Neurocircuitry underlying vocal production of the African clawed frog, *Xenopus laevis*

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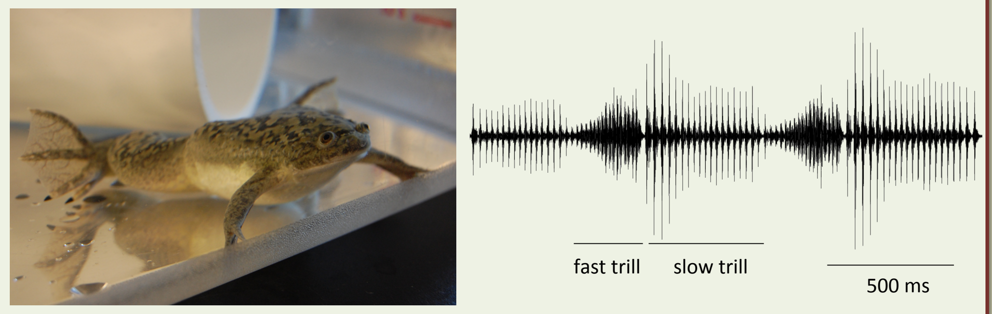
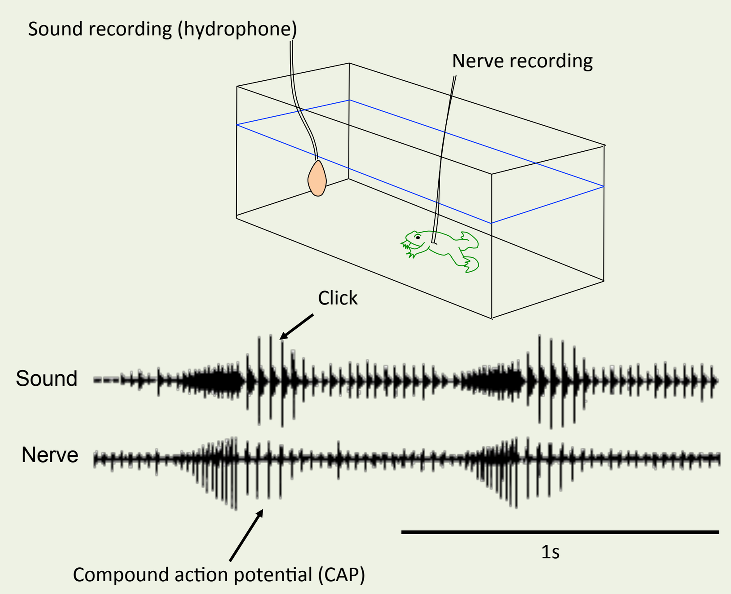
Xenopus vocal behaviors

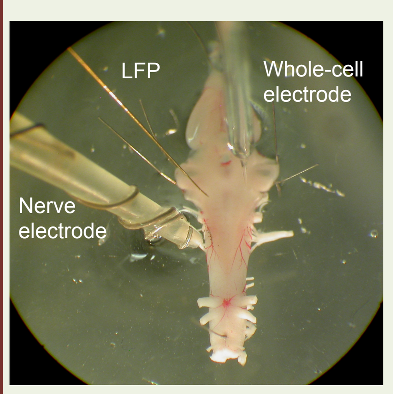


African clawed frogs, *Xenopus laevis*, are fully aquatic and call underwater during courtship. Calls consist of clicks produced by the larynx at various temporal patterns. This work focuses on the male advertisement call (see oscillogram above); this call is defined by alternating fast (~60 Hz) and slow (~30 Hz) click trills.



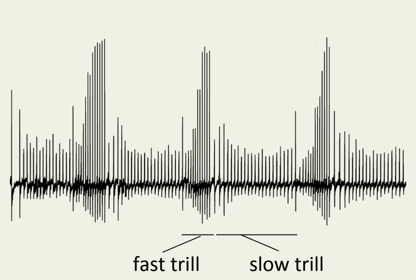
Nerve recordings from awake, calling frogs (depicted above, bottom trace), revealed that a single compound action potential (CAP) precedes every vocal click (above left; Yamaguchi and Kelley, 2000). This means that vocal patterns are produced by the brain, and that nerve activity provides a direct readout of the behavior.

