Ionotropic Receptors

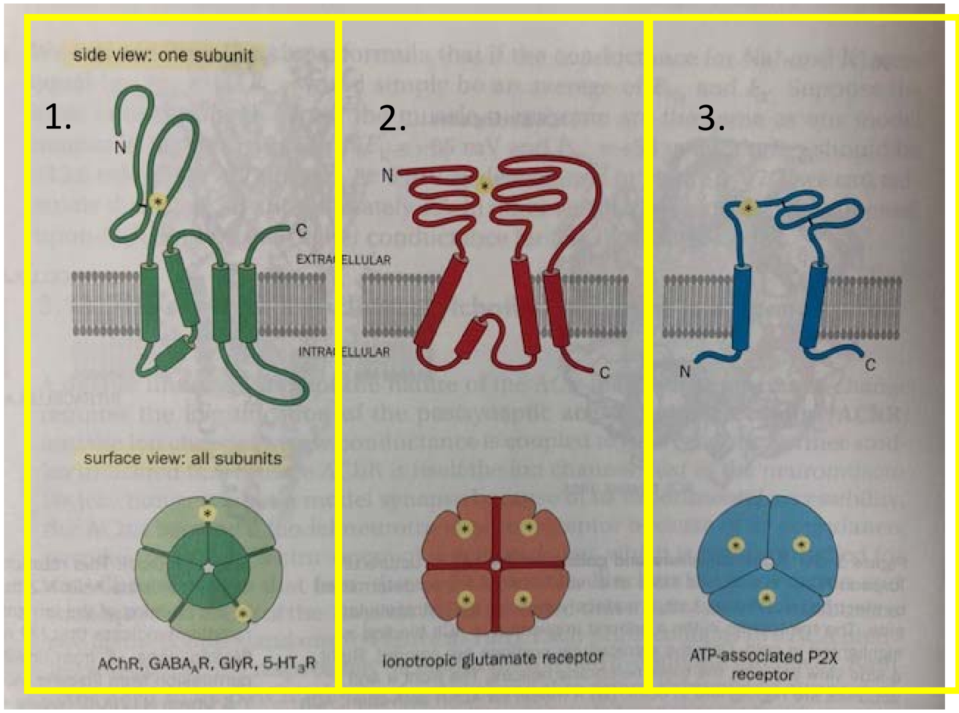
Emily Moriarty Lemmon

Ion Channels that are also Neurotransmitter Receptors

- Metabotropic neurotransmitter receptors
  - Ligand-gated (neurotransmitter) ion channels
  - Binding triggers intracellular signaling cascades to regulate conductance and and modulate membrane potential indirectly
  - Extra-synaptic
- Ionotropic neurotransmitter receptors
  - Ligand-gated (neurotransmitter) ion channel
  - Fast-acting for rapid communication across synapse (~few milliseconds)
  - Within synapse

Types of Ionotropic Receptors

- Three subfamilies of ionotropic receptors
  1. AChR/GABA/Glycine/Serotonin-gated (5 subunits, 4 transmembrane segments)
  2. Glutamate-gated (4 subunits, 3 transmemb.)
  3. ATP-gated (3 subunits, 2 transmemb.)



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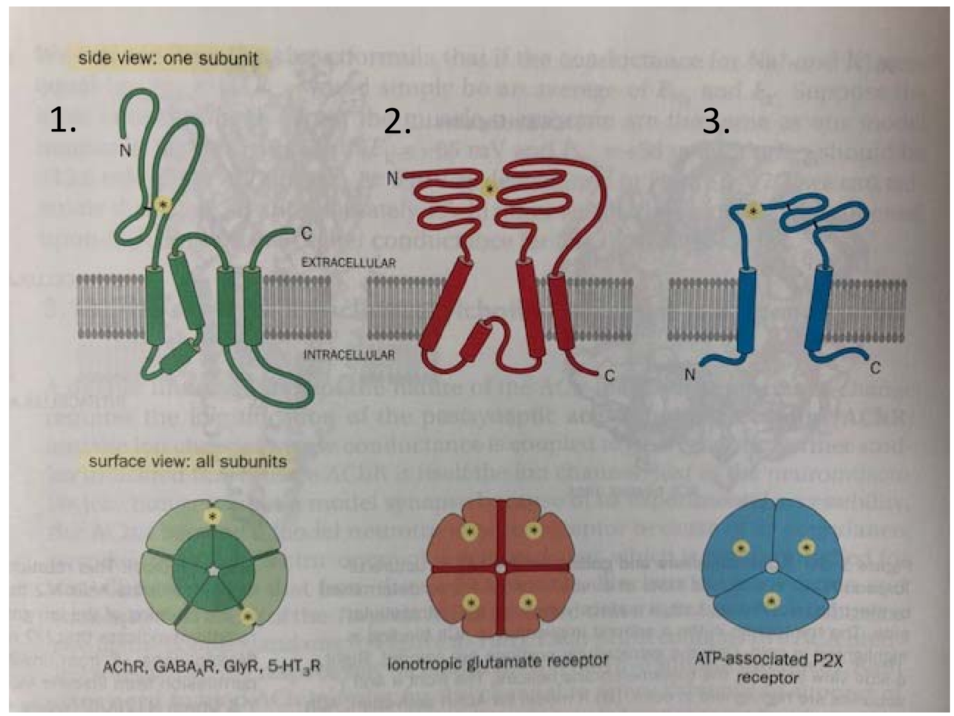


Sub-types of Ionotropic Receptors

- Glutamate receptors (excitatory)
  - NMDA
  - AMPA (non-NMDA)
  - Kainate (non-NMDA)
- GABA receptors (inhibitory)
  - $GABA_A$ (ligand-gated ion channels = ionotropic receptors)
  - $GABA_B$ G (G-protein-coupled receptors, therefore metabotropic receptors)

Sub-types of Ionotropic Receptors and Genes

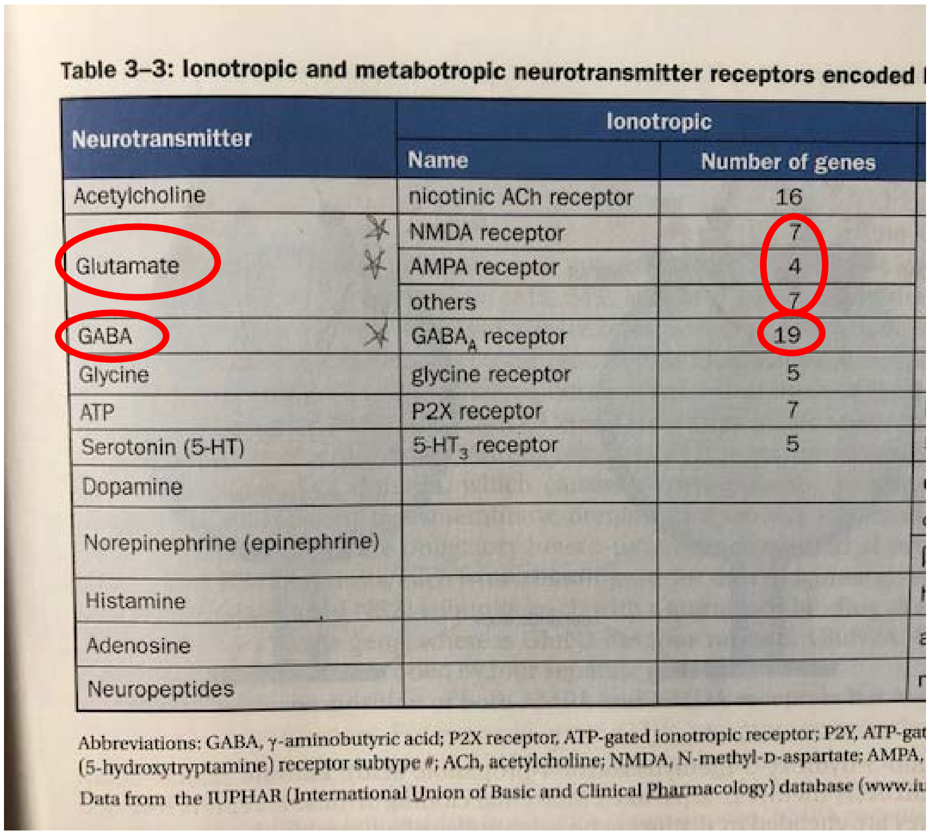


Table 3–3: Ionotropic and metabotropic neurotransmitter receptors encoded

Neurotransmitter	Ionotropic	
	Name	Number of genes
Acetylcholine	nicotinic ACh receptor	16
Glutamate	NMDA receptor	7
	AMPA receptor	4
	others	7
GABA	$GABA_A$ receptor	19
Glycine	glycine receptor	5
ATP	P2X receptor	7
Serotonin (5-HT)	$5\text{-}HT_3$ receptor	5
Dopamine		
Norepinephrine (epinephrine)		
Histamine		
Adenosine		
Neuropeptides		

Abbreviations: GABA, γ-aminobutyric acid; P2X receptor, ATP-gated ionotropic receptor; P2Y, ATP-gat
(5-hydroxytryptamine) receptor subtype #; ACh, acetylcholine; NMDA, N-methyl-D-aspartate; AMPA,
Data from the IUPHAR (International Union of Basic and Clinical Pharmacology) database (www.iu

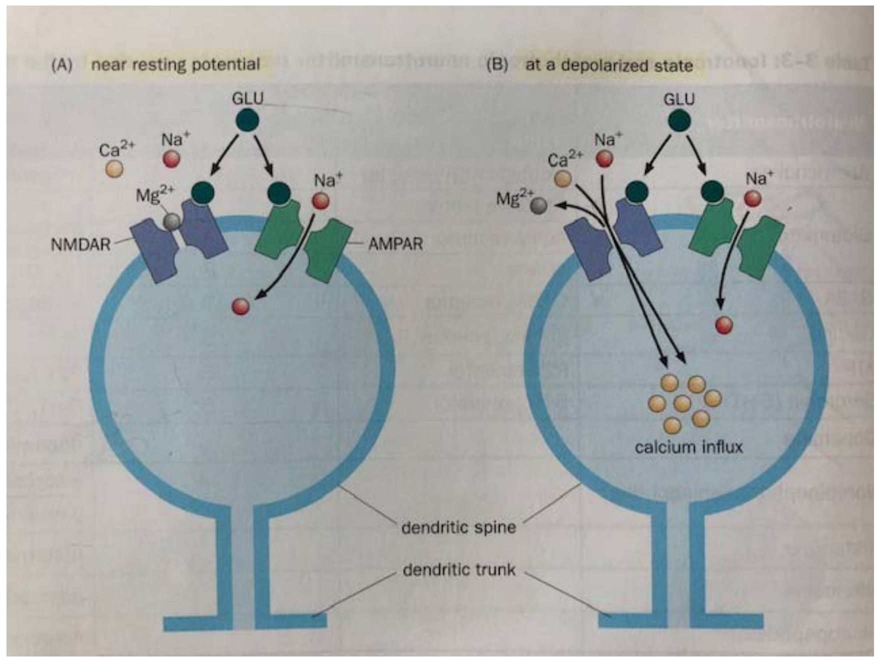
Multiple genes encode different subunits within each receptor

Properties of AMPA and NMDA glutamate receptors

AMPA—glutamate binds, channel opens, Na+ flows into cell

NMDA—glutamate (or glycine) binds, but nothing happens until neuron depolarizes via AMPA channels,

Then Mg2+ block is released, NMDA channel opens, and Ca2+ and Na+ flow inward thus contributing to stronger excitation



Activation of AMPA and NMDA receptors causes excitation

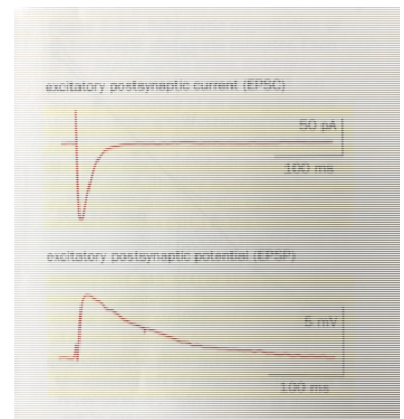
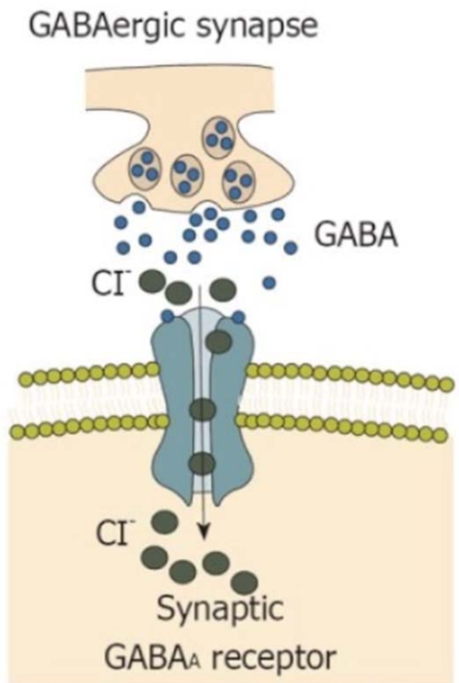


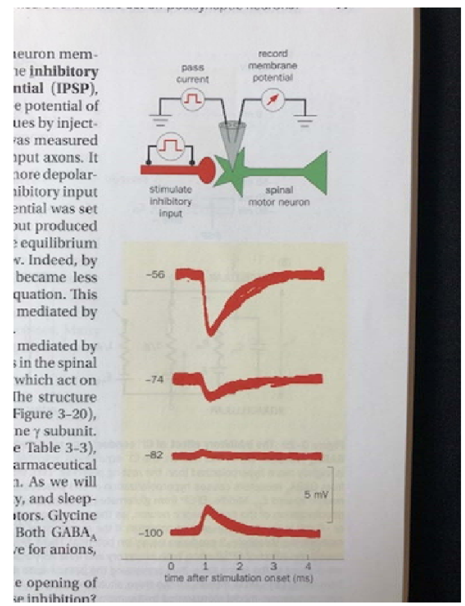
Figure 3–23 Excitatory postsynaptic current (EPSC) and excitatory postsynaptic potential (EPSP) at a glutamatergic synapse. Representative EPSC (top) and EPSP (bottom) recorded

Properties of GABA-A Receptors

GABA-A—GABA binds to receptor, Cl- ions flow inward, causing hyperpolarization and thus inhibition of the neuron



Activation of GABA-A receptors causes inhibition



Annual Review of Neuroscience

Acoustic Pattern Recognition and Courtship Songs: Insights from Insects

Christa A. Baker,[1] Jan Clemens,[2] and Mala Murthy[1]

[1]Princeton Neuroscience Institute, Princeton University, Princeton, New Jersey 08544, USA; email: mmurthy@princeton.edu

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Baker et al. (2019) Ann. Rev. Neurosci.

