Songbirds help us understand brain mechanisms of alcohol-induced speech impairment

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1 Why study alcohol effects in songbirds?

Speech impairment is a common cognitive effect of alcohol consumption, however *the neural mechanisms underlying alcohol-induced speech impairment remain unknown*. This gap in understanding exists because invasive techniques are not appropriate for use in human subjects. Additionally, **humans are vocal learners, meaning they learn speech through imitation**, and most traditional model organisms like rodents rely on innate vocalizations. Similar to a child learning to speak, juvenile **zebra finches learn to sing by imitating the song of adults**, making them a powerful model to examine the neural effects of alcohol on a learned vocal behavior. What's more, our lab recently found that zebra finches readily consume alcohol, and when they do, **alcohol alters their song** (Fig. 1), suggesting parallels to human speech impairment (Olson et al. 2014). This lead to the question explored here: **where in the brain might alcohol be acting to alter song production?**

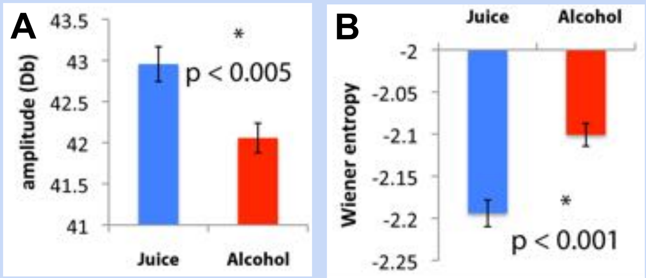


FIGURE 1. Alcohol consumption alters acoustic features of song. A) Amplitude is significantly reduced in drinking birds. **B**) Wiener entropy, a measure of how 'noisy' a sound is, is significantly higher in drinking birds. Data from Olson et al. 2014.

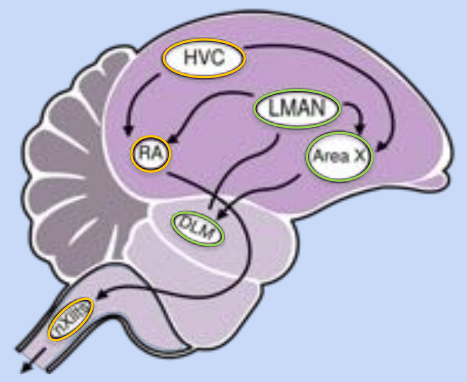
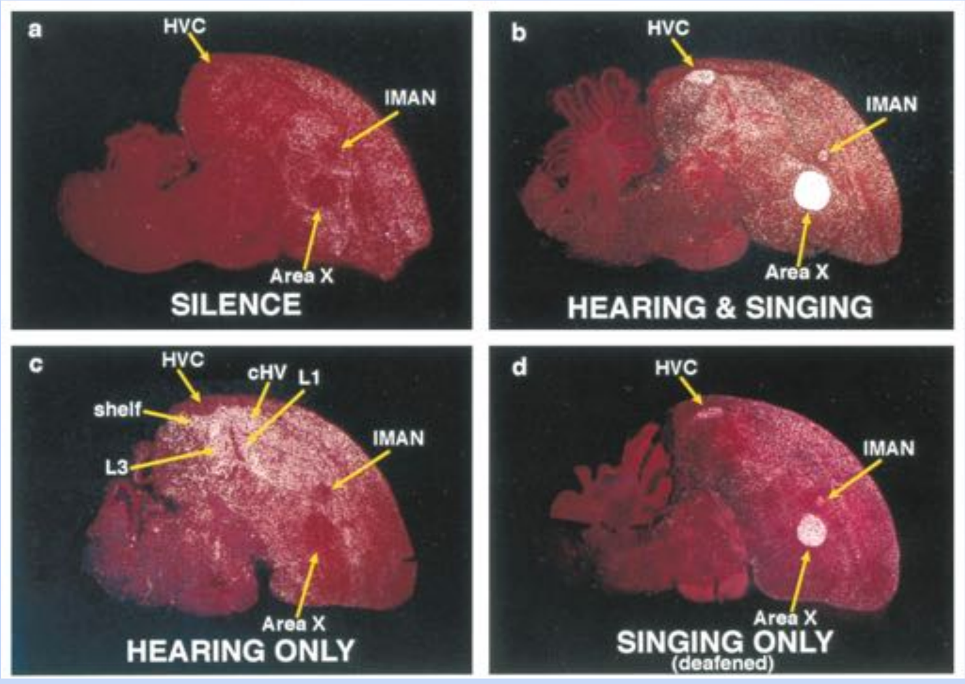


FIGURE 2. The zebra finch song system. The brain nuclei highlighted in orange constitute the direct vocal motor pathway; those highlighted in green constitute the anterior forebrain pathway.

