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REVIEW

Ultrasonic communication in rats: appetitive 50-kHz ultrasonic vocalizations as social contact calls

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Abstract

Rats are highly social animals, with a rich social behavioral repertoire, including the emission of so-called ultrasonic vocalizations (USV). Typically, three main types of USV can be distinguished based on a number of acoustic features, such as call duration, peak frequency, and frequency modulation: (I) isolation-induced 40-kHz USV in pups, as well as (II) aversive 22-kHz USV and (III) appetitive 50-kHz USV in juvenile and adult rats. In this review, evidence from selective breeding, devocalization, and playback studies is summarized and discussed, and it is concluded that appetitive 50-kHz USV serve as situation-dependent socio-affective signals with important communicative functions, for instance as play signals and/or social contact calls.

Keywords Rough-and-tumble play · Social play · Social approach · Ultrasonic vocalization · Playback

Introduction

Species differ in their auditory abilities. Humans (*Homo sapiens*) are able to hear sounds in the frequency range between 16 and 20,000 Hz, with significant variance between individuals. Other species, however, are able to perceive sounds, which humans are not able to hear; either because sound frequency is too low for the human hearing system, as for instance in elephants (e.g., *Elephas maximus*; for reviews, see: Langbauer 2000; O'Connell-Rodwell 2007), or too high, i.e., above 20 kHz in the so-called ultrasonic range, as for instance in whales or dolphins (e.g., *Tursiops truncatus*; for reviews, see: Madsen and Surlykke 2013; McGowen et al. 2014) and bats (e.g., *Rhinolophus ferrumequinum;* for reviews, see: Jones and Teeling 2006; Geva-Sagiv et al. 2015), but also mice (*Mus musculus*; for reviews, see: Wöhr and Scattoni 2013; Portfors and Perkel 2014) and rats (*Rattus norvegicus*; for reviews, see: Brudzynski 2013; Wöhr and Schwarting 2013).

First evidence that at least some rodent species are able to hear in the ultrasonic range was provided by Gould and Morgan (1941) in rats (probably *Rattus norvegicus*). They trained rats to run from one compartment of a test box to another one whenever a tone was presented in order to avoid electric shock. Gould and Morgan (1941) found that rats did not only run to the other compartment when tones were applied, which are audible to humans, with sound frequencies of 1, 2, 4, 8, and 14 kHz, but also in response to tones with sound frequencies of 21 and 40 kHz not audible to humans. They concluded: "No animal in whom hearing has been studied at all adequately presents such a disposition of auditory sensitivity as this" (Gould and Morgan 1941). The ability of voles (*Myodes glareolus*) and deer mice (*Peromyscus maniculatus*) to perceive sounds in the ultrasonic range was then demonstrated by Schleidt (1948) and Dice and Barto (1952), respectively. First comprehensive comparative analyses were performed by Schleidt (1951, 1952).

Anderson (1954) then discovered that rats (*Rattus norvegicus*) are able to produce pure ultrasonic sounds, so-called ultrasonic vocalizations (USV). He concluded that these ultrasonic sounds "may serve for communication between individual rats" and added that "it is also conceivable, though certainly not yet demonstrated, that rodents use high-frequency sounds for orientation in some manner comparable to the process of echolocation employed by bats." Shortly