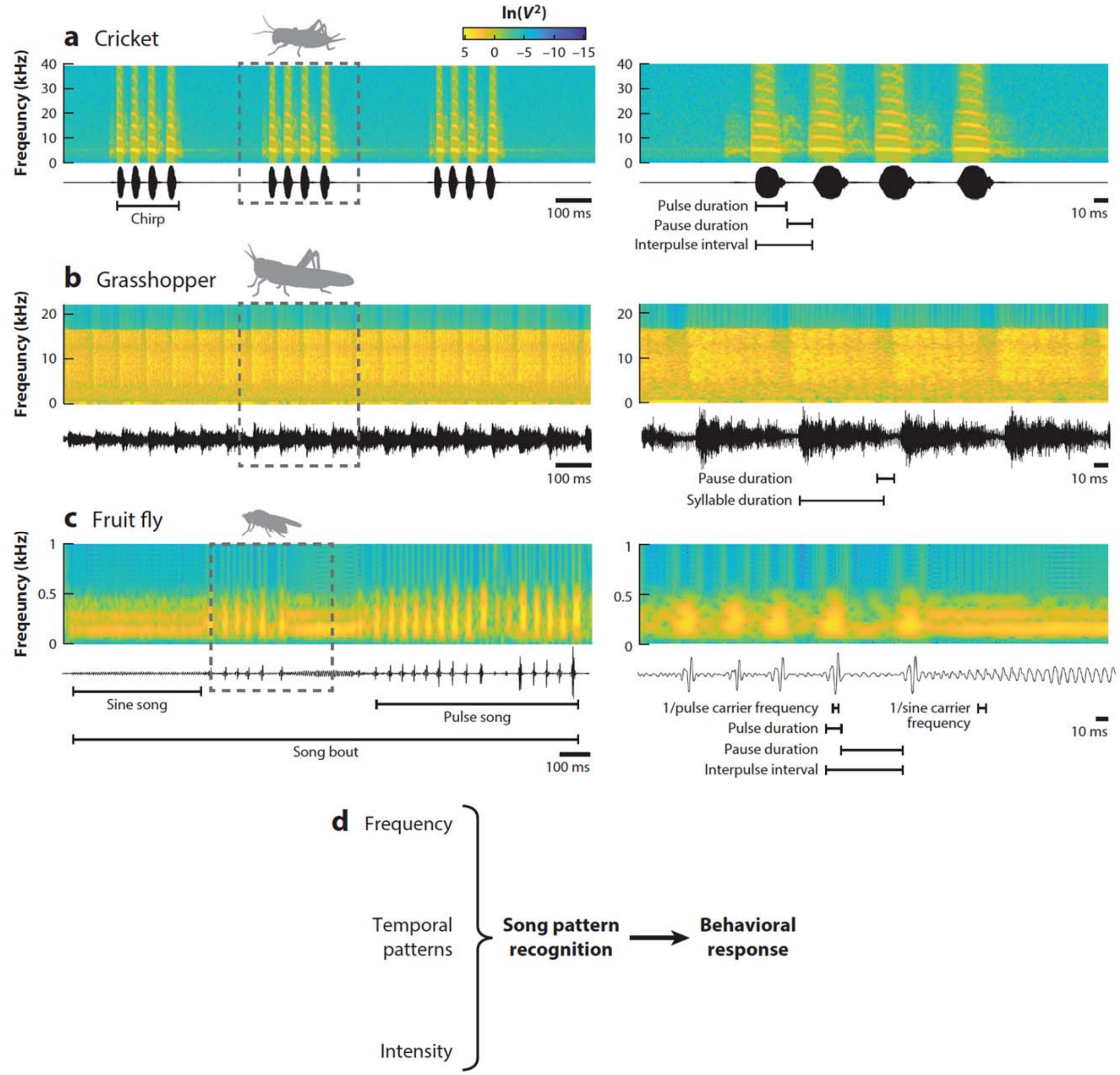
- Review of song pattern recognition in crickets, grasshoppers, *Drosophila*
- Males produce songs with time varying spectrotemporal features
- Females use this information in mate choice

Insect calling and courtship songs

Integrate different elements of information contained in song:

- frequency (pitch)
- timing
- intensity

during mate choice



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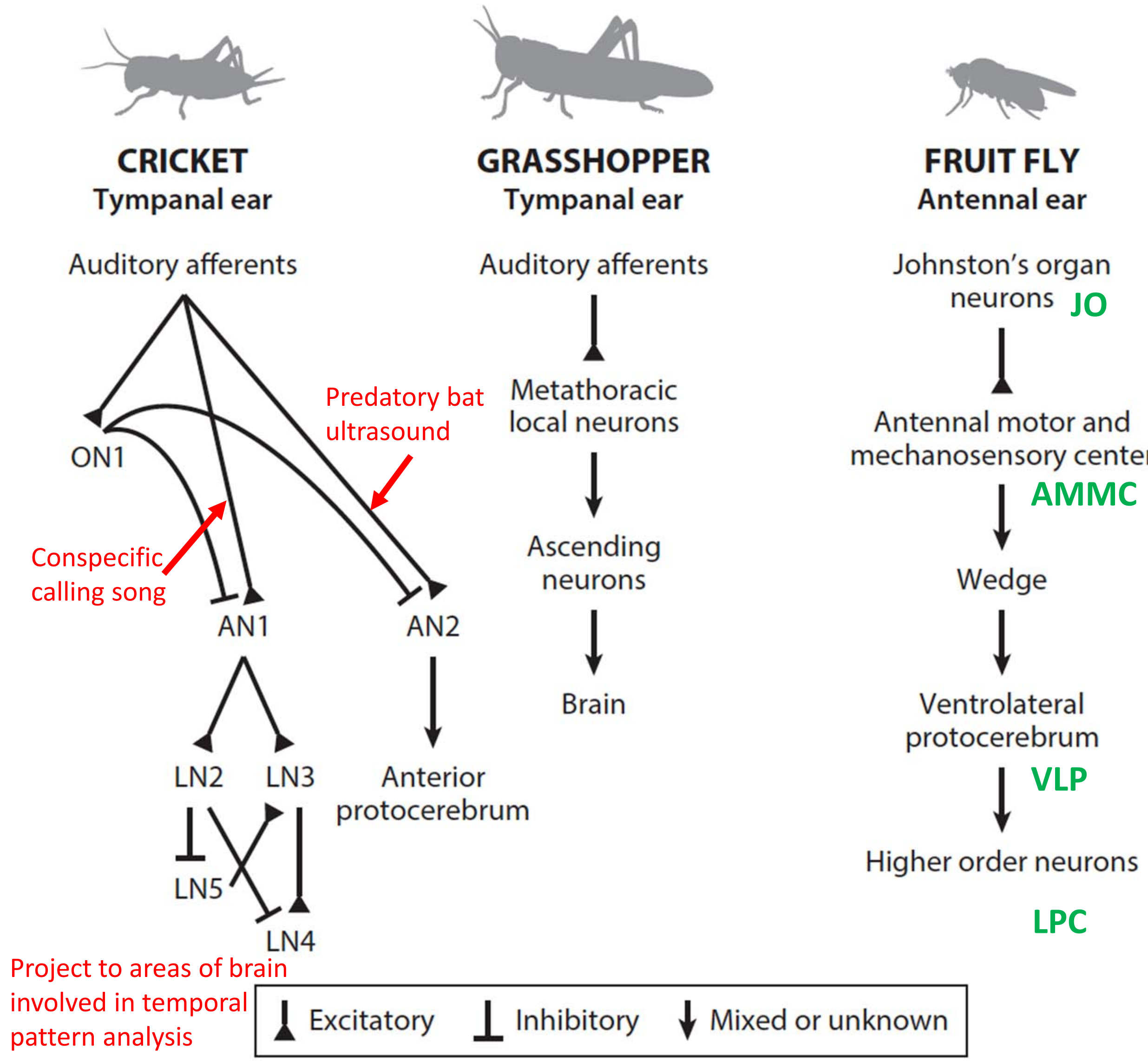
Insect auditory pathways

Prior studies elucidated the anatomy and physiology of the sound receiver and mechanosensory neurons

Recent studies are starting to explore how central neurons represent songs

Future work will decipher how these representations are decoded to change behavior



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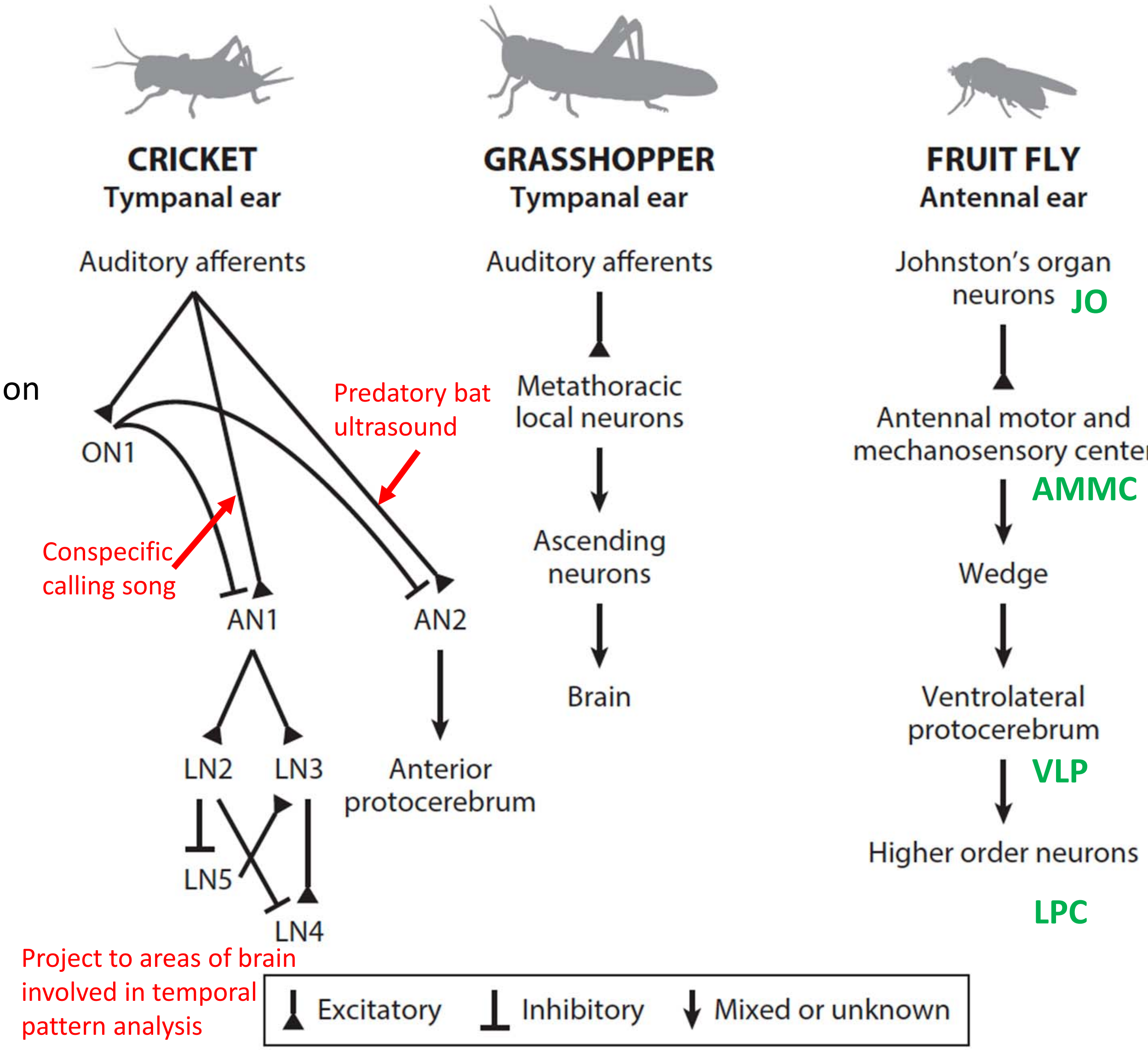
Insect auditory pathways

