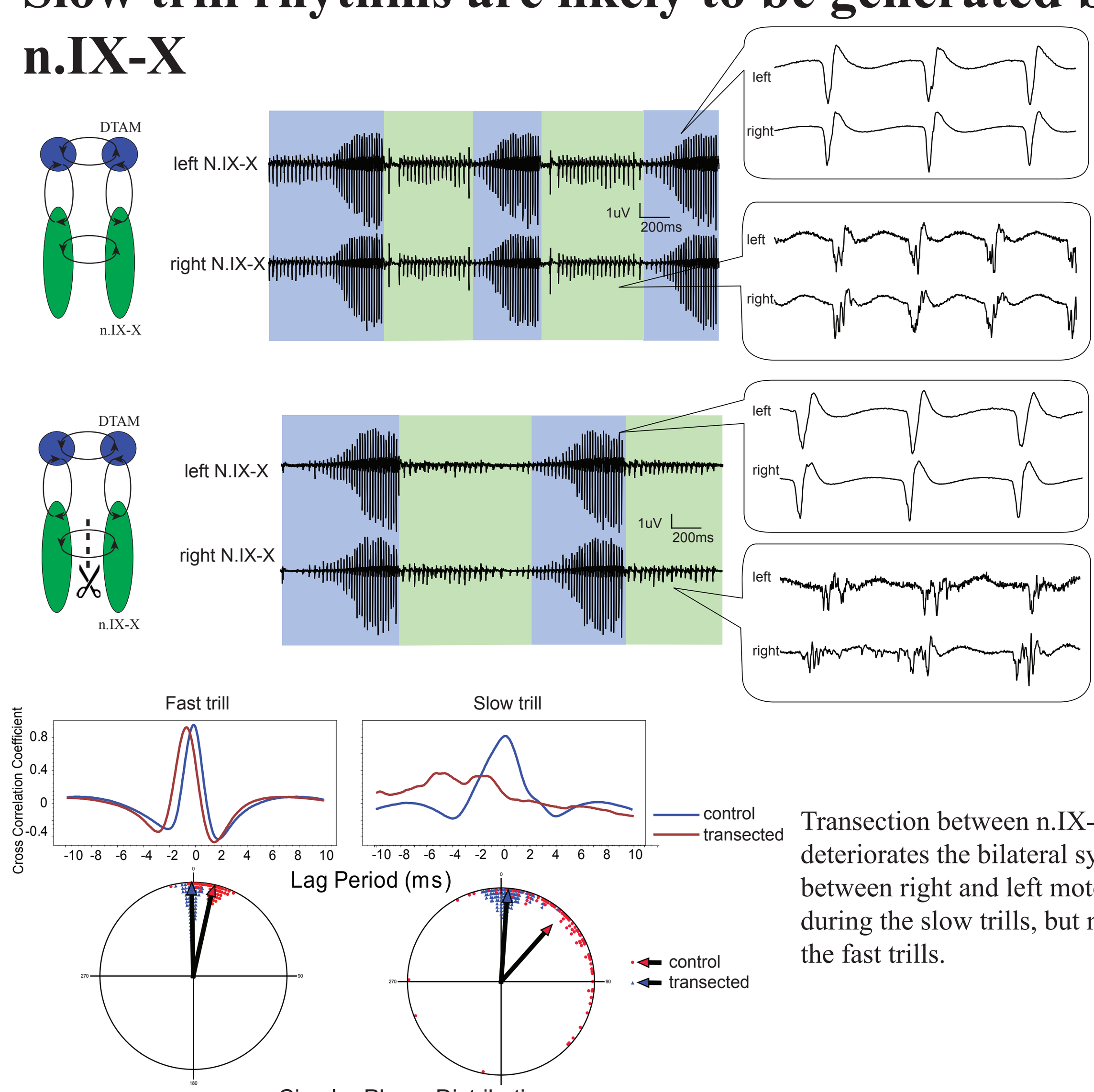
The application of serotonin to isolated brains that were bilaterally transected between DTAM-n.IX-X *never* resulted in fast or slow trills.

