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A review of ultrasonic vocalizations in mice and how they relate to human speech^{a)}

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ABSTRACT:

Mice communicate through audible vocalizations, which are within the human hearing range, and ultrasonic vocalizations (USVs), which are above the upper limit of human hearing. USVs are produced by rodents in social contexts including pup separation, territorial, and courting assays. Like birdsong, an established model for human speech, USVs in mice have been used as a model for understanding human communication. Their utility as a model of social communication is illustrated in neurodevelopmental conditions with a genetic basis, like autism spectrum disorders and Rett syndrome. As mice do not exhibit clear visual cues when they vocalize, the source of vocalization is often assumed. Therefore, there is potential to better discern the true vocal contribution of individual mice if the upper limit of human hearing were to be extended. Currently, there are efforts to increase the precision of sound-localizing technology, which will develop our understanding of communication in mice and other animal models.

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I. INTRODUCTION

Communication can be conveyed through all sensory modalities; the most ubiquitous, and arguably most important, is acoustic. Vocal communication is a critical component for establishing and maintaining complex social structures including exchange of information on social status (identification of individuals or membership in a group) (Hoffmann *et al.*, 2012), mood (dominance, submission, cooperative behavior) (Nyby *et al.*, 1976; Neunuebel *et al.*, 2015), and environmental conditions (predator presence or food source location) (Slobodchikoff *et al.*, 2012). Additionally, communication is an indicator of an animal's fitness and willingness to mate and is a key factor in cultivating mother-offspring interactions (Noirot, 1972; D'Amato *et al.*, 2005; Ehret, 2005).

Animal vocalizations are common in vertebrates including rodents, ranging from simple tonal signals to intricate songs with multiple frequency components that modulate over time (Holy and Guo, 2005). The ability of animals to perceive acoustic signals is determined by the sensory range of the underlying auditory system. The frequency range of vocalizations are often species-specific (Sales, 1972; Turner *et al.*, 2005). By understanding the biological and psychological mechanisms underlying communication, we can

better understand their contributions to behavior in the wild and the laboratory.

Most small rodent species communicate using ultrasonic vocalizations (USVs) above 20 kHz, with some species of bat producing calls with frequencies as high as 200 kHz (Heffner *et al.*, 2001; Bohn *et al.*, 2004). These frequencies are much higher than the upper limit of human hearing—the human audible range spans 20 Hz to 20 kHz (Wegel, 1922; Snow, 1931; Durrant and Lovrinic, 1984)—and cannot be detected by human ears without technological assistance. Of the common laboratory animals, mice (*Mus musculus*) communicate in both the audible (20 Hz to 20 kHz) and ultrasonic (>20 kHz) range of sound frequencies (Fig. 1), though most calls range from 30 to 120 kHz (Noirot, 1972; Sales, 1972; Gourbal *et al.*, 2004; Ehret, 2005; Brudzynski, 2007; Vogel *et al.*, 2019). The spectro-temporal components of USVs are influenced by size, age, sex, genetic makeup, social status, and reproductive state, which in turn affect vocalization rate, frequency, and duration. This review will focus on mice models.

Rodent vocalizations are of interest to animal researchers as they can provide information on socio-communicative features of disease, treatment efficacy, or experimental models. Rodent USVs are a robust outcome measure of neurological function and communicative behavior and can serve as a proxy of social interest as well as motor performance (Nyby *et al.*, 1976; Moles *et al.*, 2007; Scattoni *et al.*, 2009). For instance, USVs are produced by mouse pups when cold, removed from the home nest, or separated from their parents