In crickets, neurons in prothoracic ganglion mapped, but not higher-order neurons involved in temporal pattern analysis

In grasshoppers, higher-order auditory neurons are beginning to be identified

Drosophila auditory system remains incompletely mapped



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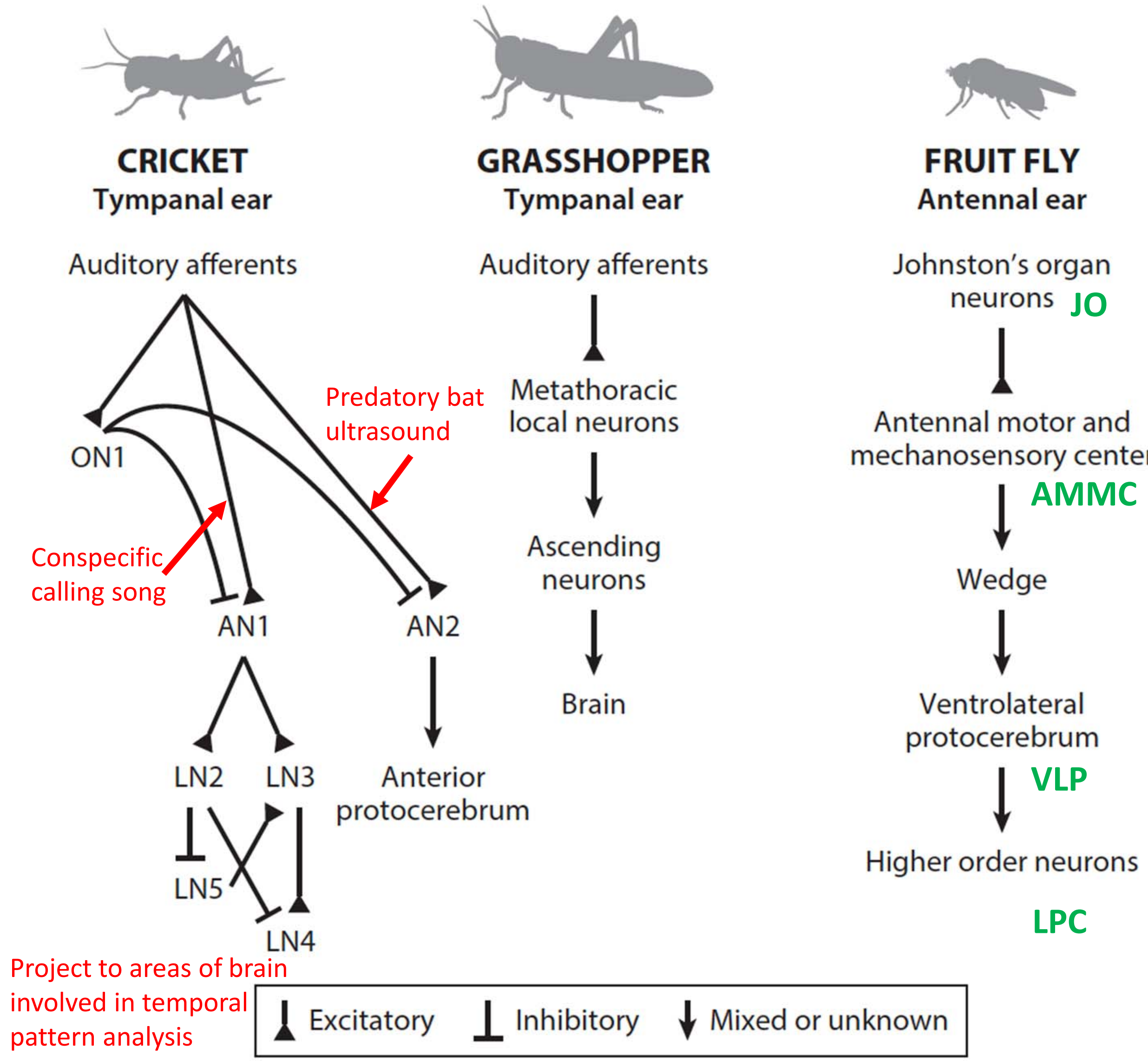
Insect auditory pathways

Need to precisely characterize each neuron's tuning, then map connectivity among auditory neurons and between auditory and motor pathways

- Whole-cell patch-clamp recordings
- Imaging pan-neuronal activity (compare across animals)
- Map connections within electron microsope data sets
- Precisely map behavioral outputs during manipulation of neural activity
- Calcium sensing to assess tuning and to identify neurons



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Mechanisms for temporal pattern recognition

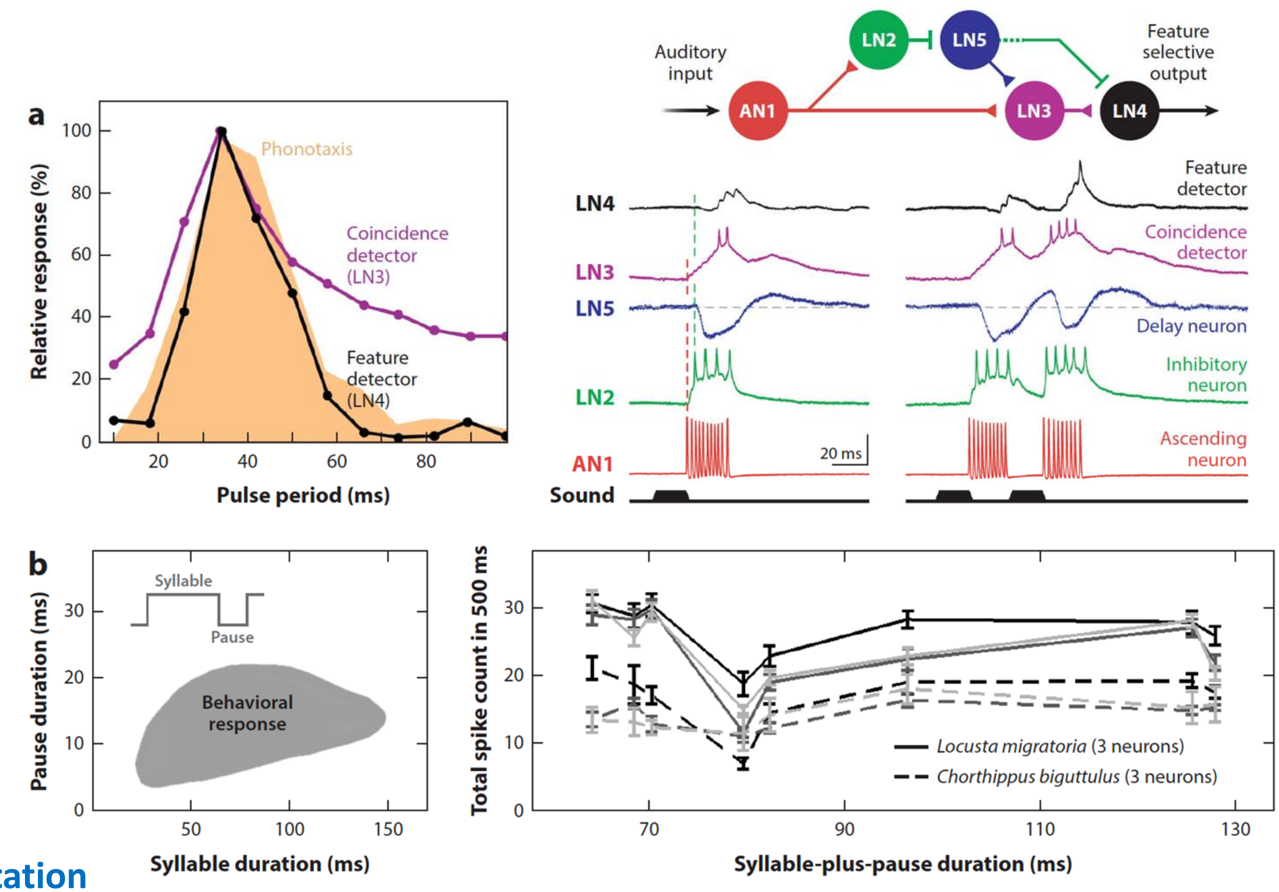
Neural tuning of coincidence detector (LN3) and feature detector (LN4) match to phonotaxis behavior

LN3 only fires when receives input via excitation from AN1 and postinhibitory rebound from delay line (LN5)

= <u>Coincidence detection mechanism</u>

Pulse interval selectivity results from interactions between inhibition and excitation

Data from crickets



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Mechanisms for temporal pattern recognition

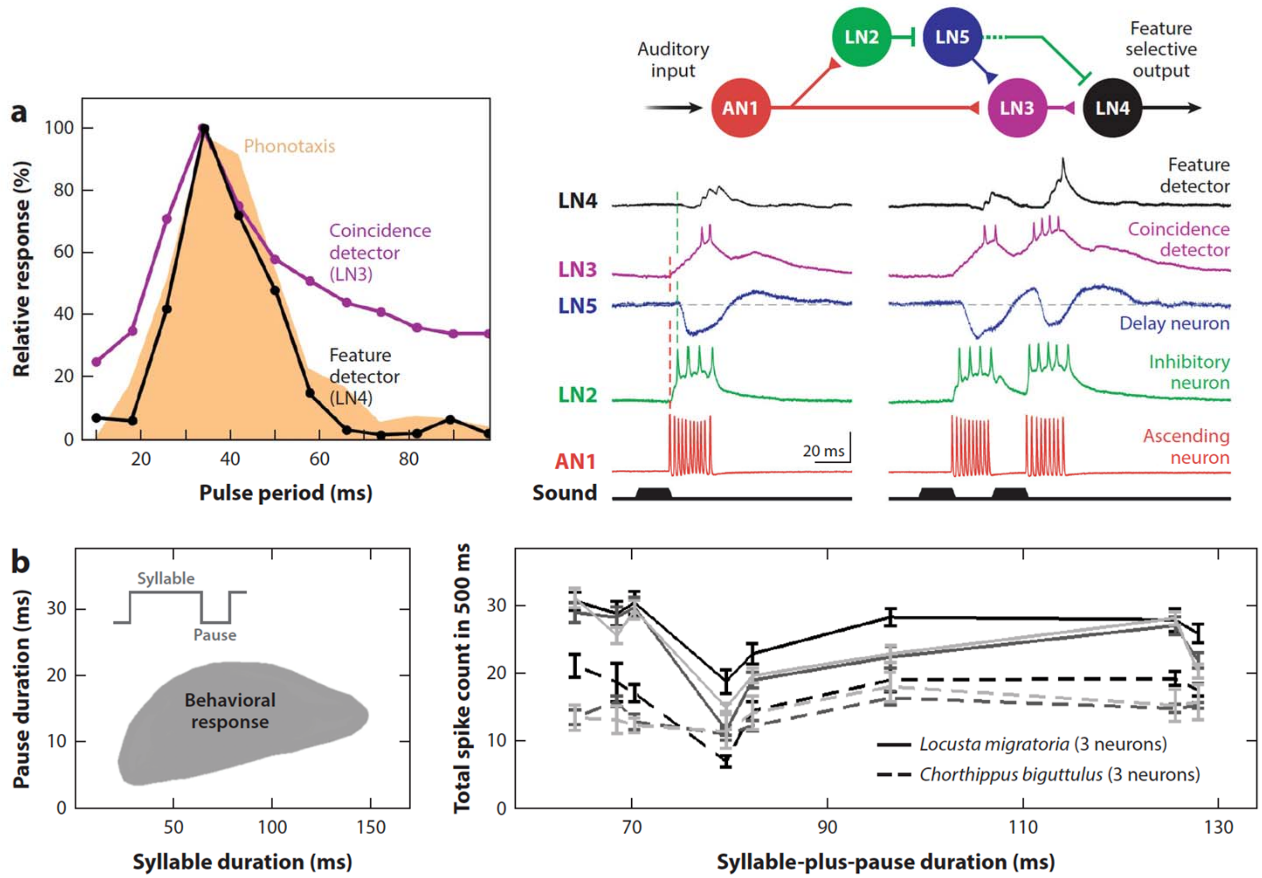
Fly auditory pathways also have interval-selective neurons

Match between neural and behavioral interval tuning gradually increases from the AMMC to the LPC of brain

In B1 of AMMC tuning results from inhibition to short responses by two **GABAergic** neurons aLN and B2 (Yamada et al. 2018).