Hemi brainstem is sufficient to generate both fast and slow trills



When the connections between left and right DTAMs and left and right n.IX-X are transected, each hemi brainstem generated both fast and slow trills, although the rhythms generated by each half are autonomous, as evident in the low cross correlation coefficient between left and right nerve traces during both fast and slow trills.

Slow trill rhythms are likely to be generated by n.IX-X

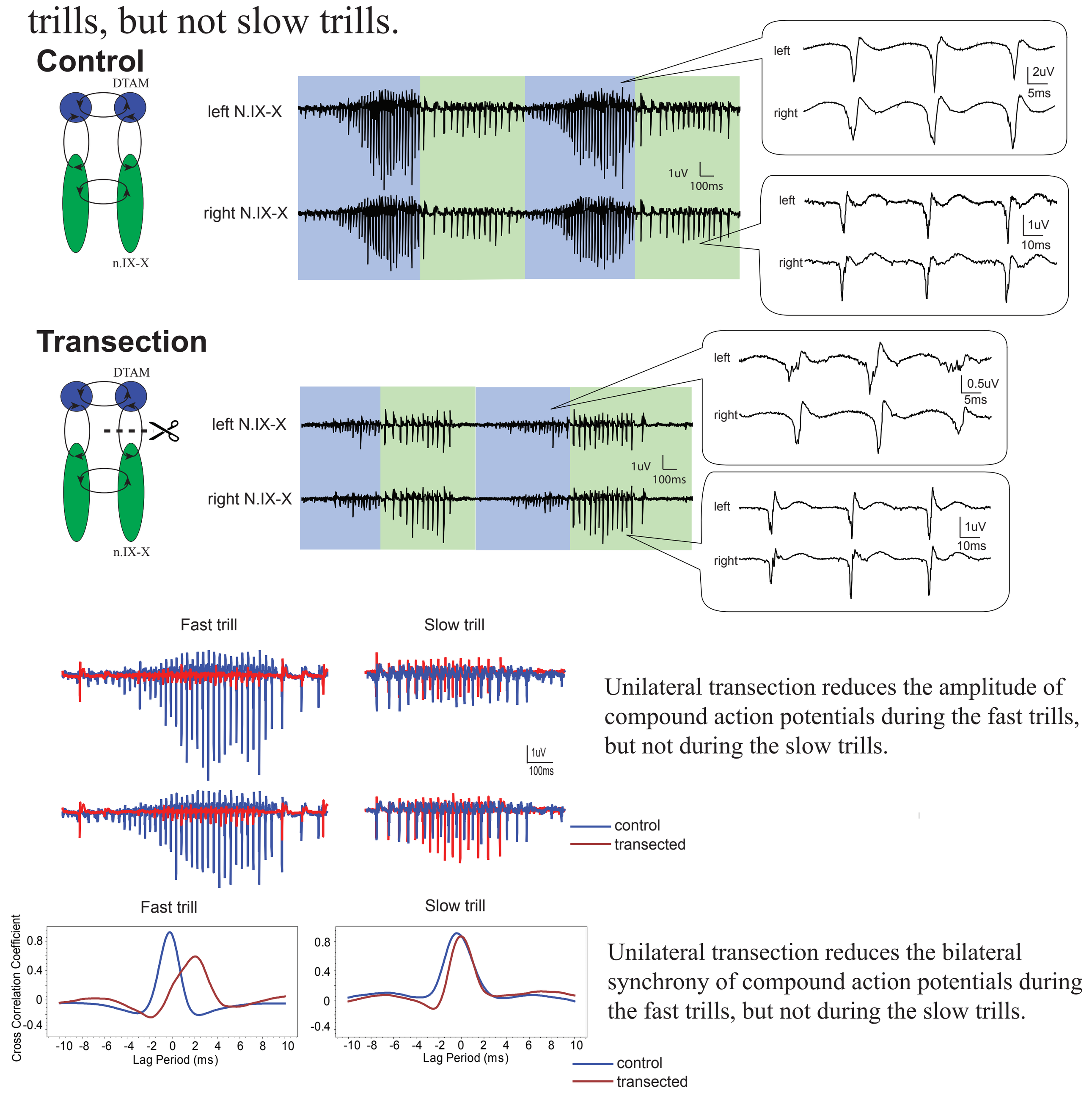


Transection between n.IX-X deteriorates the bilateral synchrony between right and left motor nerves during the slow trills, but not during the fast trills.

Given the selective effect of the transection on the slow trill synchrony, it is likely that the right and left n.IX-X function together to generate slow trill rhythms.