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after that, Zippelius and Schleidt (1956) reported that the house mouse (*Mus musculus*), the yellow-necked mouse (*Apodemus flavicollis*), and the common vole (*Microtus arvalis*) produce USV as pups when being removed from the nest and separated from dam and littermates, for which they used the term "Pfeifen des Verlassenseins" ("whistles of loneliness"), originally introduced by Lorenz (1935). For the first time, Zippelius and Schleidt (1956) thus implicitly established a link between ultrasonic calling and affect. They further performed the first series of studies on the communicative function of such isolation-induced USV emitted by pups. While Zippelius and Schleidt (1956) did not obtain evidence that the USV affect the behavior of the littermates, they observed clear behavioral responses in the dams, including search and retrieval behavior. Importantly, however, this response pattern was specifically seen when dams were exposed to pups scattered outside the nest, which were able to vocalize, but not in response to pups unable to vocalize. Zippelius and Schleidt (1956) reported that they were able to elicit search and retrieval behavior more than 100 times in a row by placing yellow-necked mouse pups able to vocalize outside the nest and concluded that this response pattern does not extinct in dams ("Die Reaktion des Weibchens auf das „Pfeifen des Verlassenseins" ist praktisch unermüdbar"; Zippelius and Schleidt 1956). The important communicative role of USV in maintaining dam-pup interactions was later confirmed by Sewell (1970) through playback experiments in wood mice (*Apodemus sylvaticus*). Sewell (1970) presented tape recordings of isolation-induced USV to lactating dams through a loudspeaker placed on one or the other side of a T partition away from the nest. Dams left the nest and displayed search behavior in response to 48 out of a total of 56 USV presentations (86%). Importantly, 38 out of these 48 responses were correct (79%), i.e., the dams entered the partition where isolation-induced USV were presented, clearly demonstrating that the dams were able to accurately localize the sound source. The dams generally reached the loudspeaker in less than a minute, yet did not respond to background noise or artificial 45 kHz pulses.

Types of ultrasonic vocalizations in rats

Today, it is known that mice and rats emit multiple distinct types of USV (for comprehensive overviews on USV in mice, see: Wöhr and Scattoni 2013; Portfors and Perkel 2014; for comprehensive overviews on USV in rats, see: Brudzynski 2013; Wöhr and Schwarting 2013). In rats, three main types of USV can be distinguished based on a number of acoustic features, such as call duration, peak frequency, and frequency modulation: (I) isolation-induced 40-kHz USV in pups, as well as (II) aversive 22-kHz USV and (III) appetitive 50-kHz USV in juvenile and adult rats (for representative spectrograms, see Fig. 1).

40-kHz USV (pups) Isolation-induced 40-kHz USV in pups are mostly characterized by call durations of ~ 100 ms or shorter, peak frequencies between 30 and 60 kHz, and are often frequency-modulated with various different call subtypes (Brudzynski et al. 1999). They occur in response to social isolation within the first 2 weeks of life and serve an important communicative function in eliciting maternal caregiving behavior, particularly search and retrieval behavior (Wöhr and Schwarting 2008a). In line with Zippelius and Schleidt's seminal work in mouse pups (Zippelius and Schleidt 1956), it