Unclear if use a coincidence detection mechanism

Data from crickets



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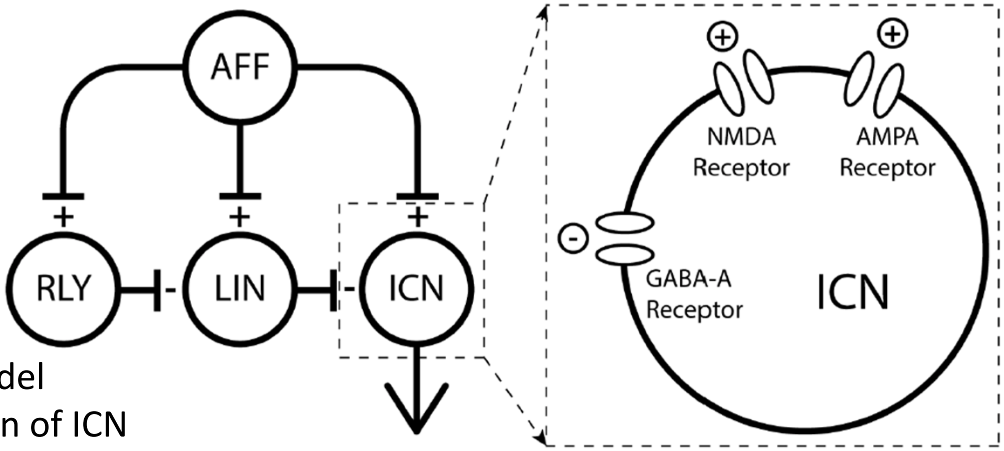
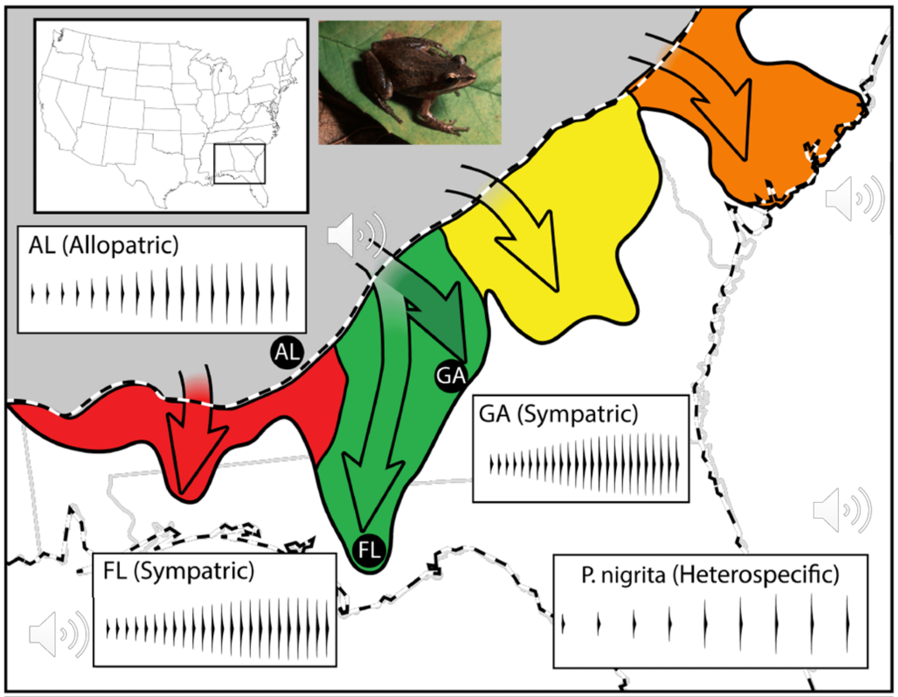
Gary Rose et al. (various papers)

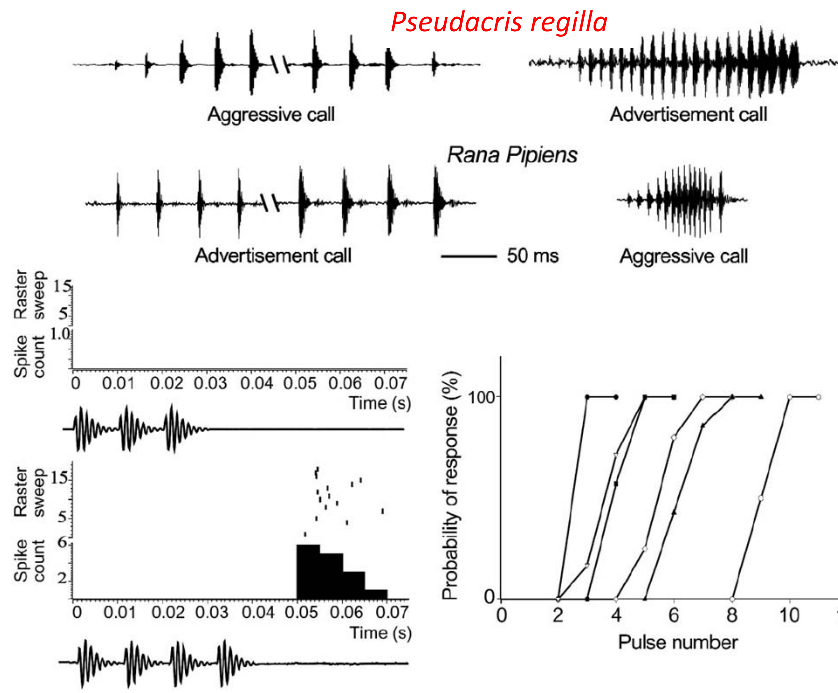
- Discovered interval-counting neurons in auditory midbrain of frogs
- Long-interval neurons (LIN) = sensitive to interval length
  - Inhibition evoked by successive short intervals coincides with excitation evoked by previous pulses, thereby preventing spikes to later pulses
- Interval-counting neurons (ICN) = count intervals (if presented at required length)
  - Optimal intervals elicit rate-dependent excitation that eventually overcomes the inhibition to produce spikes
- This information is key to species recognition!



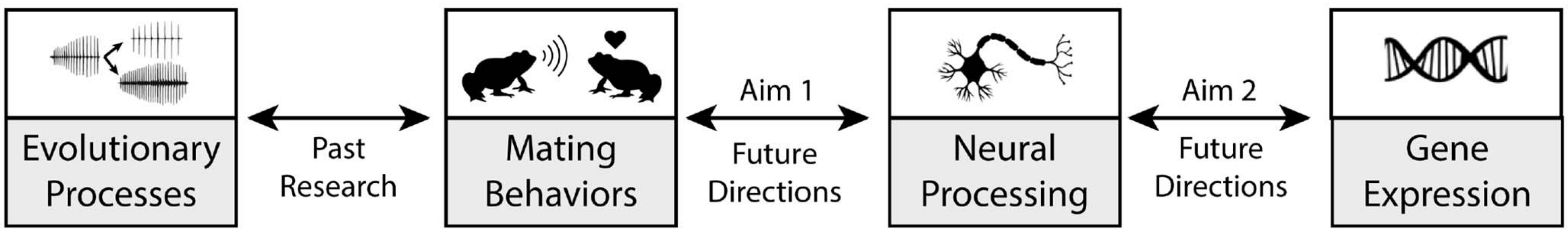
Disinhibition computational model
showing excitation and inhibition of ICN

Speciation in chorus frogs (*Pseudacris*)





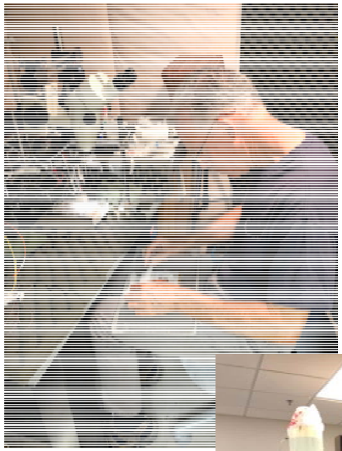
Edwards, Rose et al (2002)

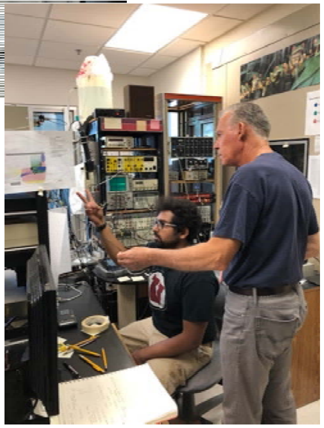

Evolutionary Processes
Past Research
Mating Behaviors
Aim 1
Future Directions
Neural Processing
Aim 2
Future Directions
Gene Expression

Alluri et al. (2016)—Phasic, suprathreshold excitation and sustained inhibition underlie neuronal selectivity for short-duration sounds

- Goal: identify the mechanism of duration-selective neurons of the anuran inferior colliculus (IC_{AN})
- Target neurons are selective for short sounds only

- Methods
  - Current patch-clamp recording (whole cell), *in vivo* of frogs
  - Extracted excitatory and inhibitory conductances
  - Employed pharmaceutical manipulations to block $GABA_A$ receptors of target neurons





Alluri et al. (2016)—Models of duration selectivity

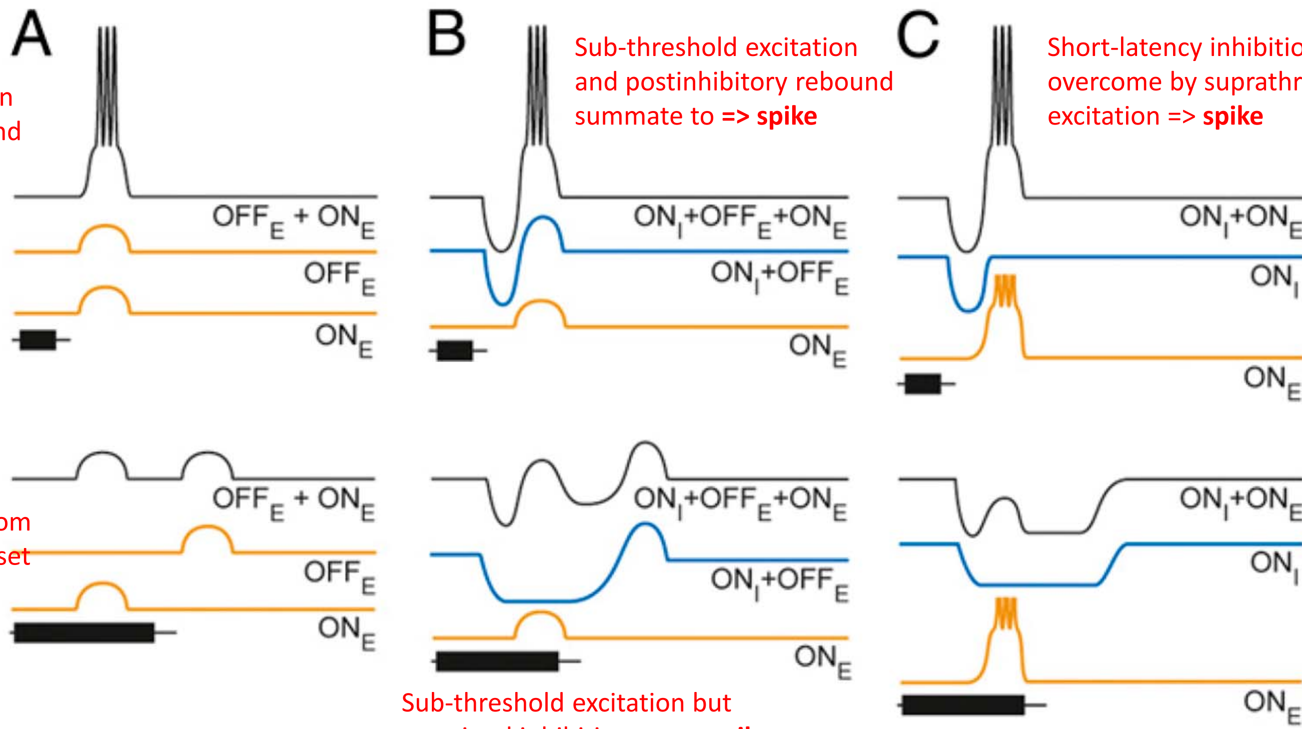
Orange = excitatory
Blue = inhibitory

A-B. Coincidence models

C. Anti-coincidence model

Relies on postinhibitory rebound

Does not require postinhibitory rebound



Subthreshold excitation from stimulus onset and offset summate to => **spike**

Sub-threshold excitation and postinhibitory rebound summate to **=> spike**

Short-latency inhibition overcome by suprathreshold excitation => **spike**

Short sound played

Asynchronous sub-threshold excitation from stimulus onset and offset => **no spike**

Sub-threshold excitation but sustained inhibition **=> no spike**

Long sound played

Sustained inhibition suppresses suprathreshold excitation => **no spike**

Figure 1.