The isolated brain



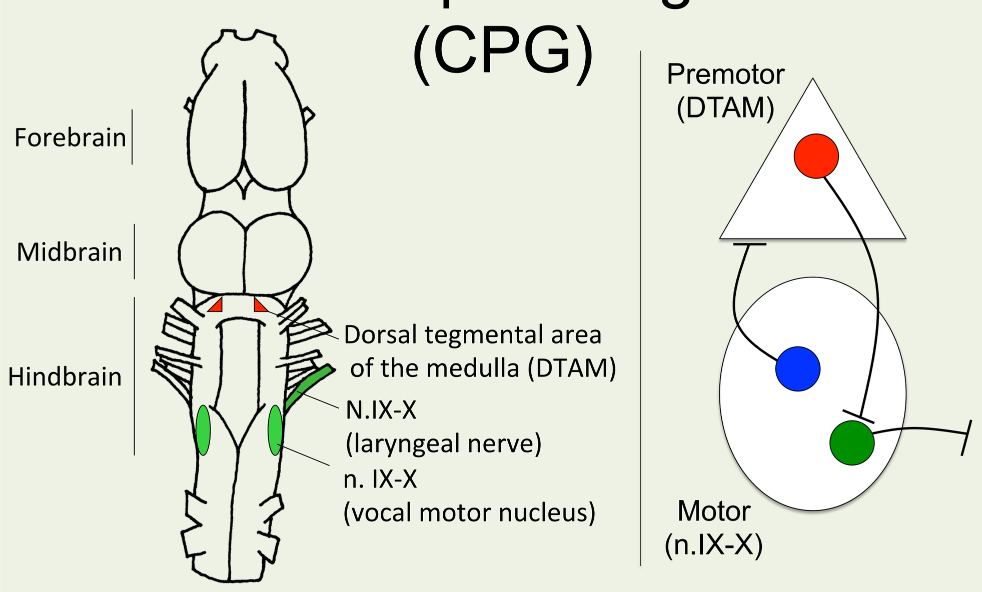
We use an *ex vivo* brain to record multiple regions. The photo shows a local field potential (LFP) electrode (left DTAM), whole-cell patch electrode (right DTAM), and a suction electrode on the vocal nerve.

The vocal CPG remains viable *in vitro*. Serotonin application elicits nerve activity that is similar to what has been recorded during *in vivo* calling. We are using the "fictively" calling isolated brain to discover how vocal patterns are generated.



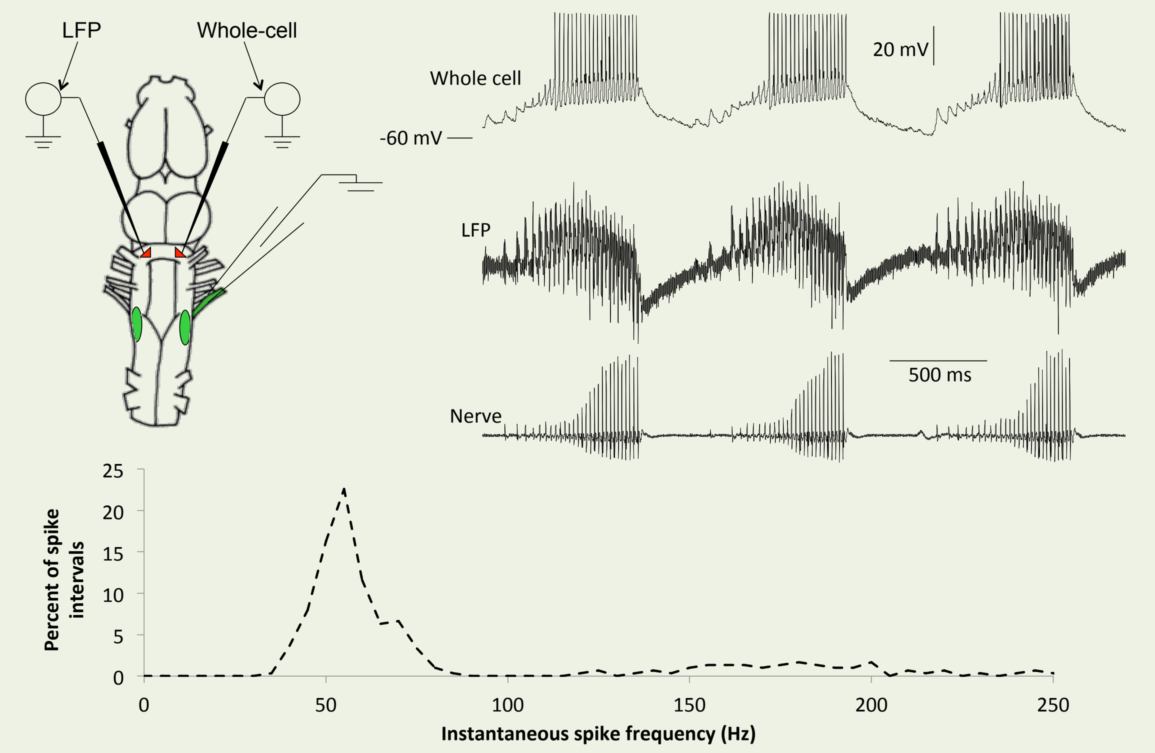
Above: a nerve recording during "fictive vocalization". Compound action potentials occur in distinct, alternating, fast and slow trills.