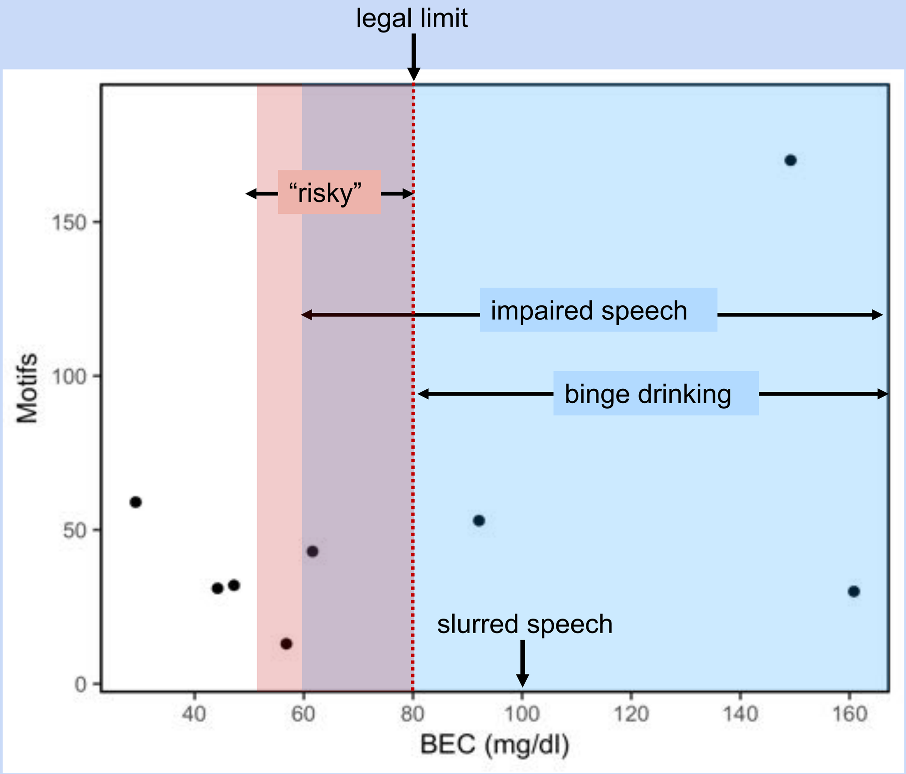
Zebra finches possess two interconnected neural pathways required for acquisition and production of song. Together these circuits make up the **song system** (Fig. 2) (Jarvis 2013). The anterior forebrain pathway (green nuclei) is required for song learning. In contrast, the direct vocal motor pathway is required for both acquisition and production of learned song (orange nuclei). In this study, **we used an immediate early gene called zenk as a proxy for neural activity to investigate how alcohol may be disrupting the song circuit** to produce observed changes in amplitude and entropy. We looked for changes in *zenk*-labeled within nuclei from the anterior forebrain pathway (LMAN and Area X) and posterior vocal-motor pathway (HVC and RA).