Alluri et al. (2016)—neurons selective for short sounds

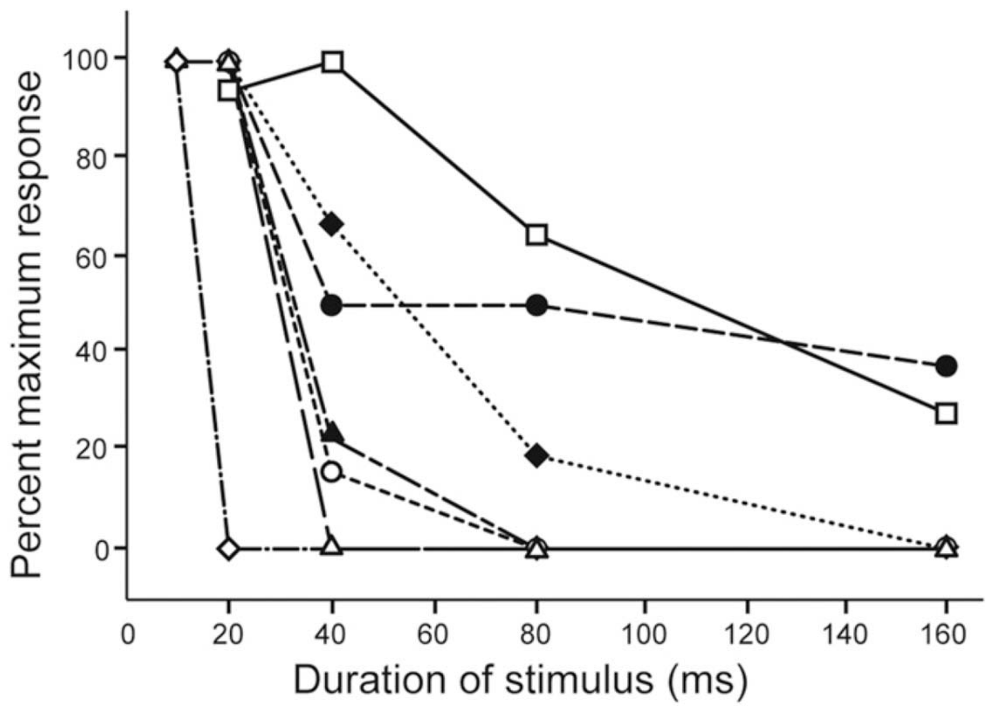


Figure 2.

Alluri et al. (2016)

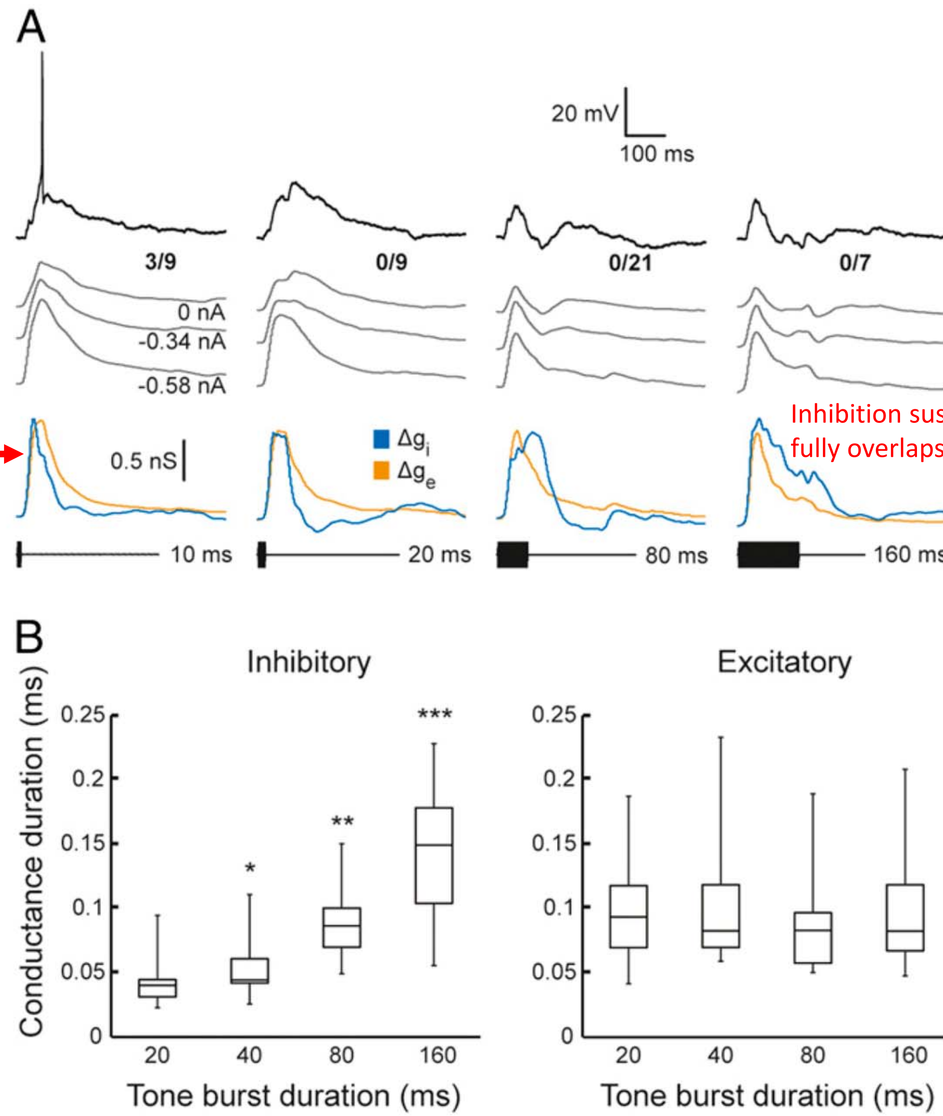
Short-pass duration-selective neuron

The longer the sound, the longer the inhibition lasts (blue line)

Inhibition brief; does not fully overlap excitation

The more negative the current injected, the stronger the response to short sounds only

Time course of inhibition, but not excitation tracks stimulus duration



Inhibition sustained and fully overlaps excitation

Voltage measured

Effect of current level on voltage

Conductances

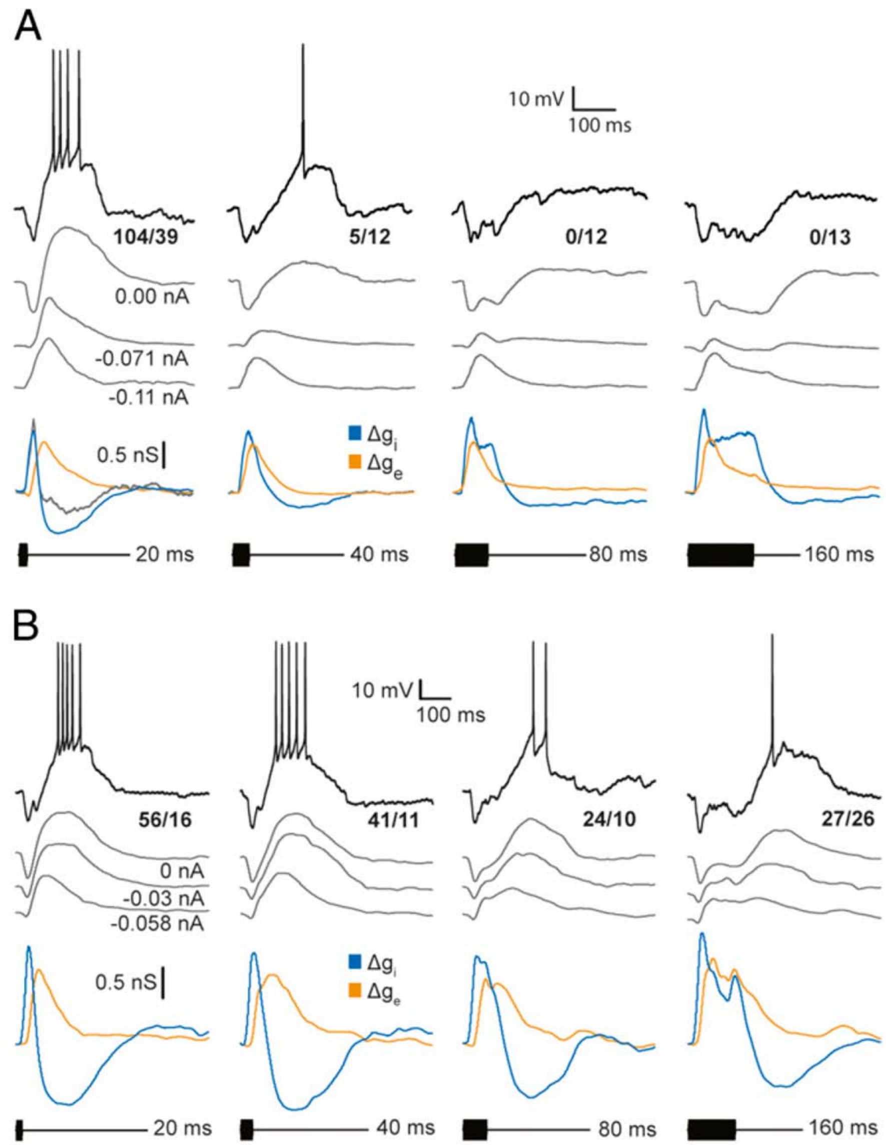
Sound length

Figure 3.

Alluri et al. (2016)

Strongly selective short-pass duration-selective neuron

Weakly selective short-pass duration-selective neuron



Conductances broadly overlap by 80-160 ms stimulus => stronger-selectivity for short sounds

Conductances do not fully overlap until about 160 ms => weaker selectivity

Figure 4.

Alluri et al. (2016)

Attenuating $GABA_A$ inhibition
(blocking the channels that inhibit excitation)
reveals suprathreshold excitation

When GABA receptors blocked, excitation takes over and neurons fire in response to any sound

When GABA receptors unblocked, the behavior of the neurons goes back to normal (baseline)

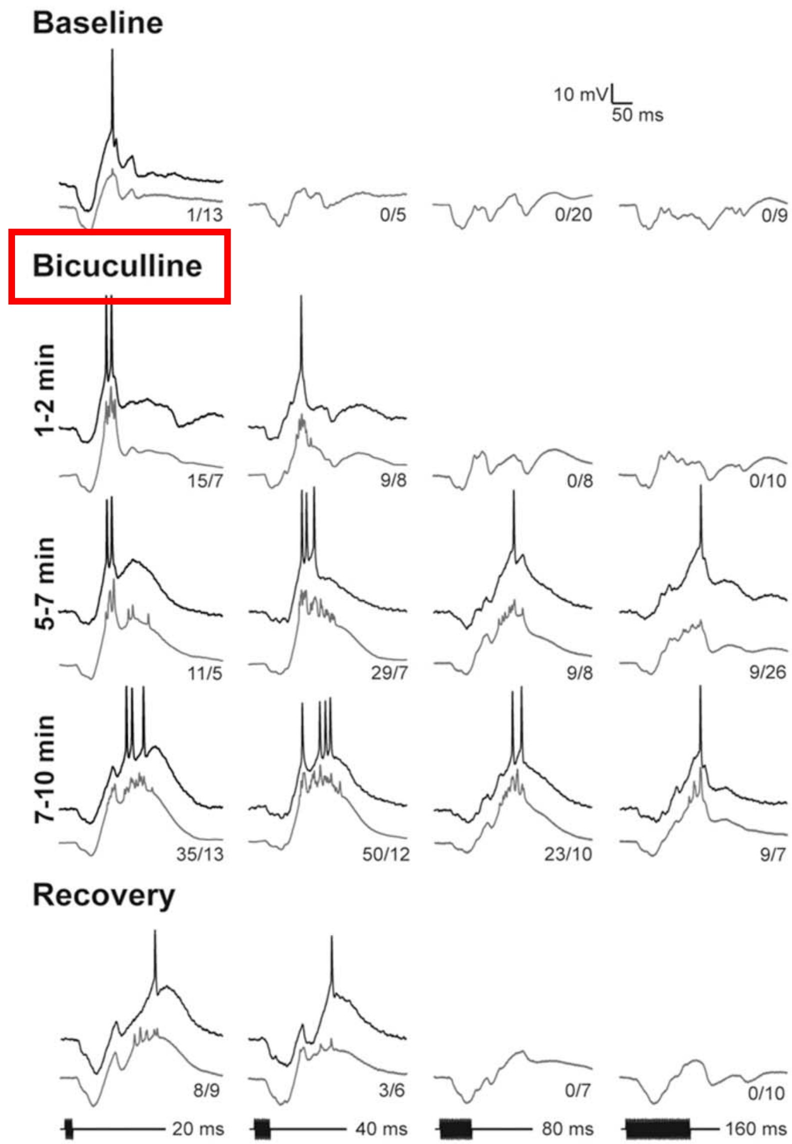


Figure 5.