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Sound Spectrum

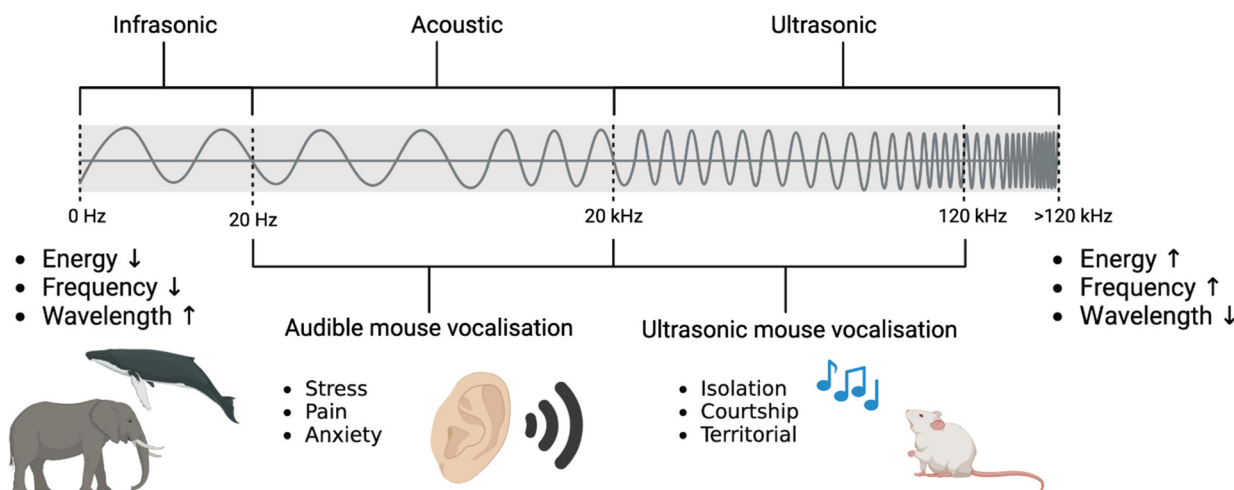


FIG. 1. (Color online) Mice produce vocalizations across a wide range of frequencies. Mice vocalize across a broad range of frequencies, extending from the human-audible range (when squeaks, peeps, and chatters are heard, below 20 kHz) to the ultrasonic range (above 20 kHz). While mice do emit audible vocalizations in response to stress and pain stimuli, their vocal communication is typically within the ultrasonic frequency range between 30 and 120 kHz. Maternal isolation-induced calls from mice pups, adult courtship and territorial disputes have been observed across this ultrasonic range. Infrasonic sounds (sound waves below audible levels) have not been observed in mice, but rather in large mammals such as elephants (Venter and Hanekom, 2010) and whales (Berchok *et al.*, 2006). Created with BioRender.com.

or littermates (Fish *et al.*, 2000; Ehret, 2005; Scattoni *et al.*, 2008); by males in the presence of females upon detection of pheromones (Wysocki *et al.*, 1982; Gourbal *et al.*, 2004); and by adult mice in aversive or rewarding contexts (Brudzynski, 2007; Arriaga and Jarvis, 2013). Isolation-induced calls from pups (Fig. 2) are an established index of pup emotional and social motivation (Ehret, 2005).

A commonly deployed assay for eliciting USVs in mice draws on male–female courtship interactions. Adult males will vocalize when near females in the estrus phase, providing a model where calls can be compared within and between litters and genotypes. USVs produced in courting mice are analogous to some birdsong where melodic changes in frequency are observed (Holy and Guo, 2005).

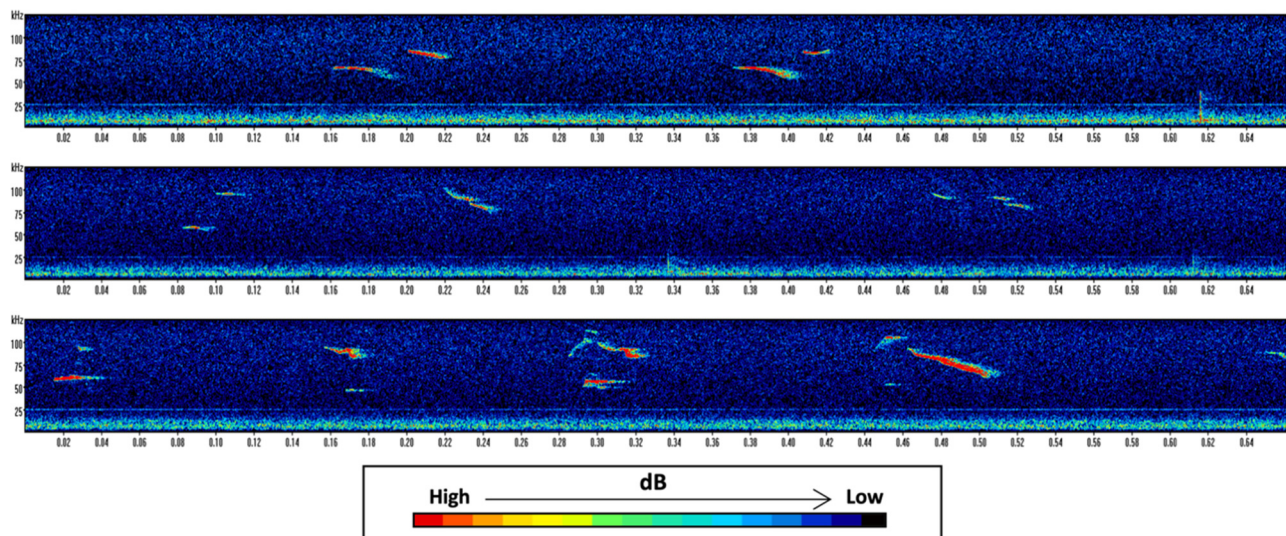


FIG. 2. (Color online) Sonograms of maternal separation-induced calls from mice pups at 4, 8, and 12 days postnatal. Example sonograms (spectrogram of sound waves) of ultrasonic vocalizations (USVs) produced by C57BL/6J mice pups at postnatal days 4 (top), 8 (middle), and 12 (bottom) after being separated from their mothers. Pups begin producing USVs from birth, increasing at 4 days and peaking 8 days postnatal, before significantly reducing their vocalizations two weeks after birth (Zippelius and Schleidt, 1956; Noirot, 1966; Yin *et al.*, 2016). Isolation-induced calls are an established index of pup emotional and social motivation (Ehret, 2005), and have been used to study neurodevelopmental disorders like autism (Scattoni *et al.*, 2008). Time (in seconds) is indicated by the X-axis, frequency (in kHz) is indicated by the Y-axis.