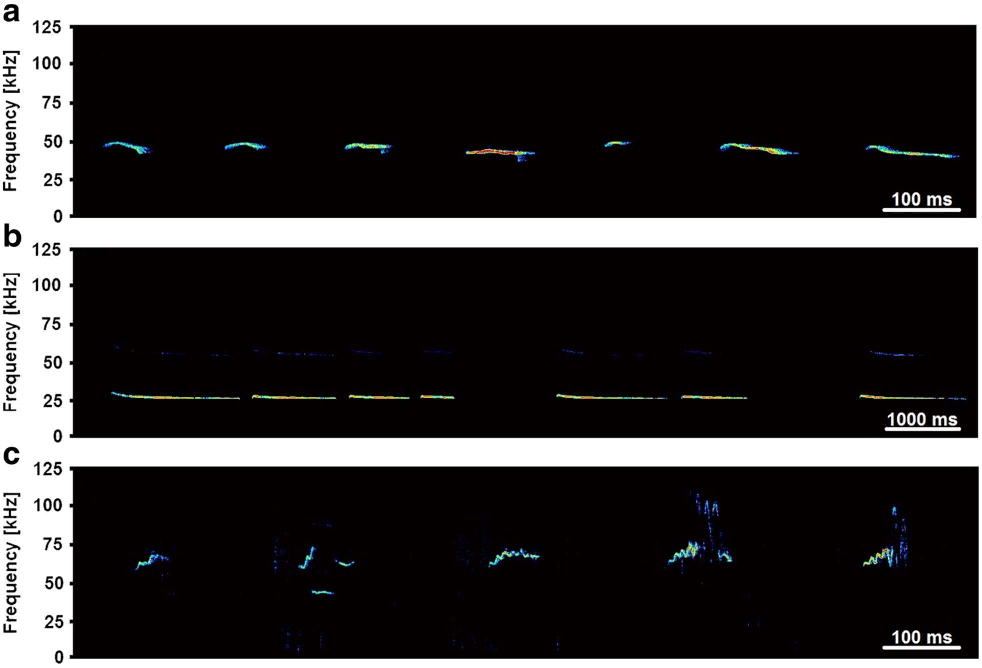

Fig. 1 Representative spectrograms of the three main types of ultrasonic vocalizations (USV) emitted by rats. **a** Isolation-induced USV emitted by a ~ 11 day old male Wistar rat during isolation from dam and littermates. **b** Aversive 22-kHz USV emitted by a ~ 3 month old male Wistar rat during fear learning. **c** Appetitive 50-kHz USV emitted by a ~ 1 month old male Wistar rat during rough-and-tumble play mimicked by a human experimenter through tickling. Please note the difference in time resolution in **b**, as compared to **a** and **c** (for other examples, see: Wöhr and Schwarting 2013)

is believed that they reflect isolation distress (for reviews, see: Brudzynski 2013; Wöhr and Schwarting 2013).

22-kHz USV (juveniles/adults) Aversive 22-kHz USV are characterized by comparatively long call durations of ~ 1000 ms or longer, a relatively narrow frequency range between 18 and 24 kHz and low levels of frequency modulation. They typically occur in bouts of 2 to 8 USV, with calls starting a bout often being characterized by higher peak frequencies and downward slope (Wöhr et al. 2005). Aversive 22-kHz USV occur in response to predator exposure (Blanchard et al. 1991, 1992) and social defeat (Sales 1972a; Lore et al. 1976), i.e., in life-threating and dangerous situations, and it is thus believed that they reflect a negative affective state akin to anxiety and fear (for reviews, see: Brudzynski 2013; Wöhr and Schwarting 2013). However, in males, they have also been repeatedly reported following ejaculation, which has a high rewarding value. It was thus speculated that such post-ejaculatory 22-kHz USV serve to maintain contact with the female and perhaps to discourage intervention by other males during periods of sexual inactivity (Barfield and Geyer 1972, 1975; Anisko et al. 1978; Adler and Anisko 1979; Thomas et al. 1982). Recently, Sachs and Bialy (2000) showed that the presence of females during the post-ejaculatory period facilitates penile erection and the emission of 22-kHz USV, and Bialy et al. (2016) challenged the idea that post-ejaculatory 22-kHz USV reflect negative affective states through applying a conditioned place preference paradigm. In the laboratory, aversive 22-kHz USV can be reliably elicited by air puffs (Brudzynski and Holland 2005; Browning et al. 2017) and electric shocks (van der Poel et al. 1989; van der Poel and Miczek 1991), as for instance during fear learning paradigms (Jelen et al. 2003; Wöhr et al. 2005). Freezing behavior, the most commonly used measure for fear in rodents, is highly positively associated with the emission of aversive 22-kHz USV (Wöhr and Schwarting 2008a). It is thus believed that aversive 22-kHz serve an alarming function and warn conspecifics about external danger, such as predators. In support of this idea, Blanchard et al. (1991, 1992) performed a series of experiments using large groups of rats living in a semi-natural environment, i.e., a visible burrow system, and found that the emission of aversive 22-kHz USV during predator exposure is potentiated by the presence of an audience, i.e., a group of familiar conspecifics living in the same environment. They further found that aversive 22-kHz USV emission by the sender led to defensive reactions in recipients not directly exposed to the predator. Under standardized laboratory conditions, it was shown that playback of aversive 22-kHz USV induces behavioral inhibition (Wöhr and Schwarting 2007) and potentiates the startle reflex (Inagaki and Ushida 2017) in the recipient. Aversive 22-kHz USV further appear to play a key role in the social transmission of fear (Wöhr and Schwarting 2008b; Kim et al. 2010).