Alluri et al. (2016)

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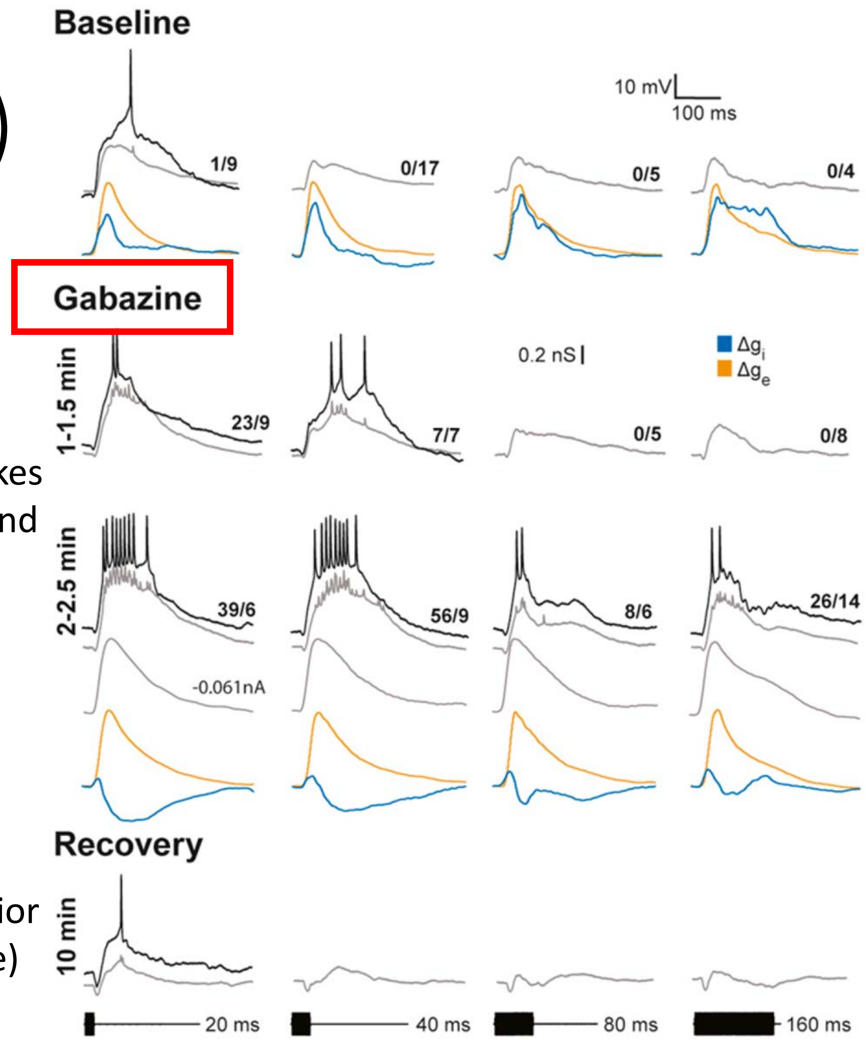


Figure 6.

Alluri et al. (2016)

Number of depolarizations increases for long sounds when $GABA_A$ receptors blocked (solid line) vs. prior to blocking (dashed line)

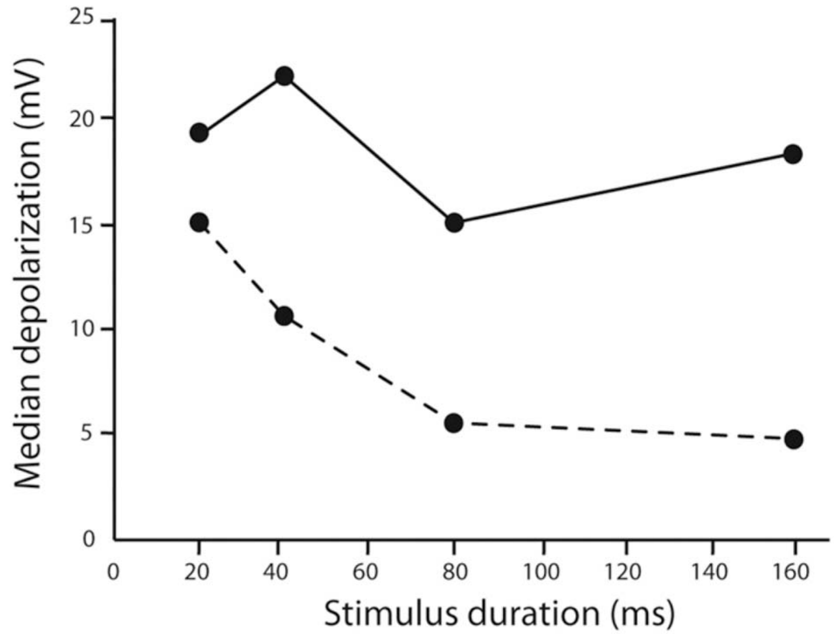
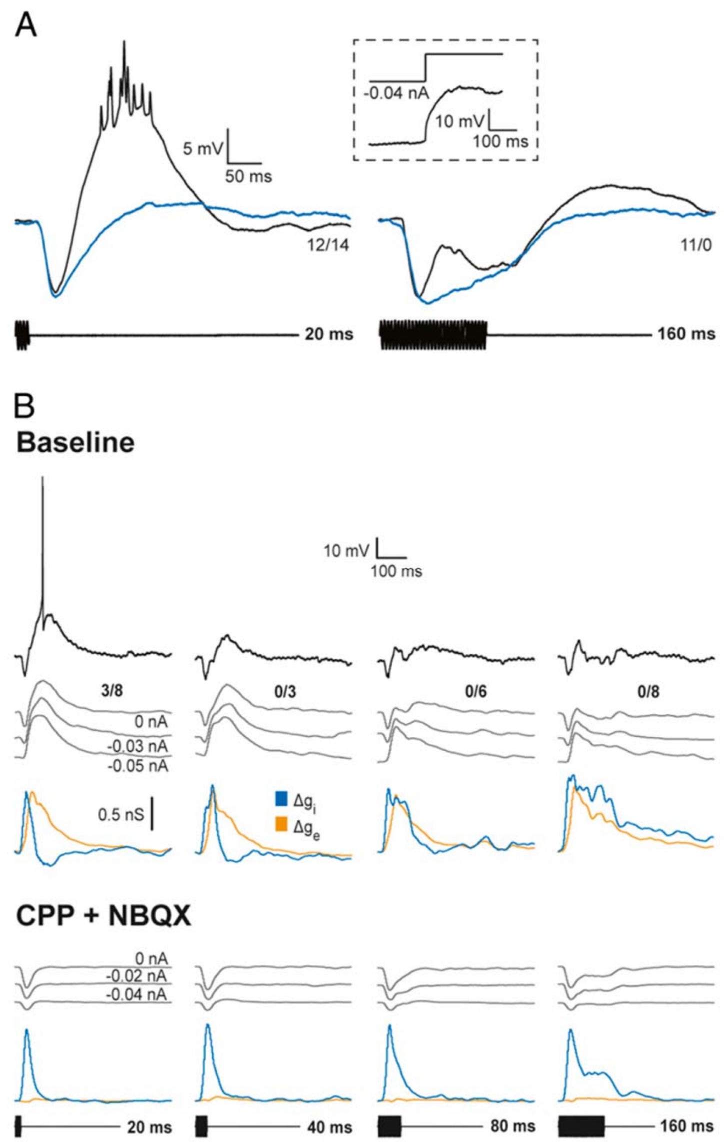


Figure 7.

Alluri et al. (2016)



Blocked excitatory receptors

NBQX blocks AMPA glutamate channels

CPP blocks NMDA glutamate channels

No evidence of postinhibitory rebound, as predicted by the second coincidence model

Figure 8.

Alluri et al. (2016)--Summary

- Short-latency, sustained inhibition and delayed, phasic excitation are integrated to generate short-pass duration selectivity
- When inhibitory and excitatory conductances do not coincide => **spike**
- Anti-coincidence model is supported over coincidence models
- No evidence for postinhibitory rebound effects
- Inhibition via GABA channels suppresses suprathreshold excitation via NMDA and AMPA glutamate channels
- Inhibition is only overcome by excitation in presence of short sounds

Yamada et al. (2018)—GABAergic local interneurons shape female fruit fly response to mating songs

- Goal: Understand the neural circuitry between auditory sensory neurons (JO) and the antennal mechanosensory and motor center (AMMC) region of the brain
- Interval-selective neurons in *Drosophila*

- Methods
  - Calcium imaging *in vivo* of JO-B and AMMC-B1 neurons
    - Expressed Ca^{2+} sensor GCaMP6f in each neuronal type to monitor neuronal activity
    - Played songs with different interpulse intervals (IPI) and measured response
    - Knocked down expresson of GABA subunit *rdl* => non-functional GABA receptors
  - Female copulation assay

Yamada et al. (2018)

In JO-B neurons, Ca^{2+} response the same across IPIs and neurons equally activated across stimuli

Suggests JO-B neurons transmit information of pulse songs without computing IPI information

In AMMC-B1 neurons, response increases monotonically from 105 to 25 IPI, then drops significantly at 15 IPI

= Selective attenuation of postsynaptic activity during signal transmission by AMMC-B1 neurons at 15 IPI

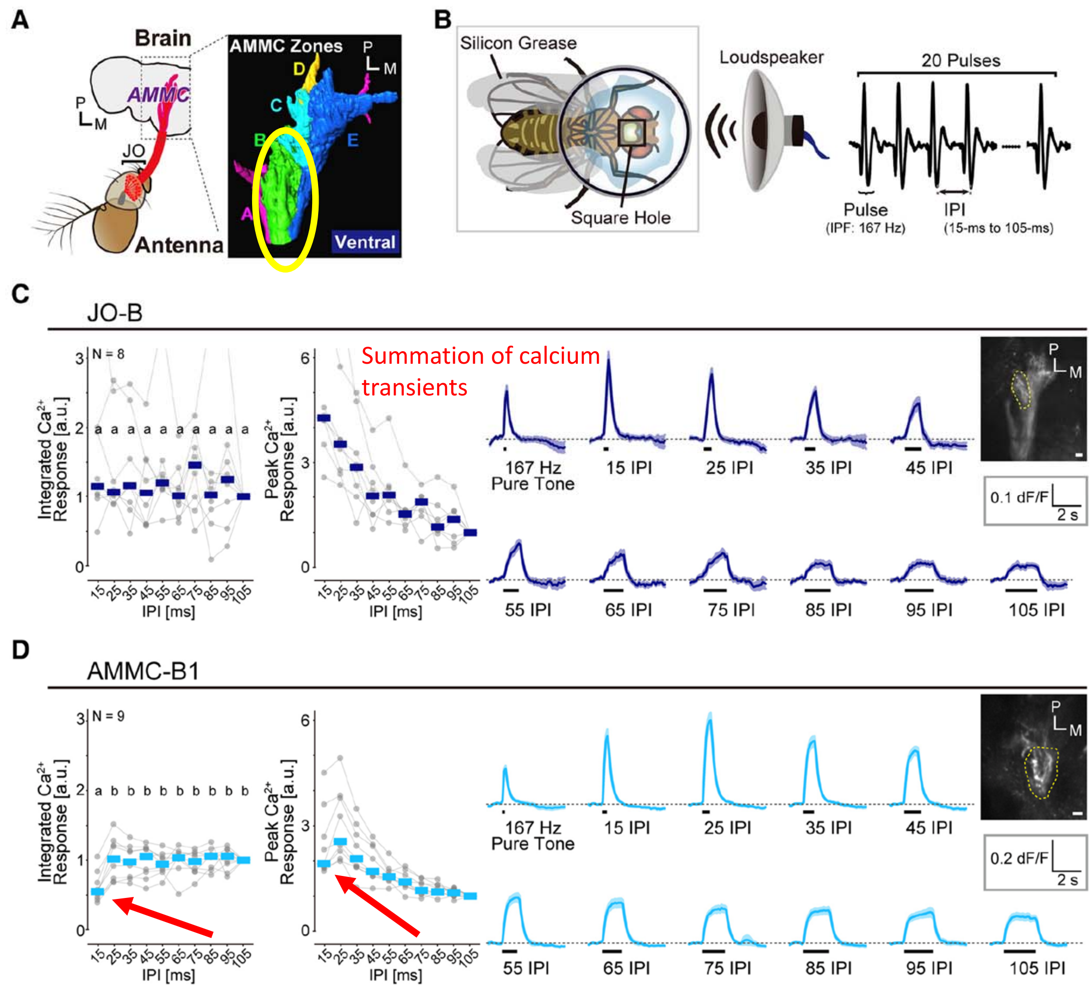
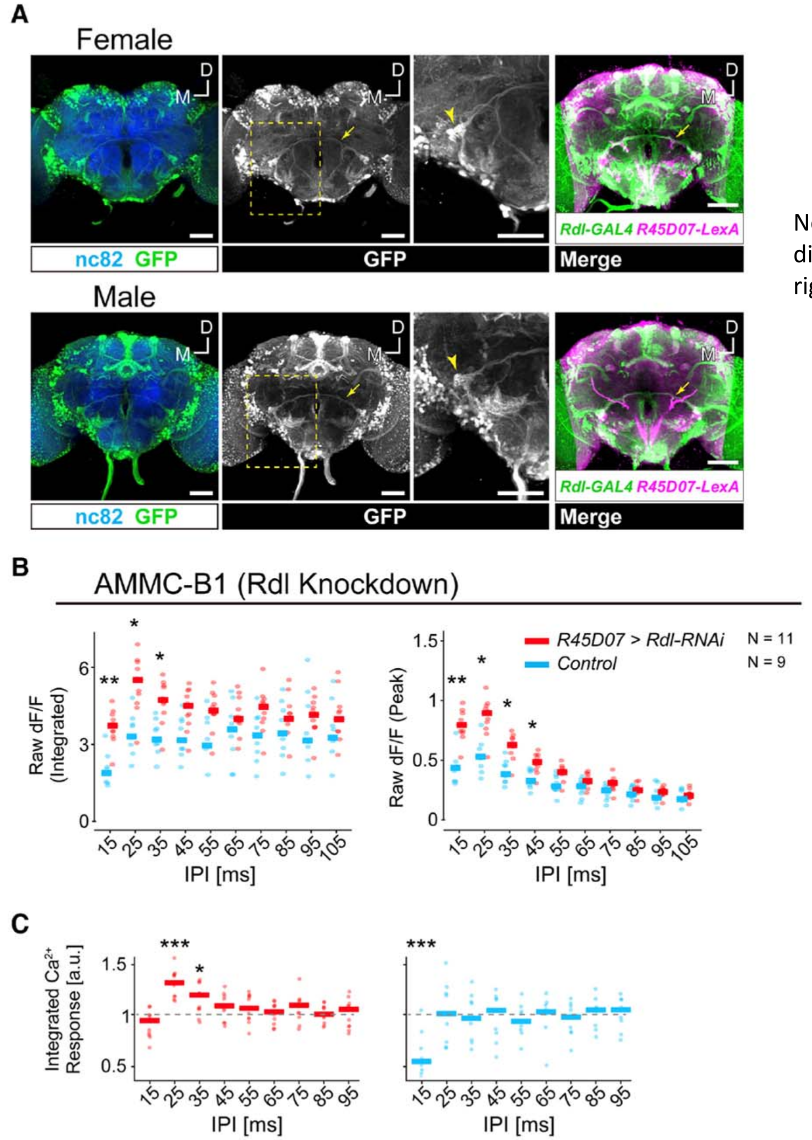