Vocal central pattern generator (CPG)



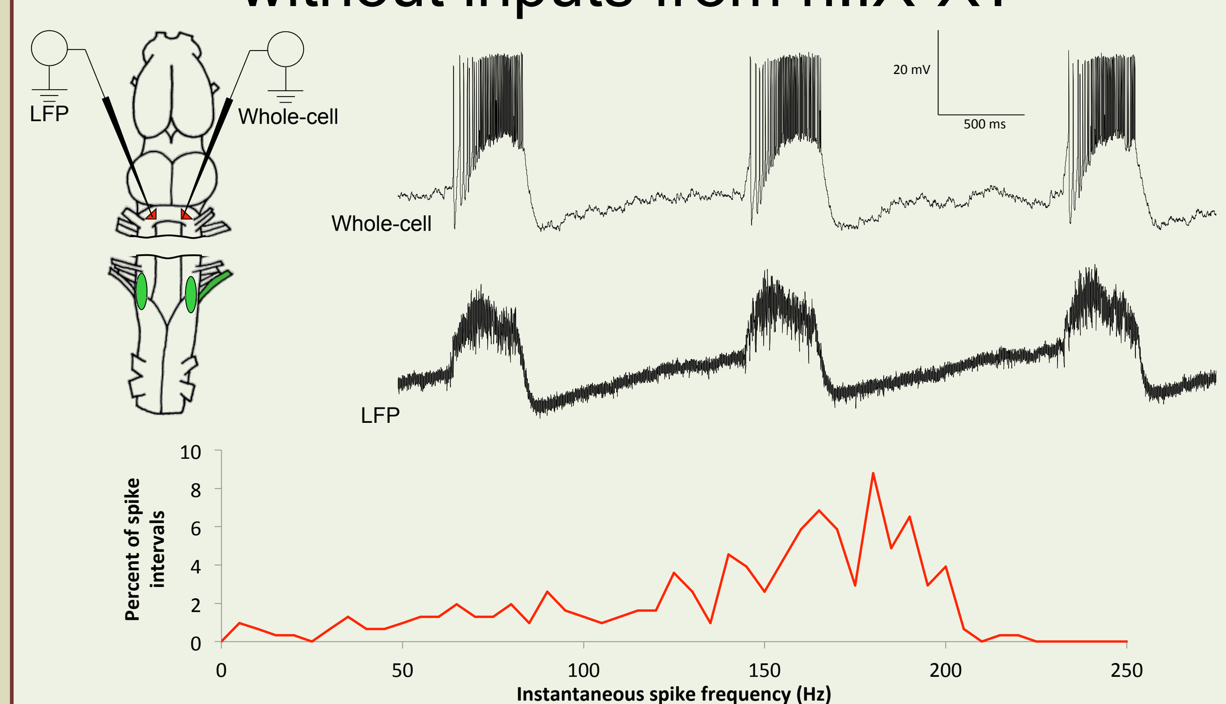
Two brainstem nuclei comprise the vocal CPG: a premotor nucleus (DTAM) and the laryngeal motor nucleus (n.IX-X). DTAM and n.IX-X are connected by reciprocal projections.