Fast trill rhythms are likely to be generated by in DTAM

1. Unilateral transection btw DTAM and n.IX-X deteriorates fast trills, but not slow trills.

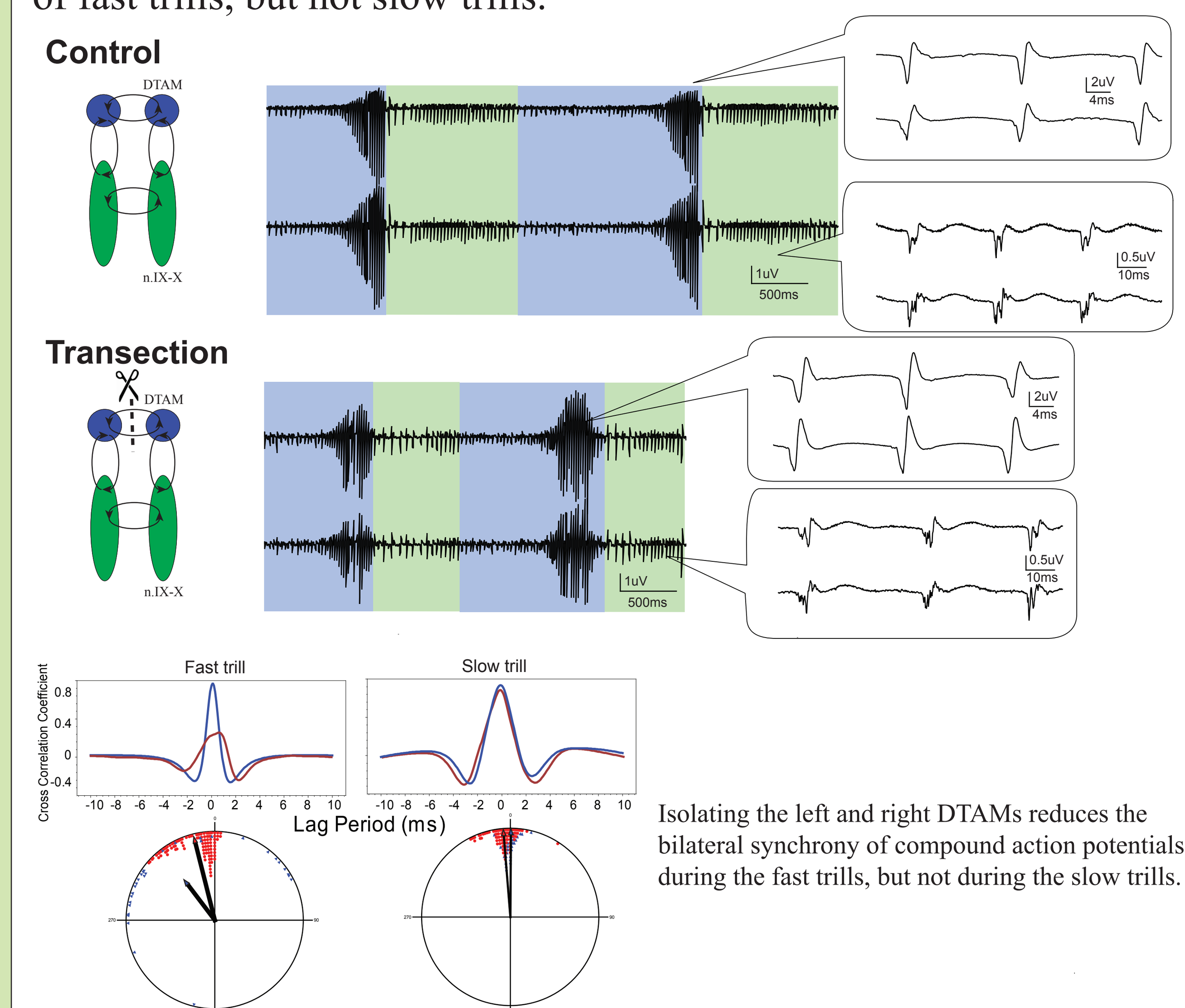


Unilateral transection reduces the amplitude of compound action potentials during the fast trills, but not during the slow trills.

Unilateral transection reduces the bilateral synchrony of compound action potentials during the fast trills, but not during the slow trills.

When the connections between DTAMs and n.IX-X are transected unilaterally, fast trill became deformed drastically while slow trill remained unchanged. The compound action potential (CAPs) recorded from