Owing to the complexity of their vocal communication, USVs from mice have also been proposed as a model for studying neurodevelopmental disorders associated with deficits in social communication including autism (Scattoni *et al.*, 2008) and speech disorders (Fischer and Hammerschmidt, 2011). USVs have also been recognized as behavioral indicators of disease progression and treatment response in some neurodegenerative diseases (Tsai *et al.*, 2012; Grant *et al.*, 2014; Mo *et al.*, 2015). As such, a better understanding of the underlying mechanisms for USV production and how they manifest across different experimental assays, conditions and methods for analysis is needed.

II. ULTRASONIC VOCALIZATIONS: LEARNED OR INNATE?

The nature of mice USVs remains an ongoing discussion requiring further investigation: are these vocalizations innate or learned? This is a critical aspect to consider when evaluating the utility of vocal communications in mice as a model for human communication, as the ability to learn is an integral component of human speech.

A. Animal models for human speech

Vocal production learning is the ability to modify vocal output by reference to an external model. This process has been identified in a range of non-human species, including bats (Prat *et al.*, 2015; Lattenkamp *et al.*, 2021; Vernes *et al.*, 2022), elephants (Poole *et al.*, 2005; Stoeger *et al.*, 2012), and a range of aquatic and semi-aquatic mammals (Miksis *et al.*, 2002; Schusterman, 2008; Janik, 2014). Of these, songbirds are the most widely studied given their vocal learning abilities, which include their capacity for modifying the spectral and syntactic composition of vocalizations and vocal mimicry using a process similar to human speech acquisition (Marler, 1970; Doupe and Kuhl, 1999; Jarvis, 2004).

Like human speech, birdsong is highly structured with multiple components—notes come together to form syllables, syllables join to form phrases, and combinations of phrases constitute the basis of song (Doupe and Kuhl, 1999). Importantly, the timing and order of syllables and phrases have been shown to follow a conspicuous set of species-specific rules (Konishi, 1985), and different songs in a bird's song repertoire convey different meanings, for instance territorial defense or mate attraction (Catchpole, 1983; Searcy *et al.*, 2000). Song complexity alone does not fully explain vocal learning. The song repertoire of a domesticated zebra finch ranges from 3 to 15 notes (Zann, 1996), while a domesticated canary's repertoire contains dozens of notes (Nottebohm and Nottebohm, 1978) that combine into syllables and phrases. Although the songs of these birds differ in syllabic complexity, both species have vocal learning trajectories that culminate in stereotyped and highly determined song structures (Williams, 2004).

Human speech and birdsong share further similarities: they depend on auditory feedback for learning and

maintenance of learned vocalizations, have critical periods for learning that are temporally restrictive, and require specialized neural networks for vocal communication (Marler, 1970; Doupe and Kuhl, 1999; Jarvis, 2004). Vocal learning processes in humans and songbirds rely on specific forebrain circuits, and these are yet to be identified in species that produce only innate vocalizations (Jarvis, 2004; Jürgens, 2009). For instance, closely related non-human primate species like squirrel monkeys (Hammerschmidt *et al.*, 2001) appear to lack both the behavioral and neural features characteristically associated with vocal learning.

The study of vocal learning in songbirds has focused on song, which is typically learned during development. Vocal learning is initiated in young birds following exposure to a song model from an adult “tutor”; this is evidenced by the rapid production of structured sound units (i.e., syllables) in the offspring. To learn song, “tutee” birds must compare these sounds with a memory template of the song model utilizing auditory feedback inputs (Doupe and Kuhl, 1999). Learning songs is fully achieved through transforming and distinguishing prototype sounds until tutee birds can replicate the different syllables of the song model demonstrated by the tutor bird. This process is analogous to that used by humans to learn spoken language, making song-producing birds a prominent model for studying vocal learning.

While a paragon for vocal learning, some songbird species have demonstrated innate components to their vocalizations. One example is the eastern phoebe, a species of small bird endemic to North and Central America. They have been shown to produce typical songs following acoustic isolation and bilateral deafening experiments via removal of the cochlea—highlighting their capacity for innate song development (Kroodsma, 1985; Kroodsma and Konishi, 1991). These experiments suggest that some birds do not require a model to produce species-typical vocalizations and can produce songs comparable to birds that do require a song model to learn typical vocalizations.