Generating vocal rhythms: fast trill neurons (FTNs)



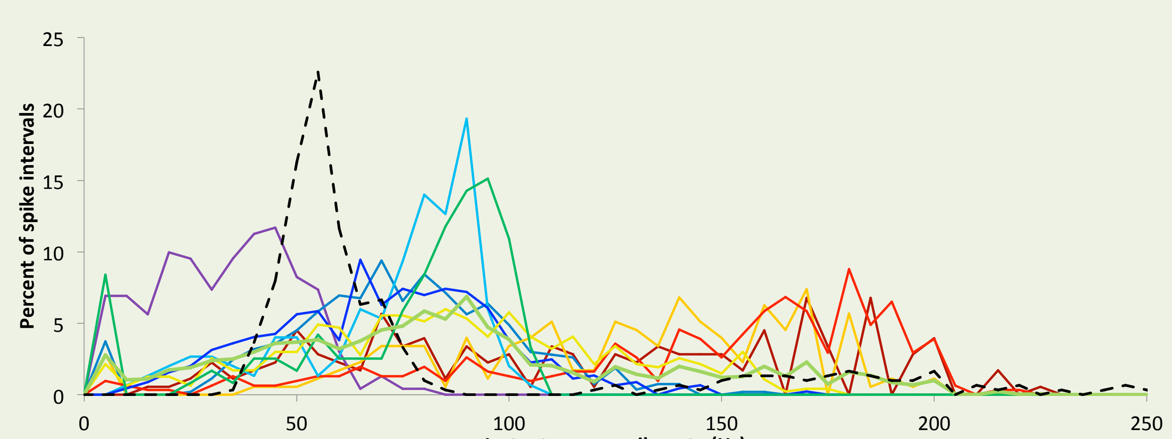
- Nerve recordings: fictive advertisement calls (bottom trace) consist of nerve CAPs that occur at advertisement call rates.
- LFP recordings: slow baseline waves are correlated with fast trills; higher frequency activity is phase-locked to nerve CAPs (middle trace).
- Whole-cell recordings: "fast trill neurons" (FTNs; top) produce spikes phase-locked to nerve CAPs near the fast trill rate of 60 Hz (spike histogram; Zornik and Yamaguchi, 2012).

Can FTNs generate fast trill rhythms without inputs from n.IX-X?



- When the connections between DTAM and n.IX-X are cut, LFP waves still occur with 5-HT application (lower trace).
- Unlike intact recordings, no fast trill rhythms occur during the wave (lower trace).
- FTNs depolarize and spike throughout each wave (top trace).
- Instantaneous spike rates of this cell were faster than FTN spike rates in the intact CPG (spike histogram).