both right and left nerve became significantly reduced in amplitude, and their timing became desynchronized. CAPs during slow trill, in contrast, were similar to those recorded from intact brains.

2. Transection between DTAMs deteriorates the bilateral synchrony of fast trills, but not slow trills.

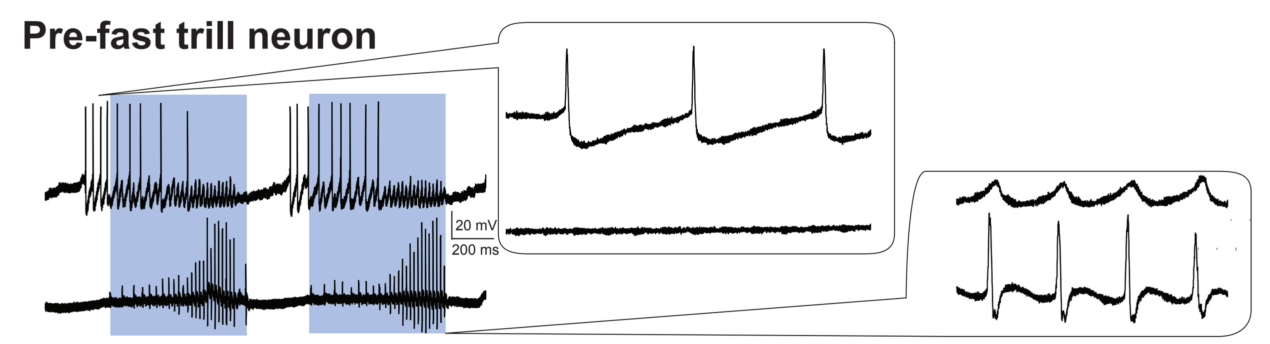


Isolating the left and right DTAMs reduces the bilateral synchrony of compound action potentials during the fast trills, but not during the slow trills.

Selective deterioration of fast trills in these experiments suggests that left and right DTAM function together to generate fast trill rhythms and drive the motoneurons.