B. Evidence for learned vocalizations in mice

USVs emitted by mice and songs produced by some songbirds share notable features. In mice, the spectro-temporal and melodic characteristics of male courtship USVs are well documented and feature reproducible sound units (Holy and Guo, 2005; Scattoni *et al.*, 2008). Hence, they have been compared to the songs produced by some birds. Songs, in this instance, can be classified as a set of often elaborate calls delivered periodically and sometimes with a rhythm (Arriaga and Jarvis, 2013).

Like other communicative species, the specific acoustic characteristics of rodent USVs vary within and between different strains of mice, but they adhere to a broad set of syllabic and temporal features that can be categorized according to their visual presentation on spectrograms (Fig. 3). Attempts to objectively quantify these calls via acoustic analysis and machine learning classification continue (Scattoni *et al.*, 2008; Van Segbroeck *et al.*, 2017; Zala *et al.*, 2017;

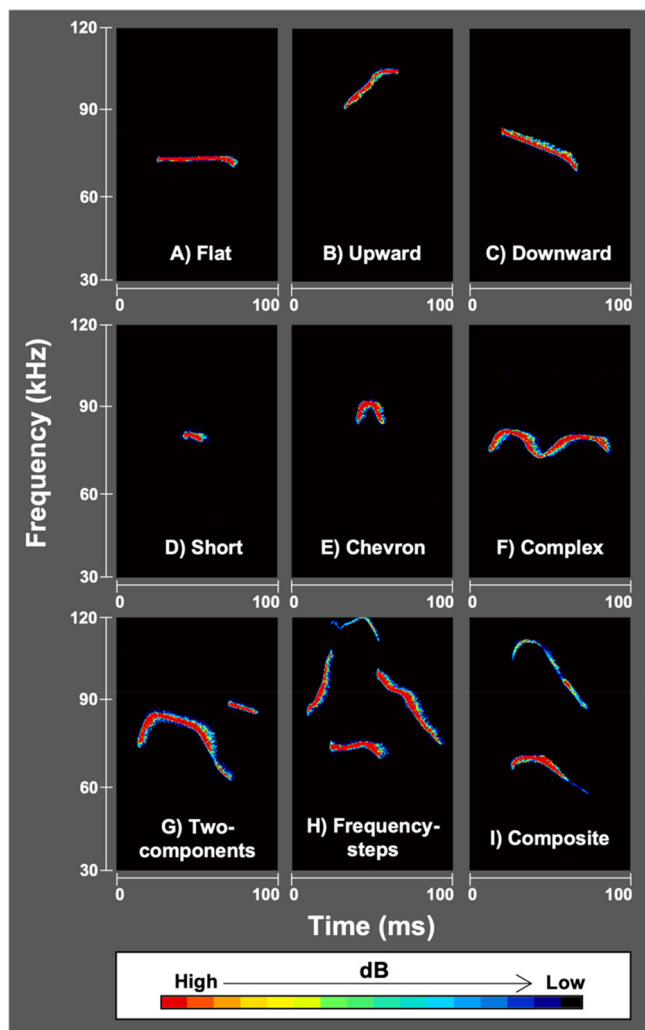


FIG. 3. (Color online) Exemplary sonograms of USVs of mouse pups highlighting nine distinct call categories. These call categories were defined by Scattoni *et al.* (2008) based on previous categorization approaches (Branchi *et al.*, 1998; Brudzynski *et al.*, 1999; Panksepp *et al.*, 2007). Ultrasonic vocalizations representing (A) flat, (B) upward, (C) downward, (D) short, (E) chevron, (F) complex, (G) two-components, (H) frequency steps, and (I) composite call categories. Time (in milliseconds) is indicated by the X-axis, frequency (in kHz) is indicated by the Y-axis.

Vogel *et al.*, 2019; Ivanenko *et al.*, 2020; Premoli *et al.*, 2021). The complexity and unique temporal structure of mice USVs has led to comparisons with those of birdsong and human speech, both of which are learned processes. These parallels have been adopted by animal researchers to explore USVs as a model of communication in conditions where the genetic basis of a disease is known and replicable in mouse models—particularly in neurodevelopmental disorders (Scattoni *et al.*, 2011; Wöhr *et al.*, 2013; Zampieri *et al.*, 2014).

Vocal motor specific activity in premotor forebrain circuits in mice is thought to be comparable to the pathways observed in the songbird vocal system during active singing (Jarvis and Nottebohm, 1997; Jarvis *et al.*, 2000; Jürgens, 2002). Neural tracing experiments show mice have developed vocal premotor neural pathways that project directly to

vocal motor neurons in regions of the brain relevant to communication, including the brainstem and thalamus (Arriaga *et al.*, 2012). These pathways, although not identical to humans or songbirds, are reminiscent of known circuits involved in vocal production across species.