2 Activity-induced genes like *zenk* reveal neural activity in the song system

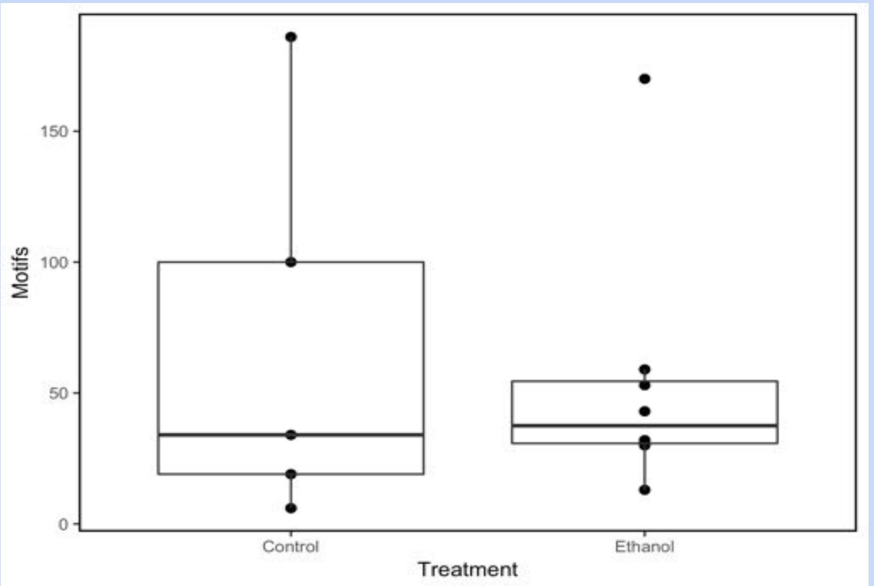


***zenk* is only induced in the song system when birds are singing.** *Zenk* signal is white. A) Birds in silence. B) Birds singing and hearing their own song. C) Birds hearing playback of song. D) Deafened birds singing but unable to hear their own song. Note the specificity of expression within song nuclei. From Jarvis & Nottebohm (1997)