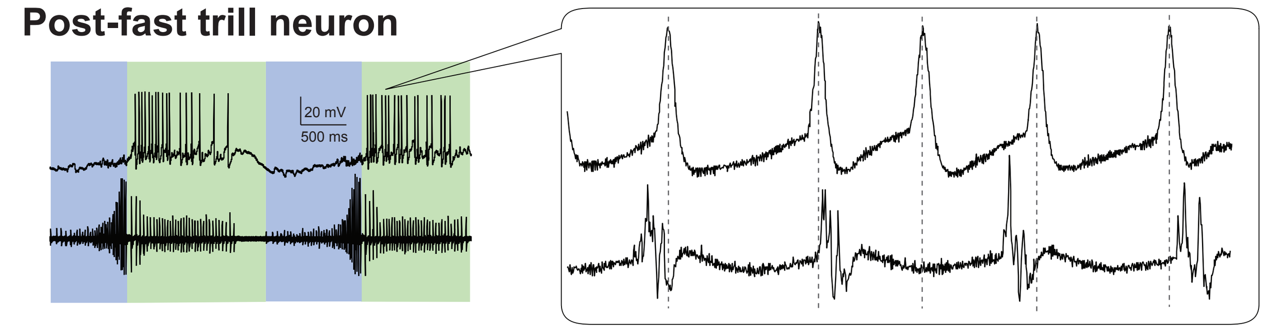
Vocal phase initiators: Neurons that could initiate fast and slow trills are found in DTAM

Pre-fast trill neuron



Pre-fast trill neurons spike before the onset of the fast trill is detected on the laryngeal nerve. As the fast trill progresses, the neuron stops spiking.

Post-fast trill neuron

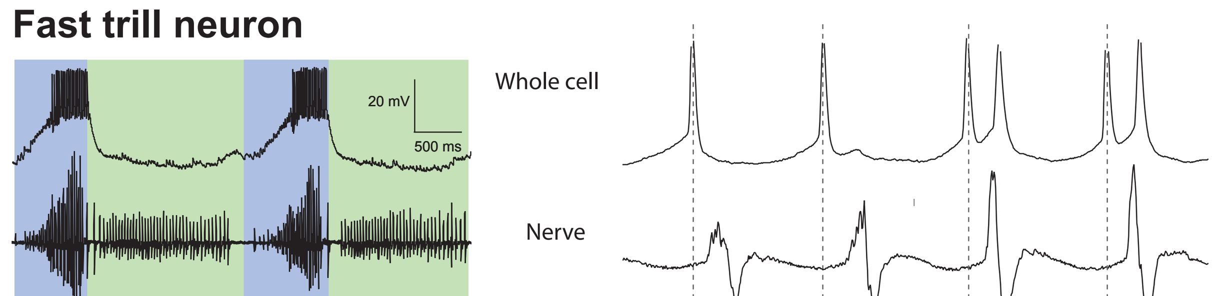


Post-fast trill neurons spike after the fast trill, even in the absence of slow trills. The spikes of the neuron has little to do with the timing of the slow trills.

These neurons may initiate both vocal phases, and may explain why no vocalizations can be activated in the absence of functional DTAMs.

Vocal rhythm generators: Neurons likely to be the fast trill rhythm generators are found in DTAM

Fast trill neuron



Fast trill neurons spike action potentials selectively during the fast trill, and each spike precede the compound action potential by ~2msec.

The fast trill neurons (FTNs) likely act as rhythm generators for the fast trill. Our previous results suggest that FTNs do not generate endogenous rhythms autonomously, but require ascending feedback from the n.IX-X. As for the slow trill rhythm generator neurons, we are currently in search for such neurons in the n.IX-X.

Conclusions

The biphasic phases of male *X. laevis* vocalizations appear to be activated by the "initiator" neurons in DTAM. Once the vocal phase is activated, fast trill rhythms seem to be generated by fast trill neurons in DTAM that receive input from both the n.IX-X and the ipsilateral DTAM. The slow trill rhythms are likely to be generated by inter-neurons in n.IX-X that interact with the contralateral n.IX-X. Phylogenetically, biphasic vocalizations are a more "derived" trait within the genus *Xenopus* , and many other species produce monophasic calls that resemble slow trills of X. laevis. Thus, understanding how neural circuits accommodate two vocal rhythms may provide us with an insight into the neural mechanisms underlying the acquisition of new behaviors over evolutionary time.

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