50-kHz USV (juveniles/adults) Appetitive 50-kHz USV are characterized by comparatively short call durations. In many cases, call duration is shorter than ~ 50 ms. The frequency range, in which 50-kHz USV occur, is very broad and ranges from 30 to 90 kHz, yet most 50-kHz USV occur between 50 and 70 kHz. Many different subtypes have been described and frequency modulation is strongly depending on the subtype, ranging from flat calls often completely lacking frequency modulation to trills with a very high level of frequency modulation. However, there is no consensus on call subtype classification, with classification systems differentiating between 2 (Burgdorf et al. 2011b), 4 (Pereira et al. 2014), or even 14 (Wright et al. 2010) subtypes. Appetitive 50-kHz USV occur at high rates during rough-and-tumble play or play fighting in juveniles (Knutson et al. 1998; Lukas and Wöhr 2015) and mating in adults (Sales 1972b; Thomas and Barfield 1985). They were also detected during aggressive encounters in adulthood, yet compelling evidence was provided indicating that the dominant rat emits appetitive 50-kHz USV, whereas the defeated one produces aversive 22-kHz USV while freezing (Burgdorf et al. 2008). In the laboratory, high rates of appetitive 50-kHz USV can be elicited by mimicking rough-and-tumble play through tickling by a human experimenter (Panksepp and Burgdorf 2000; Wöhr et al. 2009), with dorsal and ventral tickling being most efficient in eliciting appetitive 50-kHz USV (Panksepp and Burgdorf 2003; Schwarting et al. 2007; Ishiyama and Brecht 2016). Despite variations in the exact tickling procedure between laboratories, tickling-induced appetitive 50-kHz USV were consistently observed and typically associated with strong approach behavior (for review, see: LaFollette et al., 2017). Another way of inducing high rates of appetitive 50-kHz USV in the laboratory is the administration of psychostimulants, most notably amphetamine (Pereira et al. 2014; Engelhardt et al. 2017a; for review, see: Rippberger et al. 2015). It is thus believed that 50-kHz USV reflect a positive affective state akin to joy and happiness (for reviews, see: Brudzynski 2013; Wöhr and Schwarting 2013) and it was Jaak Panksepp who suggested that they share some similarities to human laughter as the "evolutionary antecedents of human joy" (Panksepp 2005; for more details, see: Panksepp and Burgdorf 2000, 2003). Consistent with the idea that appetitive 50-kHz USV reflect a positive affective state, it was repeatedly shown that aversive stimuli inhibit their production. For instance, it was found that 50-kHz USV are inhibited under bright white light conditions during rough-and-tumble play (Knutson et al. 1998) and tickling (Ishiyama and Brecht 2016).