Figure 1.

Yamada et al. (2018)



Need help digesting rightmost images

Knockdowns *rdl* ($GABA_A$ receptor subunit) showed significantly higher Ca2+ response compared to controls at low IPIs

Suggests that suppressing $GABA_A$ receptors leads to decreased selectivity at low IPIs by AMMC-B1 neurons

Figure 2.

Yamada et al. (2018)

Something is suppressing AMMC-B1 neurons via $GABA_A$ receptors

Could it be local interneurons?

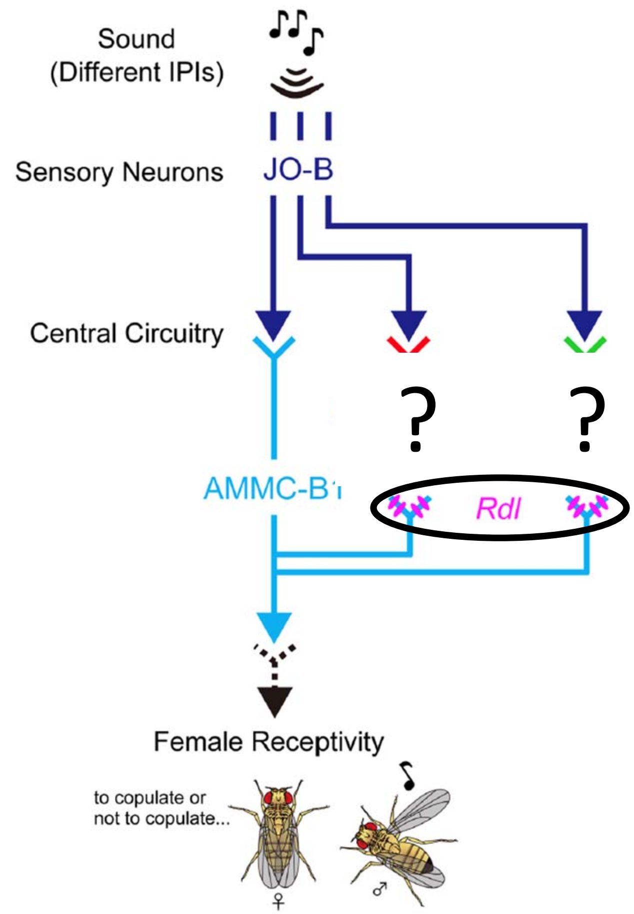


Figure 7.

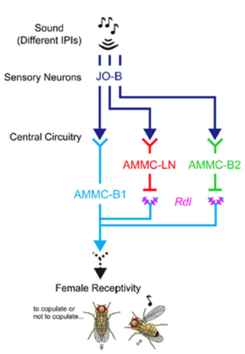
Yamada et al. (2018)

Fig 3C. Demonstrates synapses between AMMC-LN/AMMC-B1 and AMMC-B2/AMMC-B1 in the AMMC brain region

Fig 3D. Shows direction of flow of information is into AMMC zone B (not zone D)

Fig 3E. Shows presynaptic sites of candidate interneurons overlap with dendritic sites of AMMC-B1

Fig 3F-G. Reveals that JO-neurons transmit signals directly to candidate interneurons via cholinergic synapses



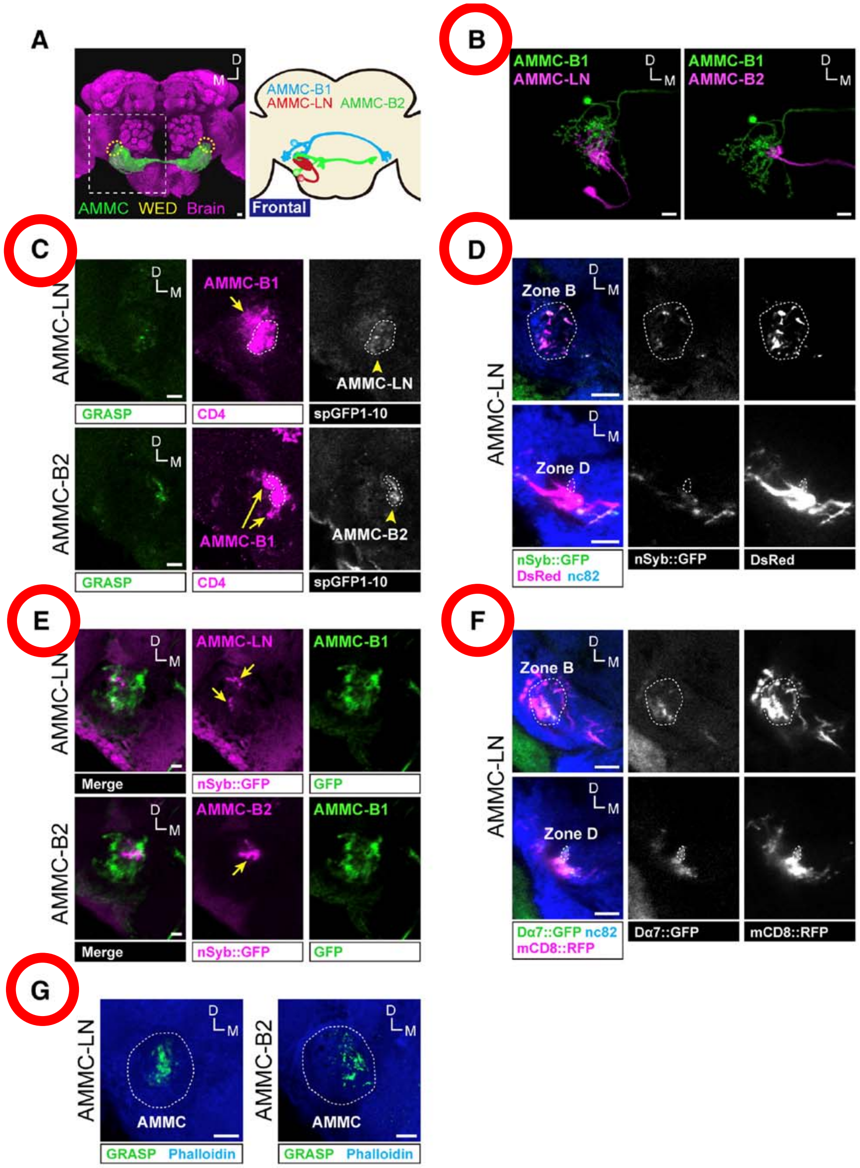


Fig. 3B. AMMC-B1 connects to candidate interneurons AMMC-B2 and AMMC-LN

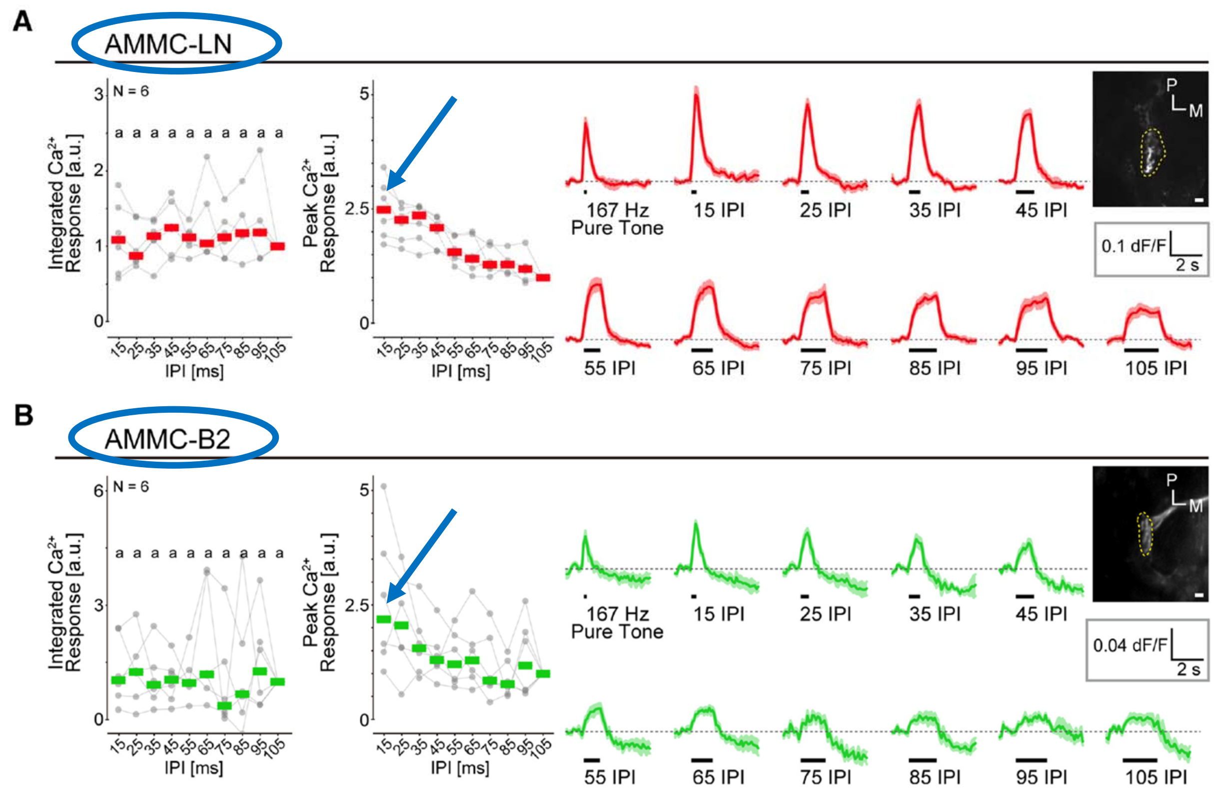
Figure 3.