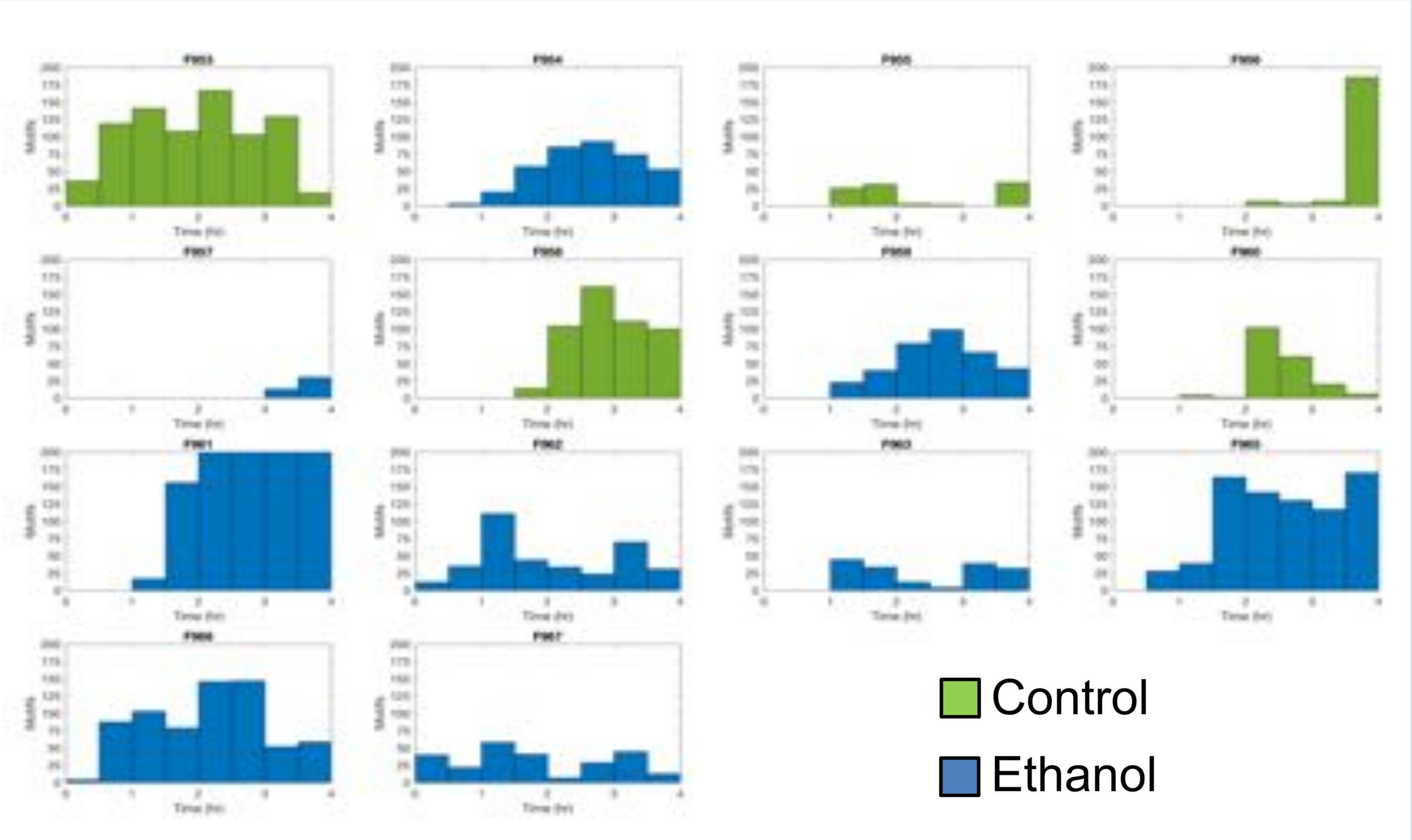
4 Birds differ in level of intoxication



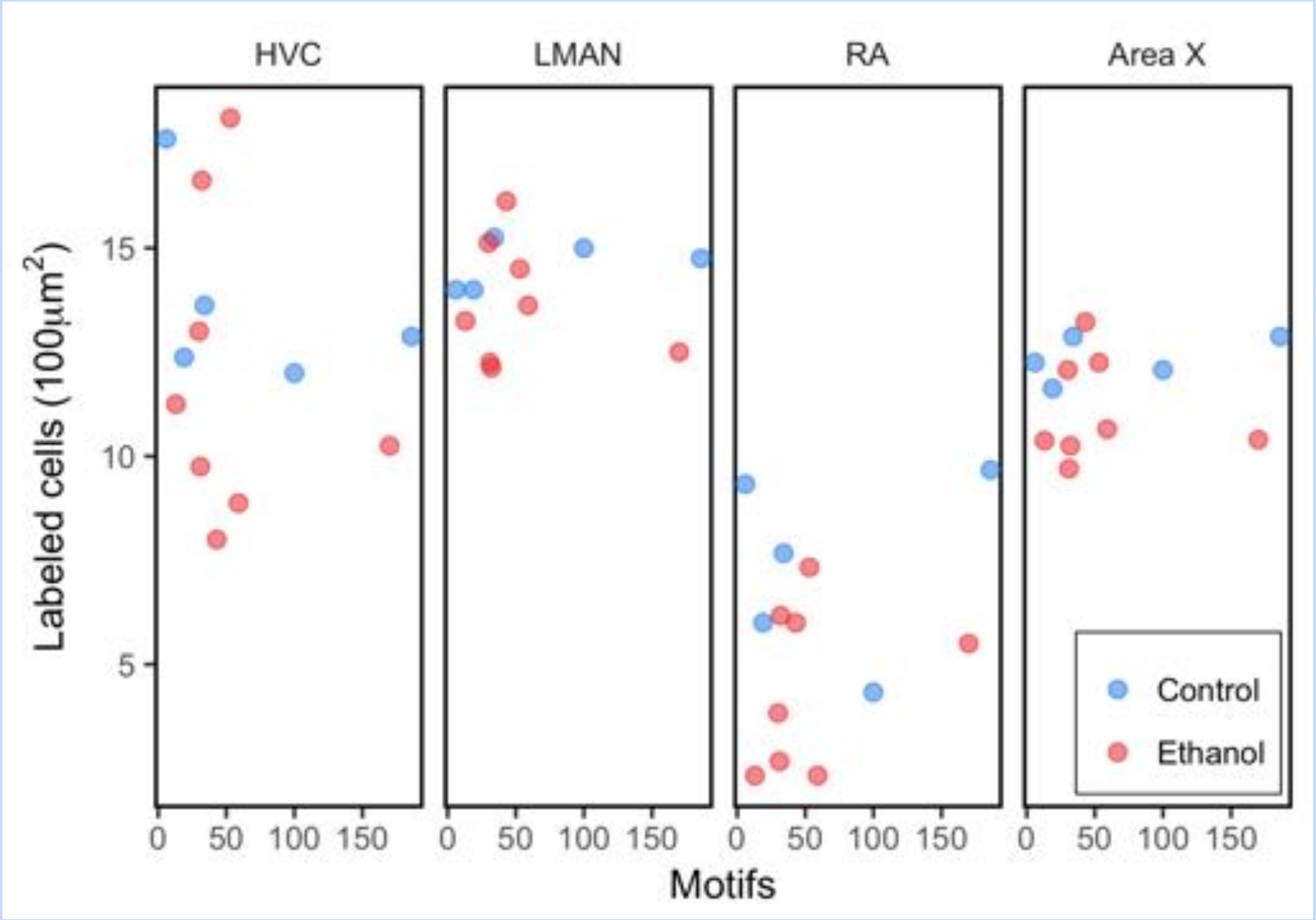
5 Alcohol does not affect amount of singing