Appetitive 50-kHz ultrasonic vocalizations serving as social contact calls

The communicative function of appetitive 50-kHz USV was extensively studied during mating (for reviews, see: Barfield et al. 1979; Barfield and Thomas 1986). In the last two decades, however, the dominant research focus changed with the discovery that rats emit appetitive 50-kHz USV during rough-and-tumble play as juveniles (Knutson et al. 1998). Knutson et al. (1998) found that 50-kHz USV emission correlated with appetitive components of rough-and-tumble play behavior, such as dorsal contacts, and that 50-kHz USV occur in anticipation of rough-and-tumble play. They further provided evidence indicating that social motivation drives 50-kHz USV emission by showing that rats exposed to single housing for about 2 weeks display more rough-and-tumble play behavior and emit more 50-kHz USV, as compared to controls housed in groups (Knutson et al. 1998). Burgdorf et al. (2006) confirmed high 50-kHz USV emission during rough-and-tumble play and showed that emission rates change as a function of rough-and-tumble play behavior. It was further shown that particularly the frequency-modulated subtype of 50-kHz USV is positively associated with appetitive components of rough-and-tumble play behavior, such as dorsal contacts (Burgdorf et al. 2008). Emission of appetitive 50-kHz USV was found to be higher in males than females, with males playing more than females (Himmler et al. 2014). Moreover, there is also evidence for strain differences. During rough-and-tumble play, more 50-kHz USV were found to be emitted by Sprague-Dawley than Wistar rats, with Wistar rats engaging less in rough-and-tumble play behavior (Manduca et al. 2014a, b). Social experiences, such as social rejection, might also have an impact (Schneider et al. 2016). Himmler et al. (2014) further performed a detailed temporal analysis and found that 50-kHz USV occur at particularly high rates before a playful interaction and that different 50-kHz USV subtypes are associated with specific types of behaviors, such as the short subtype, which was seen at high rates after evasions but not complete rotations. Finally, it was found that rats prefer to spend most of the time with a rat that had shown high levels of appetitive 50-kHz USV during rough-and-tumble play mimicked by a human experimenter through tickling, as compared to a rat that did not (Panksepp et al. 2002; Panksepp and Burgdorf 2003). Together, the data provide qualified support for the hypothesis that 50-kHz USV serve as a socio-affective signals fulfilling important pro-social communicative functions, for instance as play signals and/or social contact calls.

Experimental evidence: selective breeding studies Panksepp and Burgdorf, 2000; Panksepp et al. 2001) applied a selective breeding line approach with the aim to select rats for low or high tendencies to emit appetitive 50-kHz USV in response to rough-and-tumble play mimicked by a human experimenter through tickling. A random line was used as control. By the third generation, there was a segregation of the high line from the random line, with the high line emitting more 50-kHz USV. The low line was segregated from the other two lines from the very outset and characterized by very low 50-kHz USV emission rates. In the fourth generation, the high line exhibited more pinning behavior during rough-and-tumble play than low and random lines, with the random line displaying an intermediate pinning level (Panksepp et al. 2001). During rough-and-tumble play, 50-kHz USV emission was most prominent in the high line. In line with the idea that 50-kHz USV serve as play signals and/or social contact calls, only moderate 50-kHz USV levels were obtained in the random line, while in the low line 50-kHz USV were virtually absent (Panksepp et al. 2001). A similar result pattern was obtained during exposure to a novel unfamiliar environment, yet at much lower levels (Panksepp et al. 2001). Moreover, in the fourth generation, not only 50-kHz USV emission but also the production of isolation-induced 40-kHz USV was affected by selective breeding for 50-kHz USV. Isolation-induced 40-kHz USV were low in the low line, but high in the high line, with intermediate levels in the random line (Panksepp et al. 2001; for details, see Table 1).