n.IX-X inputs synchronize FTNs



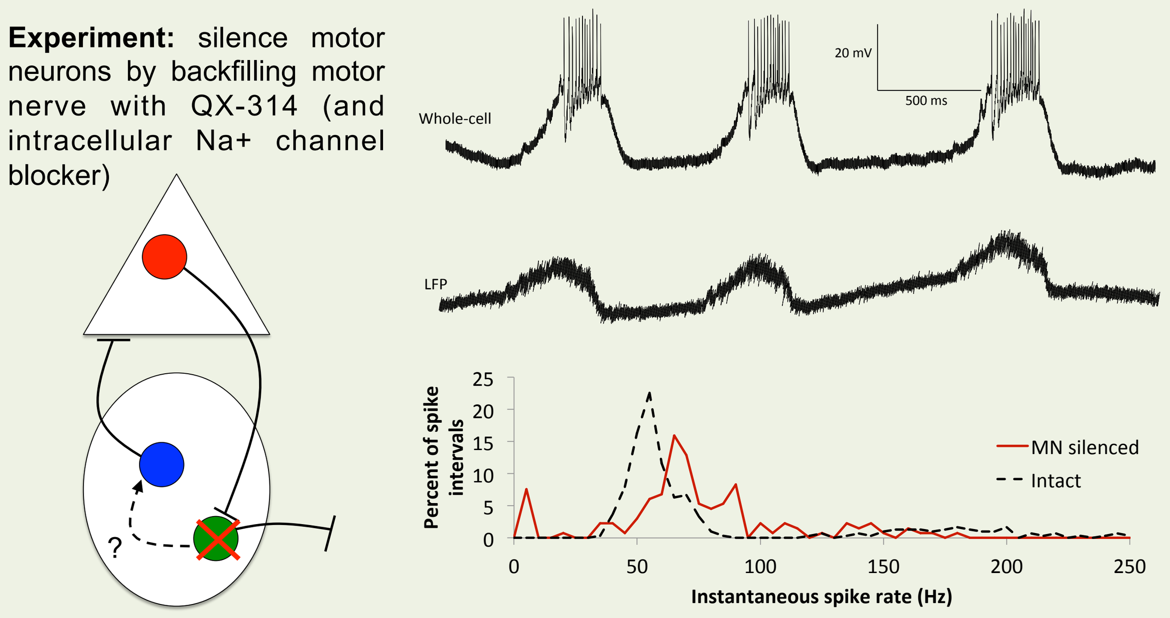
- In the intact CPG: FTNs largely spike at fast trill rates of ~50 – 60 Hz (example shown in dotted black line).
- After transections, FTN spike frequencies are more broadly tuned (each solid line represents the spike rates of one FTN).

Conclusion 1: Feedback inputs from n.IX-X to DTAM entrain and synchronize FTN firing, ensuring proper production of fast trill rhythms

Identifying the feedback circuit

Is motor neuron activity involved in generating the feedback signal?

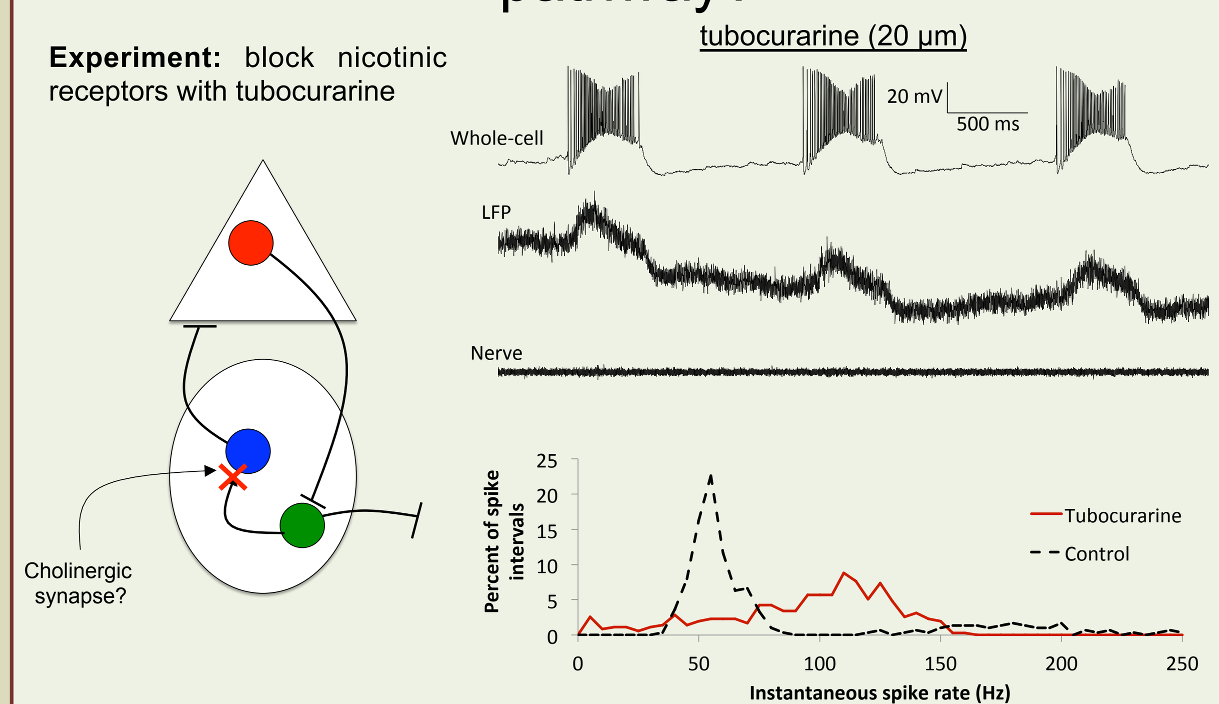
Experiment: silence motor neurons by backfilling motor nerve with QX-314 (and intracellular Na+ channel blocker)