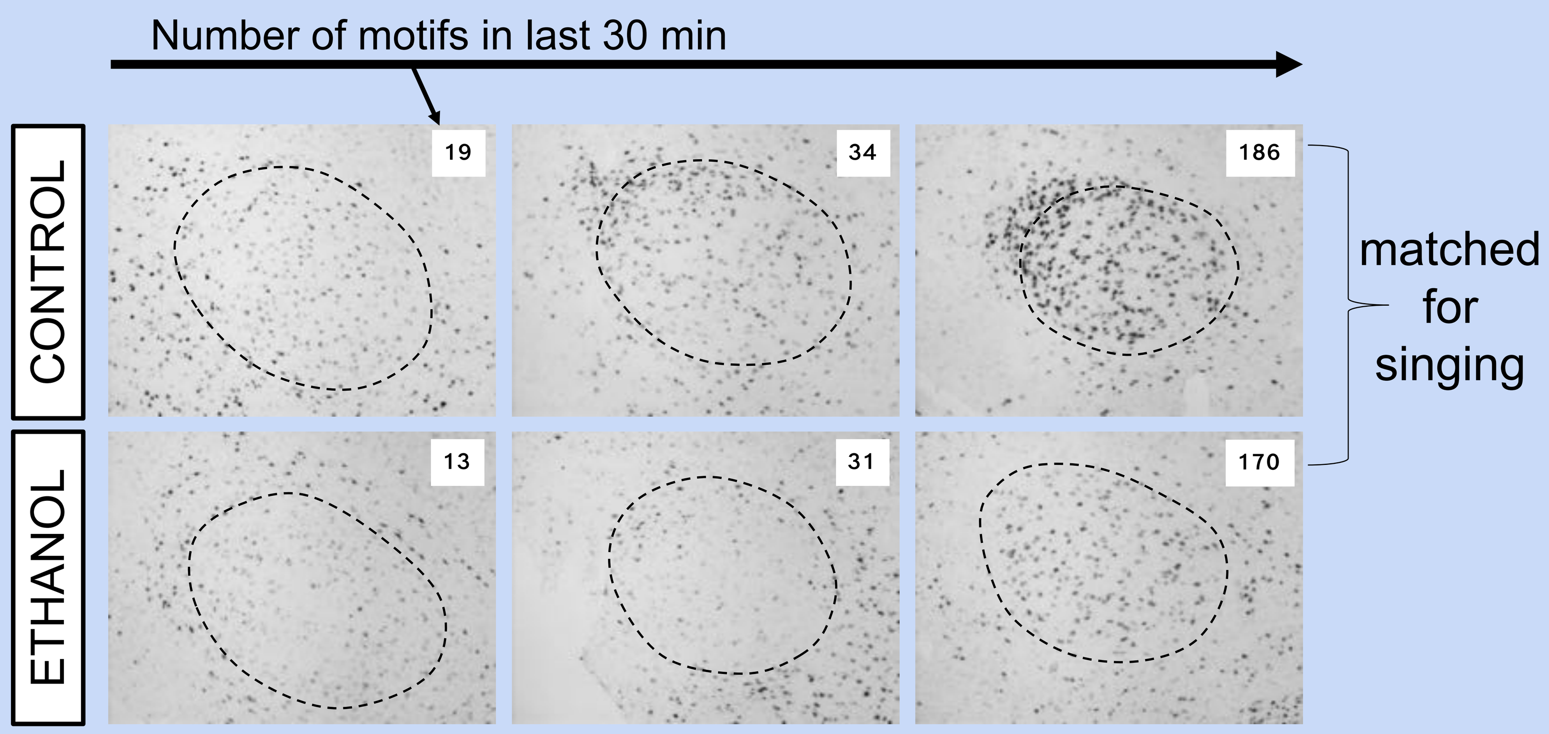
6 Singing patterns on the last day vary widely across birds