Because this first selective breeding approach could not be pursued since the rats died off, Burgdorf et al. (2005) attempted to replicate selective breeding for low or high tendencies to emit appetitive 50-kHz USV in response to rough-and-tumble play mimicked by a human experimenter through tickling. “Those original lines died off as a consequence of a laboratory shut-down for asbestos abatement and our inability to achieve a cross-fostering transfer of those lines to another facility, necessitated by our adults harboring a skin parasite that preclude the direct re-housing of our breeding stock” (Burgdorf et al. 2005). While in the first selective breeding study ultrasonic recordings by means of bat detectors were exclusively focused on 50-kHz USV, a more sophisticated approach covering all relevant ultrasonic frequency bands including the ranges of appetitive 50-kHz USV but also aversive 22-kHz USV was applied in the second selective breeding study. Similar to the first selective breeding study, four generations of selective breeding sufficed to generate constitutional differences between lines of rats in both 50-kHz USV emission and related social behavior. Specifically, there was a segregation of the high line from low and random lines by the fourth generation, with the high line emitting more 50-kHz USV (Burgdorf et al. 2005). However, random and low lines did not segregate (Burgdorf et al. 2005) and the random line was found to emit more 50-kHz USV only after the ninth generation (Burgdorf et al. 2009). Line differences in 50-kHz USV emission appeared to be mostly driven by the frequency-modulated 50-kHz USV subtype (Burgdorf et al. 2009). While no 22-kHz USV occurred in the high line, a substantial amount of 22-kHz USV was seen in low and random lines (Burgdorf et al. 2005). In the second selective breeding study, isolation-induced 40-kHz USV were found to be similar in pups from all lines under standard test conditions in the seventeenth generation (Harmon et al. 2008). In a place preference conditioning paradigm, however, the low line

Table 1 Selective breeding studies: social behavior and ultrasonic communication

First selective breeding approach for 50-kHz USV	Low line	High line	Age	Generation	References
Emission of appetitive 50-kHz USV	Low*		Adolescence	1st	Panksepp et al. 2001
Emission of appetitive 50-kHz USV	Low*	High*	Adolescence	3rd	Panksepp et al. 2001
Rough-and-tumble play	Low*	High*	Adolescence	4th	Panksepp et al. 2001
Emission of isolation-induced 40-kHz USV	Low*	High*	Infancy	4th	Panksepp et al. 2001
Second selective breeding approach for 50-kHz USV					
Emission of aversive 22-kHz USV		Low*	Adolescence	2nd	Burgdorf et al. 2005
Emission of appetitive 50-kHz USV		High*	Adolescence	4th	Burgdorf et al. 2005
Emission of aversive 22-kHz USV	High*		Adolescence	9th	Burgdorf et al. 2009
Emission of appetitive 50-kHz USV	Low*	High*	Adolescence	9th	Burgdorf et al. 2009
Rough-and-tumble play	Altered*		Adolescence	13th	Webber et al. 2012
Social interaction behavior	Low*		Adulthood	14th	Burgdorf et al. 2009
Aggressive behavior		Low*	Adulthood	14th	Burgdorf et al. 2009
Conditioned place preference for maternal odors	Low*		Infancy	17th	Harmon et al. 2008
Emission of isolation-induced 40-kHz USV	Low#		Infancy	17th	Iacobucci et al. 2013

*As compared to random line (control group)

As compared to high line

failed to display the typical preference for an odor associated with the dam and emitted more isolation-induced 40-kHz USV than high and random lines, with the latter not differing from each other (Harmon et al. 2008). More robust line differences in isolation-induced 40-kHz USV were reported by Iacobucci et al. (2013) in the seventeenth generation. Low line pups were found to emit clearly more isolation-induced 40-kHz USV. This line difference was seen irrespective of the social context. Specifically, low line pups emitted more isolation-induced 40-kHz USV no matter whether they were tested under clean bedding, familiar home cage bedding, or unfamiliar home cage bedding conditions. In contrast to low line pups, where isolation-induced 40-kHz USV emission was not affected by the social context, isolation-induced 40-kHz USV emission was modulated by social context in high line pups, with stronger responses under familiar and unfamiliar bedding conditions. In the fourteenth generation, Burgdorf et al. (2009) further showed that rats selectively bred for low rates of 50-kHz USV engage less in social interactions as adults, as compared to high and random line rats. Reduced social interaction time in the low line was paralleled by a decrease in the frequency-modulated 50-kHz USV subtype, whereas flat 50-kHz USV were found to have increased (Burgdorf et al. 2013). Moreover, the high line was found to be less aggressive (Burgdorf et al. 2009). In contrast to the first selective breeding study, however, selective breeding effects on rough-and-tumble play in juveniles were moderate in the thirteenth generation of the second study. Specifically, rats selectively bred for low rates of 50-kHz USV displayed altered rough-and-tumble play behavior, with reduced dorsal contacts but more pinning behavior (Webber et al. 2012; for details, see Table 1). In response to amphetamine and cocaine, hyperactivity and 50-kHz USV emission was reported to be more prominent in the high than the low line (Mu et al. 2009, 2010; Brudzynski et al. 2011a, b; for reviews, see: Moskal et al. 2011; Burgdorf et al. 2013).