- Silencing motor neurons disrupts fast trill rhythms in FTNs.

How do motor neurons activate feedback pathway?

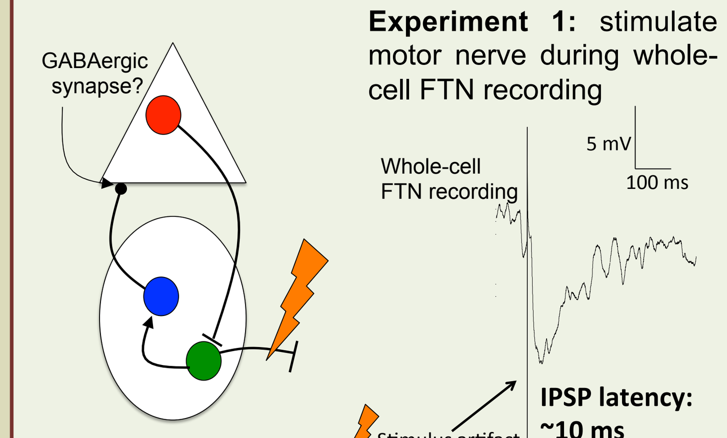
Experiment: block nicotinic receptors with tubocurarine



- Blockade of nicotinic acetylcholine receptors induces LFP waves, but 60 Hz fast trill rhythms are lost.
- FTNs spike faster than in the intact circuit, similar to transection experiments.

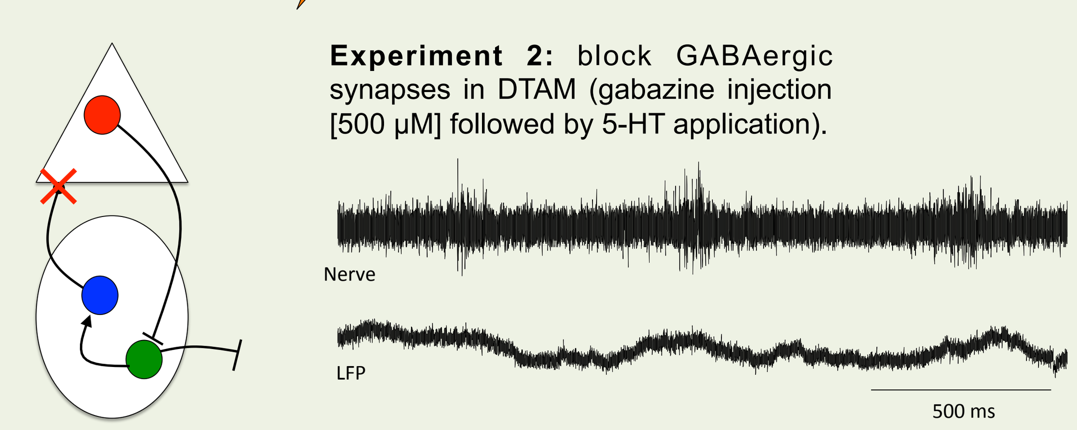
Does the feedback signal synchronize FTNs via inhibition?

Experiment 1: stimulate motor nerve during whole-cell FTN recording



- Nerve stimulation results in short-latency IPSPs in FTNs

Experiment 2: block GABAergic synapses in DTAM (gabazine injection [500 μM] followed by 5-HT application).