Disrupting audio feedback at select stages of development is known to impact vocalizations in animals and humans. In songbirds, deafening in early stages of song learning can degrade the complexity and accuracy of songs, resulting in highly variable temporal structures and unstable notes used to construct their songs (Konishi, 1965; Marler and Waser, 1977). Altering auditory feedback in mice leads to similar outcomes, lending support to the learned vocalization hypothesis. Surgical deafening experiments on adult mice have been conducted to assess whether deafening affects USV production (Arriaga *et al.*, 2012). After mice were deafened through bilateral cochlear removal, vocalizations from deaf mice were spectrally distorted with noisy syllables, reduced spectral purity, and higher pitch than those of hearing-intact control mice eight months post-surgical intervention. Based on this evidence authors concluded that USVs have a learned component, as innate vocalization would be minimally affected by altered auditory feedback.

C. Evidence for innate vocalizations in mice

Whilst there is evidence in support of learned vocalizations in the literature, recent findings have emphasized innate factors as a key component of vocal communication in mice. Mouse pups are born deaf, with their hearing developing 10 days postnatal (Ehret, 1975). During this period, they can produce vocalizations without auditory feedback (e.g., during pup-mother separation paradigm). The influence of modelling and social environments on USVs during early development has been assessed through cross-fostering experiments (Kikusui *et al.*, 2011) and deafening (Hammerschmidt *et al.*, 2012b; Mahrt *et al.*, 2013).

The role of disorders of the central nervous system in mice communication is acutely illustrated through models of disease related to social communication. C57BL/6J (B6) and BALB/cAJcl (BALB) mice strains present with unique USV phenotypes. B6 males produce vocalizations with shorter intervals between syllables and higher peak frequency of syllables, as well as different syllable composition compared to their BALB male counterparts (Kikusui *et al.*, 2011). In this experiment, male offspring of B6 mice were reciprocally cross-fostered by BALB parents (Fig. 4). That is, B6 pups were fostered by BALB parents until weaning, and vice versa. The courtship USVs emitted by the fostered male mice (e.g., B6-foster) were subsequently compared to those of naturally reared control male mice (e.g., B6-son) once they matured into adults at 10–20 weeks of age. Cross-fostered offspring produced USVs with acoustic characteristics that resembled those of their genetic parents rather than their foster parents. These strain-specific characteristics included marked frequency modulation patterns and syllable

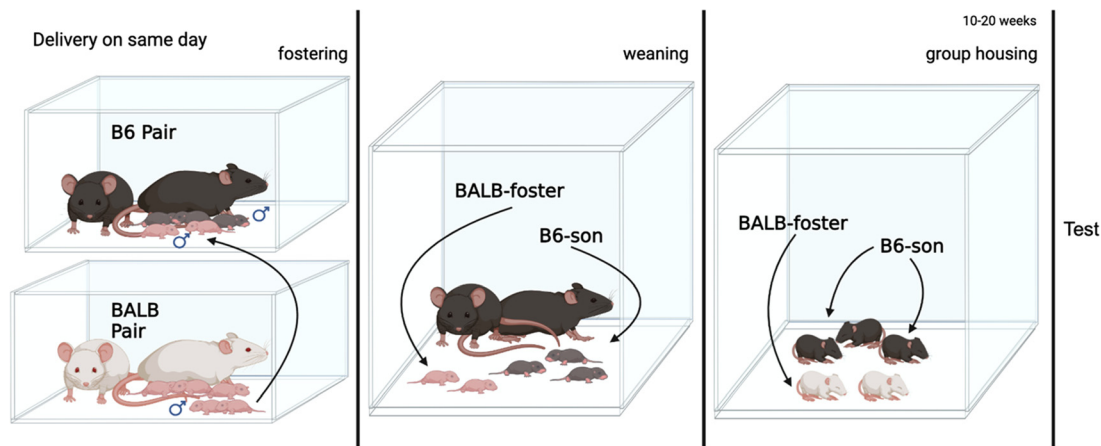


FIG. 4. (Color online) Cross-fostering experiments using C57BL/6J (B6) and BALB/cAJcl (BALB) mice strains. Illustrative diagram demonstrating the experimental design of cross-fostering BALB mice pups to B6 parents (Kikusui *et al.*, 2011). A few BALB pups from a newly born litter were removed from their BALB birth parents and placed in the care of B6 foster parents (BALB-foster). Similarly, a few B6 pups were reciprocally removed from their B6 birth parents and given to BALB foster parents (B6-foster, not depicted). The control mice (BALB-son and B6-son) were handled in the same manner but returned to their birth parents. All litters remained undisturbed until weaning (21 days postnatal), wherein the foster parents were removed, and both foster and control pups were housed together (i.e., BALB-foster with B6-son, B6-foster with BALB -son). USVs of the mice were recorded between 10 and 20 weeks of age. Created with BioRender.com.

intervals, in addition to differences in the temporal structure of vocalizations (Kikusui *et al.*, 2011). Data suggests that USVs may have a strong genetic component, not necessarily requiring imitative vocal learning in the same way that birds typically learn songs and humans learn speech.

