Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Insights from a Nonvocal Learner on Social Communication

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Review of Mahrt et al.

Language is unique to humans. As a result, the neurobiological underpinnings of language are difficult to study in animal models. Fortunately, components of language, such as vocal learning, occur in other animals, including cetaceans, pinnipeds, elephants, bats, and several classes of birds, including songbirds. Many of these animals are not amenable for laboratory study, however, and the ones that are well suited (e.g., birds) are difficult to genetically manipulate. Stereotactic injections of virus to alter songbird gene regulation are possible, but there is limited reach with this method, including the inability to interfere before hatching or early in development before song learning. Given these challenges, determining the capacity for vocal learning in traditional genetically tractable animal models, such as rodents, is important.

Male mice emit quantifiable ultrasonic vocalizations (USVs; 30–125 kHz) throughout their lifespan; pups call to signal distress and adult males call during courtship. There is ongoing scientific debate as to whether mice learn these vocalizations and the relevance of rodent models to vocal learning. Mahrt et al. (2013) present rigorous data that suggest rodent vocalizations are innate, not learned, but that rodents can nonetheless be valuable for elucidating genetic control of the brain circuitry underlying vocal motor function.

There are three well known experimental paradigms that test for vocal learning in animal species. In social isolation experiments, animals are reared in the absence of tutors or auditory models. Vocalizations from isolated animals are compared with normally reared animals to determine whether vocalizations are innate, or rather require memorized templates. In juvenile zebra finches, isolation from a tutor song results in disordered and abnormal singing behavior in young birds that persists into adulthood (Doupe and Kuhl, 1999). In many songbird species only the male sings, which allows young to be reared by females without exposure to song. Social isolation experiments in mice are difficult, if not impossible, to perform because both males and females emit USVs, and maternal care is critical for pup survival (Bowers et al., 2013).

A second test for vocal learning is cross-fostering. Vocal learning animals generate vocalizations that mimic the social environment in which they were raised, as opposed to vocalizations that characterize their genetic background. Cross-fostering experiments in mice suggest that mouse vocalizations are innate (Kikusui et al., 2011), because vocalizations of adult animals more closely resemble their genetic parentage than the vocalizations of the animals with whom they were raised. However, as Mahrt et al. (2013) indicate, cross-fostering studies in mice are confounded by restriction of high-frequency hearing in inbred mouse strains.

Perhaps the most compelling manipulation that can be used to determine the capacity for vocal learning is auditory deprivation during the sensory phase (a time period in which an animal is exposed to auditory stimuli from conspecifics to derive its later vocalizations; Konishi, 1965). Songbirds that are deafened before sensory acquisition (song memorization) never acquire or learn to sing a song. However, auditory deprivation studies have come to opposing conclusions regarding vocal learning in mice. First, Hammerschmidt et al. (2012) used Otoferlin knock-out mice to assess differences in USV acoustic structure between deaf and hearing mice. These knock-out mice model human deafness resulting from deficits in the inner hair cell synaptic vesicle protein otoferlin. No differences in USV spectral features from deaf and wild-type (WT; hearing) littermates were observed in either young or adult animals. However, one potential limitation of this study is that calls were classified into 2–3 major categories, as opposed to the 10 categories that have been described and quantified by Scattoni et al. (2008). A second deafening study found "striking" alterations in USV structure of pup vocalizations in caspase 3 (*Casp3*) knock-out mice (Arriaga et al., 2012). *Casp3* mice are born congenitally deaf because of the loss of inner ear hair cells shortly after birth. This study sorted calls into 11 categories, quantified differences between hearing and deaf animals for each call type, and concluded that auditory experience is important for strain-typical

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vocal production in both mouse pups and in mice mechanically deafened as adults. One major caveat to these data is that *Casp3* knock-out mice have abnormal brain morphology that could result in altered vocalizations independent of hearing loss (see Mahrt et al., 2013).