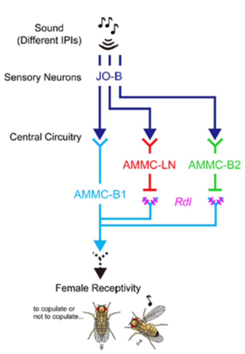
Yamada et al. (2018)

Same Ca^{2+} imaging techniques as in Figure 1 auditory neurons

No drop off in response at low IPIs

Interneuron patterns resemble JO-B neurons

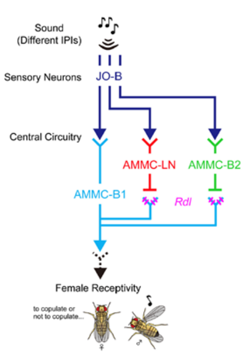


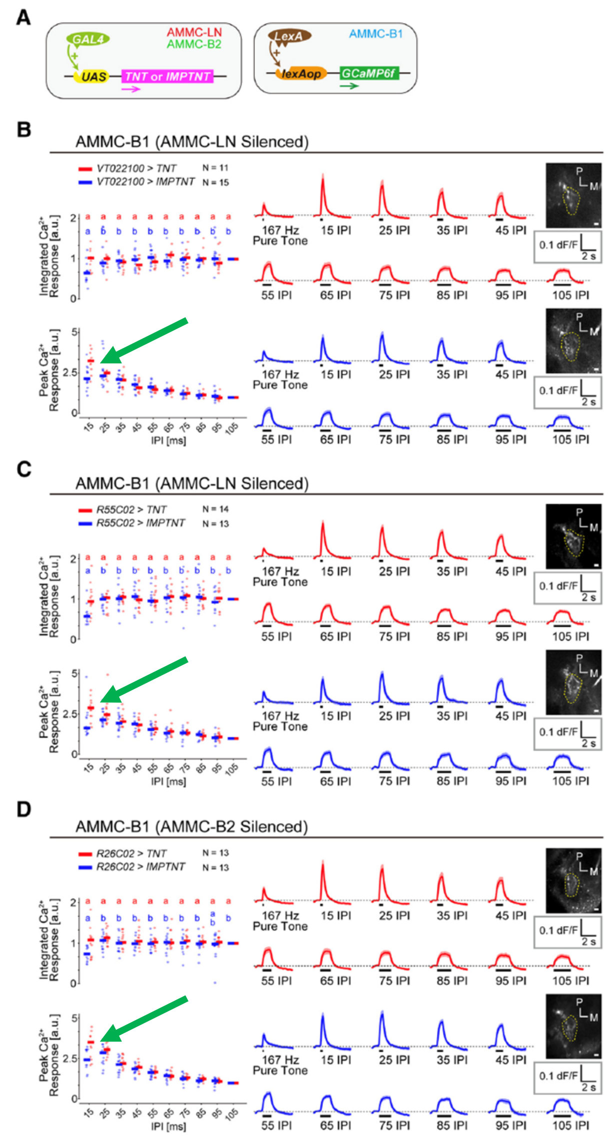


Yamada et al. (2018)

When either of the two candidate interneurons are silenced, AMMC-B1 loses selectivity at low IPIs

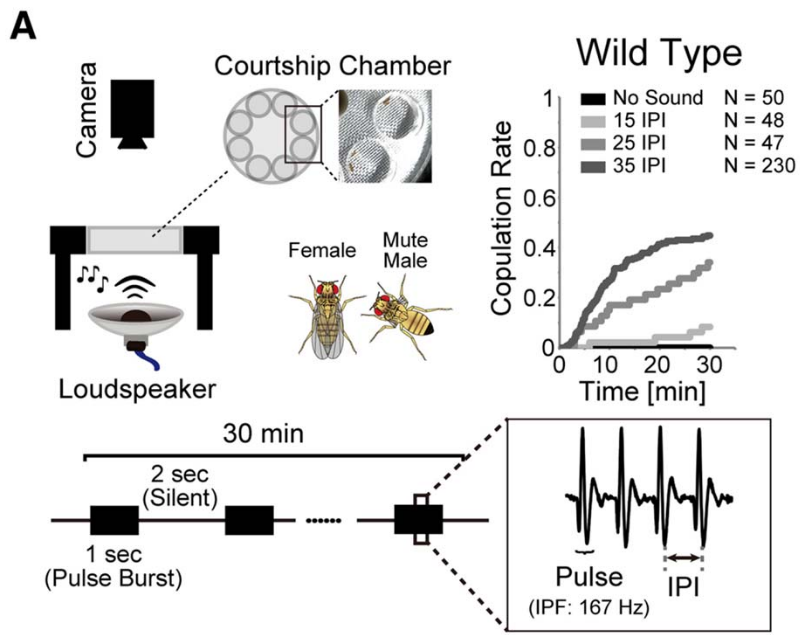
Red = TNT (tetanus toxin) expressing neurons
Blue = IMPTNT (inactivated tetanus) expressing neurons



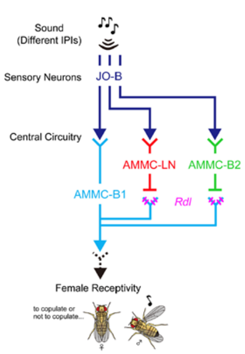


Yamada et al. (2018)

Copulation Rate Experiments



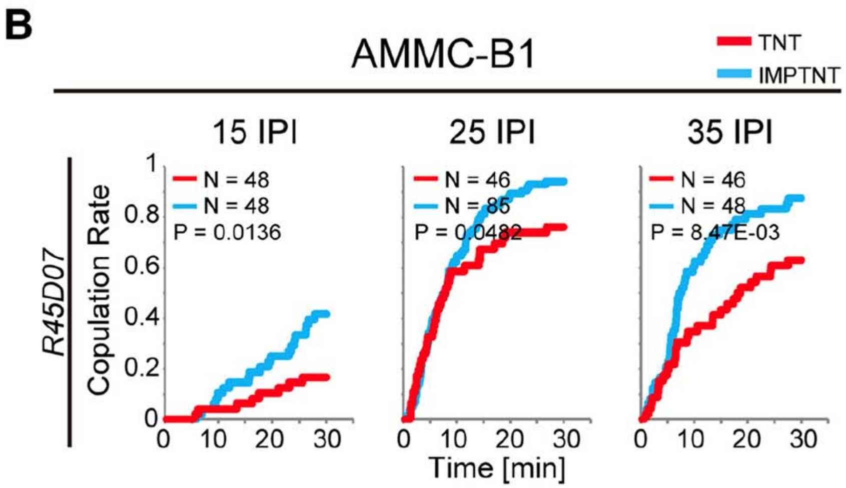
Played different IPI sounds to female in presence of mute male

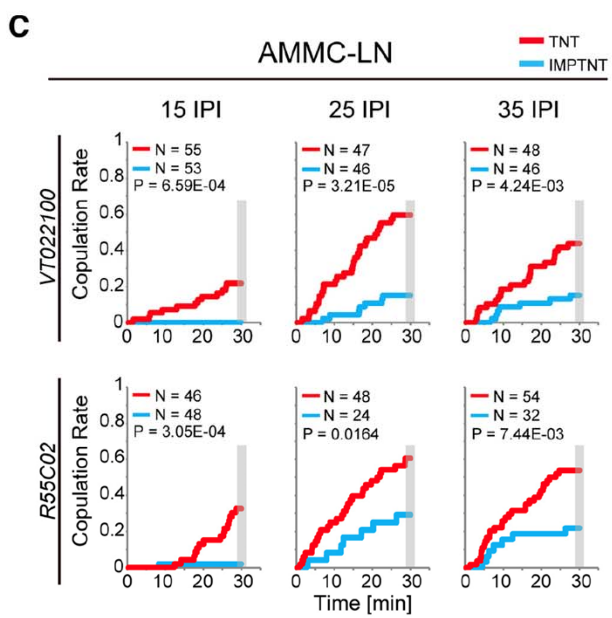


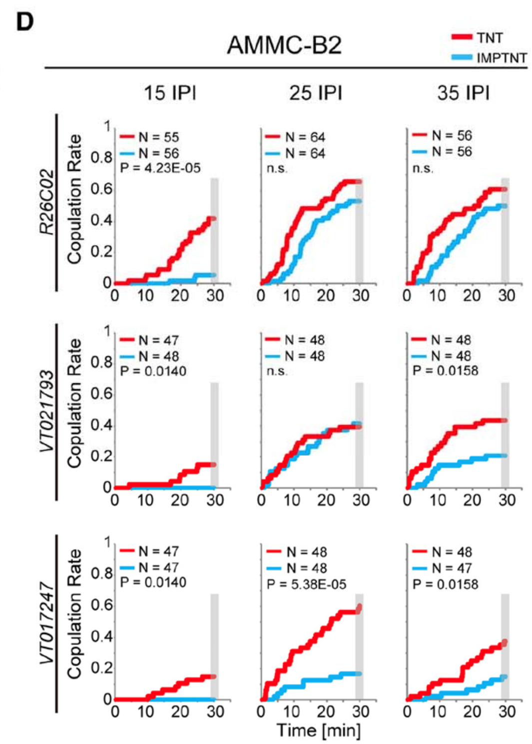
Yamada et al. (2018)

If inactivate AMMC-LN or AMMC-B2 with TNT, then mating <u>increases</u> at short IPIs

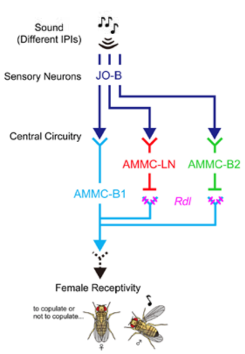
Indicates both GABAergic interneurons normally suppress female response to short IPIs







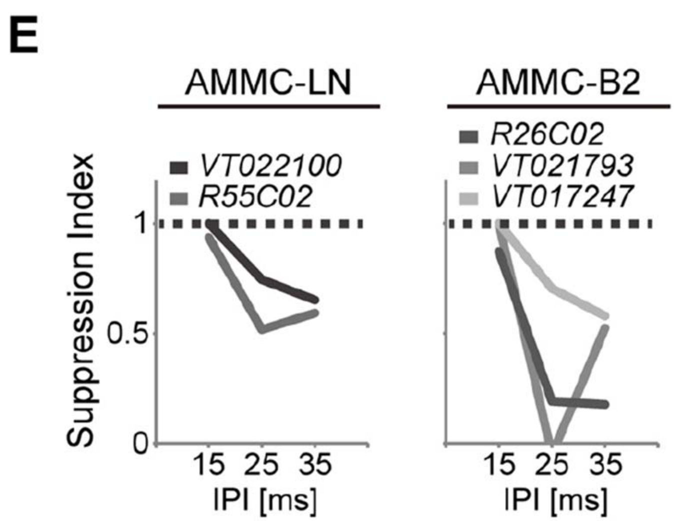
If inactivate AMMC-B1 with TNT, then mating <u>decreases</u> at short IPIs

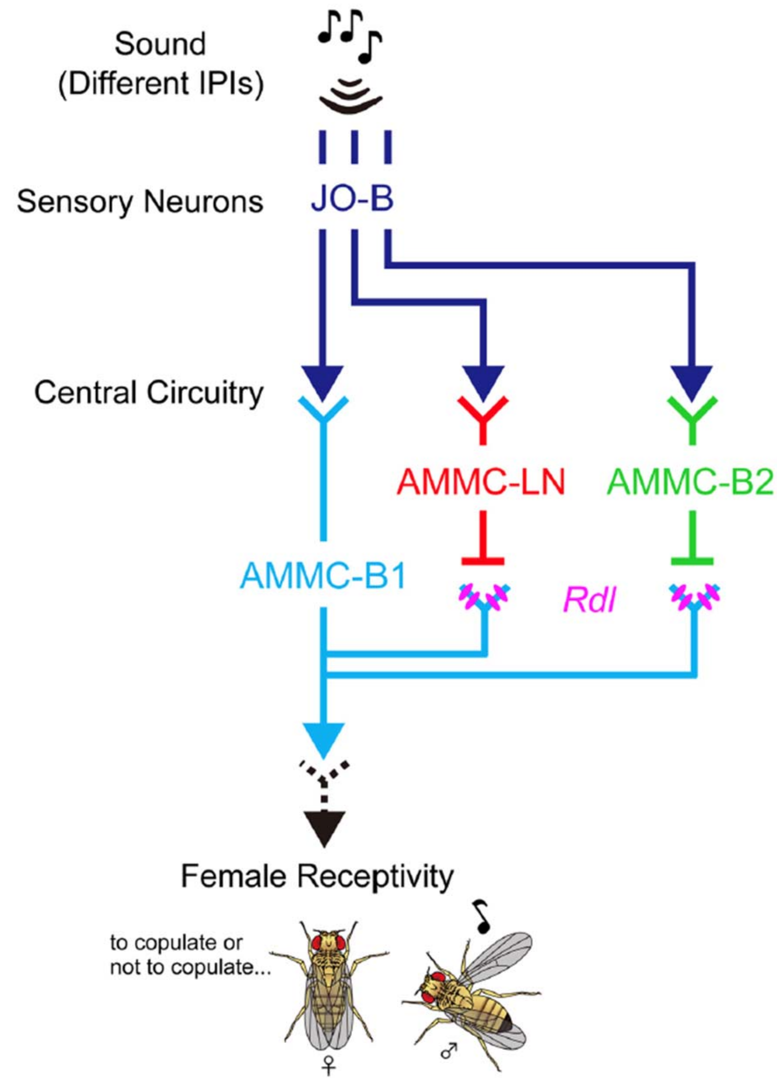


Indicates these neurons contribute positively to behavioral response of females to courtship song

Yamada et al. (2018)

Interneurons suppress mating most effectively for 15 IPI songs





Yamada et al. (2018)--Summary

- Auditory circuit identified that contributes to recognition of temporal song elements
- Involves excitation and inhibition of auditory neurons
- Inhibition occurs through GABAergic local interneurons of the AMMC
- Suppression of these interneurons directly affects female mating behavior