Subsequent work explored the use of deaf mice to delineate the innate nature of murine vocalizations. Investigation of the occurrence and structure of USVs from otoferlin-knockout mice, a model for human deafness, in comparison to those of hearing wildtype and heterozygous littermates (Hammerschmidt *et al.*, 2012b) showed that USVs produced by deaf genetic knockout mice, hearing wildtype, and heterozygous littermates did not significantly differ in number and nature of acoustic features. Similarly, comparisons of chronically deaf and normal hearing adult CBA/CaJ male mice revealed minimal differences in vocalization profiles (Mahrt *et al.*, 2013). Complementary work examining the importance of cortical structures on song development through cerebral cortex removal also showed that mice were able to produce typical songs (Hammerschmidt *et al.*, 2015).

A limitation of using mice strains like C57BL/6 for studying vocalizations is that age-related hearing loss associated manifests with the strain [i.e., rapid hearing deficits by 8 weeks (Zhu *et al.*, 2007)]. Yet mice pups begin producing USVs from birth, increasing at 3–4 days and peaking 8 days postnatal before dramatically reducing their vocalization rate, nearing 0, two weeks after birth (Zippelius and Schleidt, 1956; Noirot, 1966; Yin *et al.*, 2016). As such, this age-related hearing loss is less relevant to vocalizations studies completed in mice <6 weeks old. If considering innate USV production, the impact of hearing loss in older mice is less relevant to the assay.

Taken together, these findings suggest the use of mice as a model of mammalian vocal learning needs to be considered carefully. Rodent models of disease assist in elucidating the molecular and neural foundation of social and

communicative behavior. The utility for investigating USV and vocal behavior requires a clear understanding of the research aims and the mice model in question.

D. Sex differences in social communication

USVs are a key form of social communication. Interactions have been studied across specific life phases and contexts, including pup isolation (Fish *et al.*, 2000; D’Amato *et al.*, 2005) and female-male mice courtship assays (Wysocki *et al.*, 1982; Gourbal *et al.*, 2004; Holy and Guo, 2005). Female mice can distinguish male courtship songs from pup isolation calls, and selectively approach the source of the courtship USVs rather than the isolation USVs (Hammerschmidt *et al.*, 2009). Female mice appear to be more attracted to USVs of unfamiliar non-kin male mice versus familiar siblings (Musolf *et al.*, 2010).

Adult female-female interactions have also been used to elicit USVs, which are thought to serve territorial functions (Hammerschmidt *et al.*, 2012a) and to establish social hierarchies (Moles *et al.*, 2007; Zala *et al.*, 2017). Female resident mice often produce more USVs when female intruders are present than male intruders, whereas male resident mice can be more responsive to female intruders than male intruders (Hammerschmidt *et al.*, 2012a). Female resident mice can be more vocal toward female intruders than their male resident counterparts, indicating a sex-dependent territorial aspect to the USVs produced. Parallel to these phenomena, anesthetized intruders can elicit acoustically different USVs in resident mice (Hammerschmidt *et al.*, 2012a), suggesting that mice differentiate between behavioral states of the intruders through USVs.

Altogether, these findings corroborate the hypothesis that USVs are unique and distinguishable, and contribute to social (individual, sibling) recognition, inbreeding avoidance, as well as other social and reproductive functions.

III. EMPLOYING ULTRASONIC VOCALIZATIONS IN CLINICAL PRACTICE

USVs are a core component of mice social behavior. Mice produce the highest number of USVs with the largest variety of call types when interacting socially (Chabout *et al.*, 2012; Zala *et al.*, 2017; Burke *et al.*, 2018). The importance of communication in behavioral assays suggest USVs are a suitable feature to explore in neurodevelopmental, affective, and psychiatric conditions where sociability and communication are impacted.

A. Model for neurodevelopmental disorders

USVs are a valuable tool for studying pathologies associated with neurodevelopmental disorders [e.g., autism spectrum disorders (ASD)] (Scattoni *et al.*, 2008; Fischer and Hammerschmidt, 2011; Binder and Lugo, 2017; Tesdahl *et al.*, 2017; Simola and Granon, 2019; Yang *et al.*, 2021). Neurodevelopmental disorders are an umbrella term for conditions that may be congenital or manifest in childhood and are defined by neurological or behavioral delays, and/or deficits during early developmental periods. They are also characterized by abnormal cognitive, language, social, affective, and motor behaviors (Hansen and Rogers, 2012; Homberg *et al.*, 2016).

B. Understanding autism spectrum disorders

ASD are a heterogenous group of disorders characterized by two behavioral symptoms that appear in early childhood—the first being abnormal social interactions, and the second being restricted, repetitive behavior and interests (American Psychiatric Association, 2022). ASD can result in significant reductions in quality of life for the individual and their community (Leigh and Du, 2015; Brown *et al.*, 2019; Rogge and Janssen, 2019).