In this most recent contribution to the debate about vocal learning in rodents, Mahrt et al. (2013) used conditional cell ablation to selectively kill all hair cells before the onset of hearing at postnatal d9 (P9), thereby avoiding any confounds present in previous studies (Kikusui et al., 2011; Arriaga et al., 2012). The mouse strain, CBA/CaJ, had human *DTR* (diphtheria toxin receptor) inserted into a gene (*Pou4f3*) found exclusively in hair cells. As a result, when diphtheria toxin was injected into P2 mice, all *Pou4f3*$^{+/DTR}$ mice were rendered deaf, and all WT littermates were spared hearing loss. All animals were raised within litters and subsequently lived within mixed-genotype colonies to control for exposure to the acoustic environment. Male USVs were recorded in the presence of a female for 15–20 min between 1 and 5 times between P60 and P70 to assess differences in adult courtship vocalizations between deaf-reared and control animals. After behavioral testing, auditory brainstem responses were recorded to verify that injected *Pou4f3*$^{+/DTR}$ animals were functionally deaf, and that WT animals exhibited normal hearing (Mahrt et al., their Fig. 2). Additionally, intact cochleae were examined using immunohistochemistry to ensure that the manipulation eliminated hair cells from the inner ears of deafened animals only (Mahrt et al., their Fig. 3).

The authors analyzed USVs by categorizing calls into 12 different groups based on previously described criteria (Scattoni et al., 2008). Deaf and hearing animals emitted the same types of syllables and at approximately the same rate (Mahrt et al., their Fig. 4). Calls within each category were subjected to rigorous quantification (up to 50 parameters were used to quantify syllables within each class; Mahrt et al., their Tables 2–3). The described methodology permitted precise measurements of multiple aspects of each syllable, using software developed to semi-automatically categorize syllables. Importantly, no statistically significant differences between hearing and deaf animals in number, duration, frequency, spectral, or temporal aspects for calls within each USV category were observed (Mahrt et al., their Figs. 8–9), indicating that mouse vocalizations are innate and not learned.

Though the conclusions in this paper are well supported, replication of these results in another mouse strain will be critical, because different mouse strains exhibit different calling behavior (Kikusui et al., 2011). Furthermore, in songbirds, subtle differences in timing and variability of courtship song, which are difficult for humans to detect, greatly impact zebra finch female preference and partner choice. Therefore, it would be worth determining whether subtle changes to vocalizations from deaf mice detract from the overall reproductive success of the animal.

Overall, the study by Mahrt et al. (2013) provides strong support for the innate capacity for vocal production, yet clearly suggests that CBA/CaJ mice do not learn their vocalizations. Although the lack of vocal learning in mice may limit their use for studies pertaining to human speech learning, mice may still be useful for studying the general mechanisms of vocal communication, and perhaps more importantly, the molecules putatively involved in vocalization. For example, mouse pup isolation calling appears to be related to *FOXP2* function, a gene essential for language in humans (Lai et al., 2001) and song learning in zebra finches (Haesler et al., 2007). Male mouse pups call more than females, tend to be retrieved by their dams preferentially, and have higher Foxp2 levels (Bowers et al., 2013). Expressing the human-like form of *FOXP2* in mouse pups resulted in changes to ultrasonic calling behavior (Enard et al., 2009). Despite the inability of rodents to acquire socially learned vocalizations, examining vocalizations in rodent models may underscore the relevance of Foxp2 and other molecules that affect vocal output across both vocal learning and nonvocal learning animals.

Analyzing similarities between rodents, songbirds, and humans will elucidate shared neuromolecular mechanisms of vocal learning and social communication. The parallels between bird song and human speech learning include reliance upon corticobasal ganglia-thalamic loops, social interactions that occur early in life, and similar neuromolecular mechanisms. Where one model falls short (i.e., molecular manipulation in songbirds or innate courtship vocalizations in rodents), the other model can compensate. Effects of genes such as *FOXP2* on vocal behavior across species strengthen the case that it is essential to vocal communication. Because the evolution of language and vocal learning are likely to rely on genes and molecules already in place in nonvocal learning species, a complementary panel of both songbird and rodent might best characterize a gene's contribution to vocalization. The findings of Mahrt et al. (2013) underscore a weakness in the established rodent model and open the door for cross-species comparisons in the future.

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