7 Variability in *zenk* labeling is higher in nuclei of the direct motor pathway (HVC and RA)

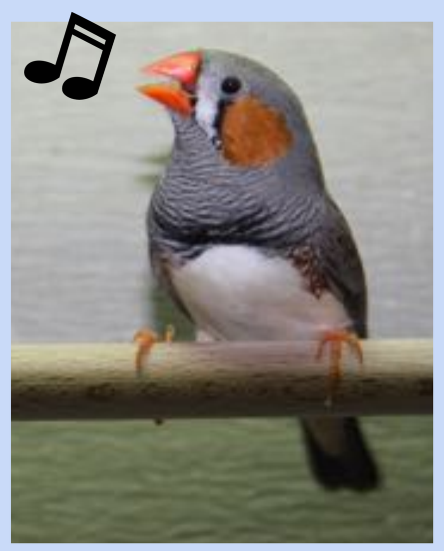


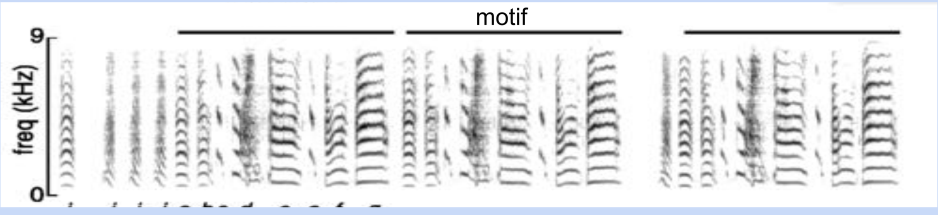
8 Decreased *zenk*-labeled cells in nucleus RA of birds consuming alcohol



3 Methods Overview

1. Record song from birds drinking alcohol or juice
2. Measure blood ethanol concentration (BEC) and sacrifice animals
3. Cryosection brains
4. Synthesize *zenk* probe
5. *In situ* hybridization
6. Count labeled cells in song nuclei

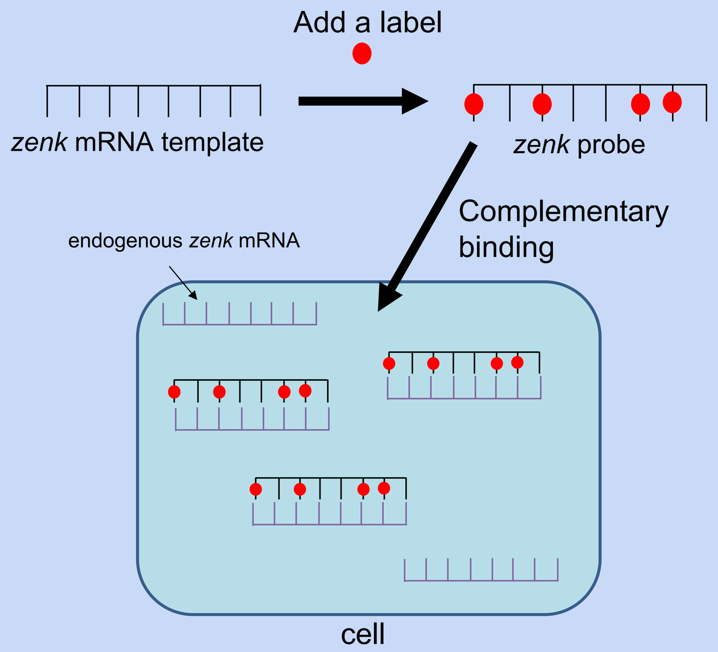


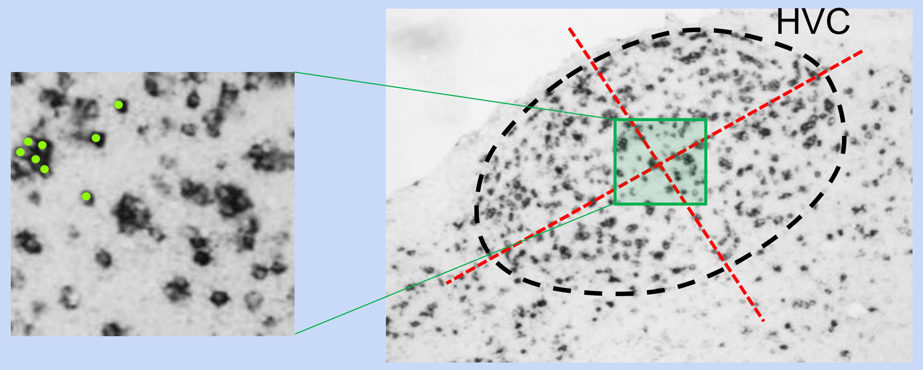


Scheduled access to alcohol

	Habituation	4 hr access	4 hr access
Alcohol n = 9	water	50% juice	6.5% alcohol in 50% juice
Control n = 5	water	50% juice	50% juice
# days	1	4	4

In situ hybridization





Counting labeled cells

Conclusions

1. Zebra finches are an advantageous model to study how alcohol alters neuronal control of learned vocalizations.
2. High variability in a spontaneous behavior was mirrored by high variability in *zenk* induction within nuclei of the direct motor pathway.
3. Song nucleus RA, an analogue for human laryngeal motor cortex, is a candidate site of action at which alcohol disrupts vocal behavior.

References

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