There are no established bio-physiological measures (e.g., blood test or imaging procedure) that characterize ASD at present. The types of biological markers under investigation include genetic, prenatal history, neurological (e.g., neuroimaging), metabolic (e.g., abnormal mitochondrial pathways), immune (self-antibodies and cytokine dysregulation), and nutritional (Jensen *et al.*, 2022). Many biomarkers show promising preliminary evidence (Frye *et al.*, 2019), yet individuals with ASD are diagnosed, treated, and understood through their associated behavioral symptoms. The lack of biomarkers, combined with the heterogeneity of ASD highlights the need for phenotypically strong animal models of disease to maximize opportunities for treatment development. In that context, studying mouse USVs can provide an ecologically valid means of evaluating pharmacological and behavioral therapies for these conditions.

C. Using mice to explore communication disorders

Mice engage in social behaviors from birth as pups all through their adult life. The changes that occur across the

lifespan provide an opportunity to interrogate therapies and underlying mechanisms are crucial stages of each disease. Individuals with ASD can present with different speech characteristics compared to typically developing speakers, including monotonic or machine-like intonation, atypical phonation, impaired use of pitch and loudness, and use of aberrant stress patterns (Tager-Flusberg, 1981; Sheinkopf *et al.*, 2000; Oller *et al.*, 2010; Bonnef *et al.*, 2011; Morgan *et al.*, 2021).

Studying murine USVs in neurodevelopmental disorders may improve our understanding of the speech deficits observed in humans, especially in minimally verbal children with ASD (Shu *et al.*, 2005; Young *et al.*, 2010; Ey *et al.*, 2012; Roy *et al.*, 2012; Yang *et al.*, 2015; Yang *et al.*, 2021). SHANK3 is a scaffolding protein that regulates the formation, maturation, and maintenance of synapses between neurons in the human brain (Lim *et al.*, 1999). Mutations in SHANK3 have been identified in individuals with ASD (Durand *et al.*, 2007; Moessner *et al.*, 2007) and the gene has been implicated in the pathogenesis of ASD (Sykes *et al.*, 2009; Cooper *et al.*, 2011). Mice deficient in the *Shank3* gene have presented with abnormal USV emission patterns and social behaviors, as well as repetitive behaviors (Bozdagi *et al.*, 2010; Wang *et al.*, 2011; Sungur *et al.*, 2016; Sungur *et al.*, 2018) classically observed in individuals with ASD.

Rett Syndrome (RS) is another neurodevelopmental condition leading to profound communication impairment (Burford *et al.*, 2003; Lee *et al.*, 2013; Einspieler and Marschik, 2019; Bartl-Pokorny *et al.*, 2022). RS is caused by *de novo* mutation in the methyl CpG binding protein MECP2 (Amir *et al.*, 1999; Zoghbi, 2005) and *Mecp2* mutant mice serve as a model for the disorder. The mouse model, akin to humans with the variant, show abnormal communication patterns in early postnatal periods (De Filippis *et al.*, 2010), as well as in response to social isolation (Picker *et al.*, 2006).

D. Ultrasonic vocalizations: From bench to bedside?

Mice pup USVs have been likened to the cries of human infants, particularly in the context of neurodevelopmental disorders like ASD (Scattoni *et al.*, 2008). Isolation-induced USVs from mice pups are an established index of pup emotional and social motivation (Ehret, 2005), while infant cries are one of the first affective social behaviors perceptible in human development (Schaffer and Emerson, 1964; Furlow, 1997). Both patterns of social behavior are acts of communication, and so the acoustic and functional features of mice USVs have been suggested to reflect the same purpose as infant cries in their capacity to elicit responses from caregivers (Scattoni *et al.*, 2008; Scattoni *et al.*, 2011). Cry patterns of infants with ASD are thought to differ in some circumstances to typically developing infants and those with other types of developmental delays (Esposito and Venuti, 2008, 2010). Similar work in mouse models corroborate

these findings (Scattoni *et al.*, 2009; Esposito *et al.*, 2017; Shekel *et al.*, 2021).

Investigation of features core to human disease phenotypes in mice is an established approach designed to substantiate animal models for these conditions. It also serves to use these features as indicators of differential responses to environmental changes from early postnatal periods prior to the typical period when more complex aspects of behavior, such as social interactions and information processing. Hence, USVs serve as a useful parameter for assessing vocalization and vocal behavior and may indicate early signs of cognitive abnormalities that portend later deficits.