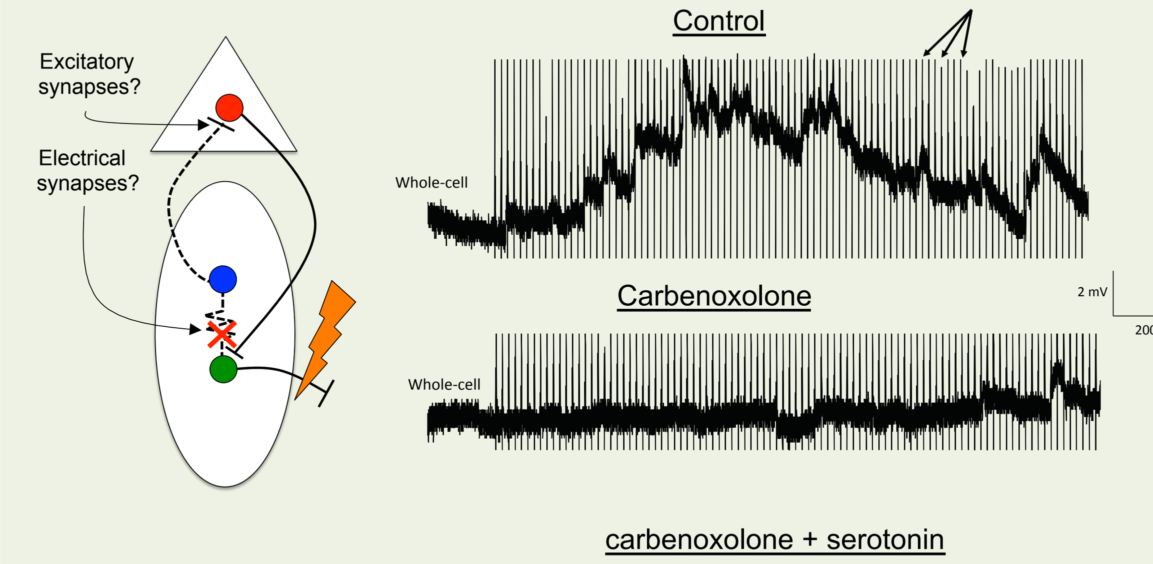


- Blocking GABA-A receptors in DTAM results in abnormal fictive vocal behaviors.
- Nerve and LFP activity lack correlates of synchronous fast trill rhythms.
- This supports the hypothesis that synchronous premotor activity requires inhibitory feedback inputs.

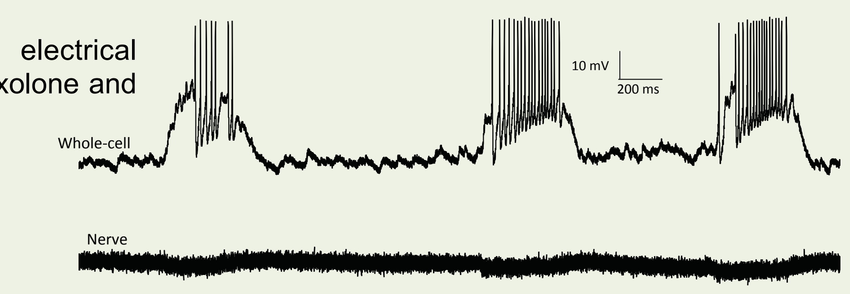
Conclusion 2: FTNs are entrained by feedback inhibition. These signals arise as an efference copy of motor output, and are transduced through an intervening inhibitory interneuron.

Do feedback signals include excitatory inputs?

Experiment 1: block electrical synapses with carbenoxolone while stimulating the vocal nerve.

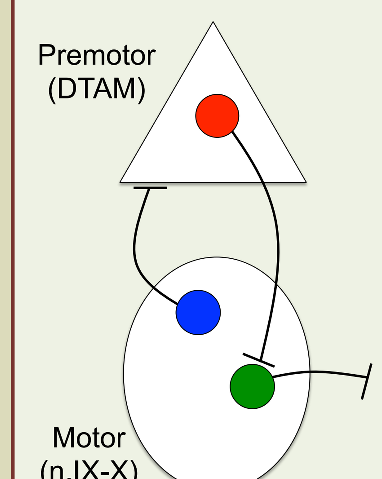


Experiment 2: block electrical synapses with carbenoxolone and apply serotonin.