IV. EXTENDING THE UPPER RANGE OF HUMAN HEARING

The study of USVs in rodents has been facilitated by advances in audio recording and analysis technology, which have allowed researchers to make detailed measurements of the acoustic properties of these sounds (Vogel *et al.*, 2019; Fonseca *et al.*, 2021; Abbasi *et al.*, 2022). This has led to a better understanding of the role that USVs play in rodent communication and behavior, as well as their potential use as markers of stress (Yin *et al.*, 2016; Feifel *et al.*, 2017; Schmidt *et al.*, 2017), anxiety (Budylin *et al.*, 2019; Yamauchi *et al.*, 2022), pain (Jourdan *et al.*, 1995; Williams *et al.*, 2008; Kurejova *et al.*, 2010; Smith *et al.*, 2020) and other behavioral states. USVs are typically above the frequency range of human hearing (typically around 20 Hz to 20 kHz), yet interest in the acoustics of USVs in rodents that could still fall within the upper range of human hearing endures.

A. Currently audible mouse vocalizations

Mice produce vocalizations audible to humans. Audible calls are emitted in stress and pain contexts, such as during handling and restraint situations (Whitney, 1969), or surgical procedures like tail snipping and ear notching (Jourdan *et al.*, 1998; Williams *et al.*, 2008). The specific vocalizations audible by humans depend on several factors, including the species producing the calls, the frequency range of the calls, and the listener's hearing ability. In general, USVs that are lower in frequency are more likely to be audible. Even within this frequency range (<20 kHz), there is variability in the ability of individuals to hear USVs. The effects of high-frequency sound exposure in humans suggests there are some adverse effects including fatigue, headache, tinnitus, and pain (Ueda *et al.*, 2014; Leighton, 2016; Fletcher *et al.*, 2018).

B. Potentially audible mouse vocalizations

Our understanding of USVs and the role they play in communication may change if our hearing range was extended to reliably detect ultrasonic sound in other mammals. An important challenge currently limiting USV experimental work is accurate attribution of vocalization to the emitter. Mice do not exhibit obvious movements or other

visual cues when they vocalize (Chabout *et al.*, 2012), and vocal behaviors are similar across individual mice, both within and between sexes (Hammerschmidt *et al.*, 2012a). The source of vocalization is often assumed rather than known; for example, courtship USVs are often attributed to the male mouse in a male-female encounter (White *et al.*, 1998; Holy and Guo, 2005). Control experiments with anesthetized or devocalized male mice do seem to suggest that female mice rarely vocalize or are often completely silent (Whitney *et al.*, 1973; Warburton *et al.*, 1989; Sugimoto *et al.*, 2011) during these interactions. However, this does not mean that females do not contribute to these interactions—it is possible that female mice may still produce vocalizations but only in response to male calls.

If we were to extend our hearing into the ultrasonic range, we could better discern the true vocal contribution of individual mice. It is possible that the putatively defined male courtship calls may be intertwined with female USVs. Though we could study social interactions with male-male paradigms and thus definitively exclude the emission of female USVs (Hammerschmidt *et al.*, 2012a), the vocalizations produced would no longer be comparable to male courtship USVs because of changes in the behavioral state of the male mice.

Efforts to spatially localize sounds with higher precision (Zhang *et al.*, 2008) in mice vocalizations (Neunuebel *et al.*, 2015; Heckman *et al.*, 2017) have employed microphone arrays and estimation techniques for localizing and assigning USVs. These results have illustrated that female mice do indeed produce vocalizations during male-female courtship interactions (Neunuebel *et al.*, 2015). Additionally, differences in vocalization pitch, duration, frequency range, and energy between male and female mice were detected—contrary to some earlier work (Hammerschmidt *et al.*, 2012a).

The utility of wearable spatial hearing technology for ultrasonic frequencies allowing the listener to localize ultrasonic sources of interest in real-time (Pulkki *et al.*, 2021). There are prototypes designed to render an ultrasonic sound source audible to human ears after shifting pitch, as well as permitting the listener to localize it in the correct direction under both laboratory and field conditions. The development of this technology could change how we understand vocal communication and behavior in a variety of animal models, not just in rodents.

V. CONCLUSION

Vocalizations are an important mode of communication in both human and non-human species. Mice are a common animal model for a variety of experimental contexts, and USVs provide a rich source of information that we continue to study. Mice USVs can be classified into several types, including social calls, isolation calls, and courtship calls. Social calls are produced in response to the presence of intruding mice and to establish social hierarchies. Isolation calls are produced by mice pups that are separated from their mothers and diminish with age. Courtship calls are

produced by male mice during mating behaviors and are characterized by complex and highly structured acoustic patterns. While some findings suggest mice USVs have a learned component, recent evidence endorses the larger contribution of innate factors. Their utility in studying neurodevelopmental disorders with genetic components like ASD remains high when strong animal models exist. Improvements in recording technology and analytics have helped elucidate some aspects of vocal behavior in mice. Perhaps creating a stronger link between USVs and optimized human hearing will enhance our understanding of human-animal models and provide more opportunities for discovery work across treatment and underlying neurobiology.

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