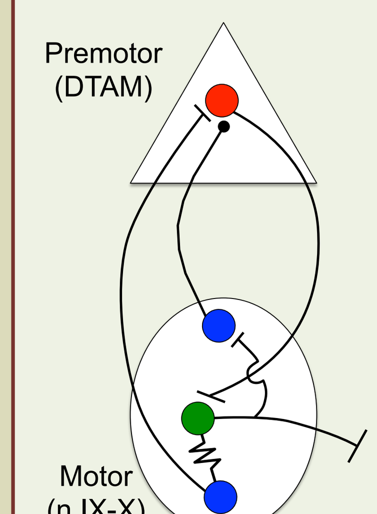
- Stimulating the motor nerve at fast trill rates (60 Hz) induces EPSP summation; blockade of gap junctions eliminates these EPSPs.
- Blockade of electrical synapses abolishes fictive advertisement calls and induces fast FTN spiking.
- The results support the hypothesis that ascending excitatory feedback from n.IX-X to DTAM mediated by electrical synapses are critical for the generation of advertisement calls.

A model of the vocal CPG



Previous model:

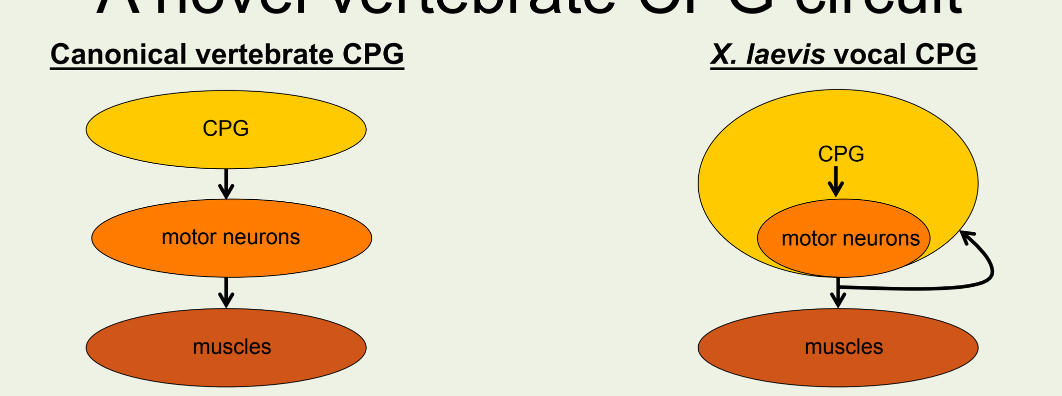
1. FTNs directly activate vocal motor neurons (Zornik and Kelley, 2007).
2. n.IX-X interneurons project to DTAM (Zornik and Kelley, 2008), transmitter unknown
3. FTNs receive phasic IPSPs, but the source is unknown (Zornik and Yamaguchi, 2012).



Updated model:

1. Motor neurons provide an efference copy of their activity to interneurons in n.IX-X.
2. n.IX-X interneurons are activated by motor neurons via a cholinergic synapse.
3. Feedback inhibition entrains and synchronizes FTN spiking, resulting in fast trill rhythm generation.
4. Without feedback inhibition, FTNs spike at faster and broader range of rates (as observed following transection, motor neuron silencing, and blockade of nicotinic receptors)
5. Motor activity also induces an excitatory feedback signal to contralateral FTNs that requires gap junctions.

A novel vertebrate CPG circuit



Vertebrate CPGs are thought to function in a top-down manner: CPG output activates motor neurons that, in turn, induce muscle activity. Motor neurons play little, if any, role in generating behavioral rhythms.

The *Xenopus* vocal CPG is an exception to this top-down rule. Motor neurons act as a critical component of the vocal CPG by entraining the activity of premotor neurons.