A different but related selective breeding line approach was applied by Brunelli et al. (2006). They compared 50-kHz USV emission during rough-and-tumble play in rats selectively bred for low or high rates of isolation-induced 40-kHz USV as pups to an unselected random control group. They found that rough-and-tumble play behavior and 50-kHz USV emission is reduced in both lines, as compared to the random control group. This indicates that not only high trait anxiety levels, as seen in the rats selectively bred for high rates in isolation-induced 40-kHz USV as pups (Dichter et al. 1996), but also low social motivation leads to a reduction in appetitive 50-kHz USV in a social context (for review, see: Brunelli 2005). Moreover, in rats selectively bred for high anxiety-related behavior on the elevated plus maze, 50-kHz USV emission is almost completely absent during rough-and-tumble play behavior (Lukas and Wöhr 2015). As the breeding lines differ in their hypothalamic vasopressin availability and vasopressin is strongly implicated in the regulation of social behavior, Lukas and Wöhr (2015) further tested whether manipulating the vasopressin system alters rough-and-tumble play behavior and concomitant 50-kHz USV emission. While the administration of synthetic vasopressin had no effect, blocking the central vasopressin system by means of a vasopressin 1a receptor antagonist resulted in lower levels of rough-and-tumble play and fewer 50-kHz USV, indicating that the central vasopressin system is involved in the regulation of affiliative communication in rodents (Lukas and Wöhr 2015). Other studies on 50-kHz USV emission during rough-and-tumble play focused on the effects of acute or prenatal ethanol (Willey et al. 2009; Wellmann et al. 2015; Waddell et al. 2016), morphine (Manduca et al. 2014a), amphetamine (Manduca et al. 2014a), endocannabinoid signaling modulators (Manduca et al. 2014b), and valproic acid (Wellmann et al. 2014; Raza et al. 2015). There is also a number of studies specifically targeting the glutamatergic neurotransmitter system (Burgdorf et al. 2011a, b; Moskal et al. 2011) or the insulin-like growth factor I (Burgdorf et al. 2010). Together, the selective breeding studies (Panksepp and Burgdorf 2000; Panksepp et al. 2001; Burgdorf et al. 2005, 2009, 2013; Brunelli et al. 2006; Harmon et al. 2008; Mu et al. 2009, 2010; Brudzynski et al. 2011a, b; Webber et al. 2012; Iacobucci et al. 2013; Lukas and Wöhr 2015) demonstrate that it is possible to select for appetitive 50-kHz USV, that such a selection process affects related components of the social behavioral repertoire, and that, in turn, selective breeding for anxiety-related traits alters 50-kHz USV emission, supporting the nation that 50-kHz reflect a positive affective state akin to joy and happiness.

Experimental evidence: devocalization studies As described above, multiple evidence was provided that appetitive 50-kHz USV promote and maintain playful social interactions, in line with the idea that 50-kHz USV serve as play signals and/or social contact calls. Supporting this view, rough-and-tumble play behavior was found to be altered following deafening. In deafened rats, the close quarter wrestling component of rough-and-tumble play was found to be diminished, yet play initiation remained unchanged (Siviy and Panksepp 1987). To further test the hypothesis that appetitive 50-kHz USV serve as play signals to promote and maintain playful social interactions, Kisko et al. (2015a, b, 2017) performed a series of experiments using rats unable to vocalize due to surgical devocalization. If appetitive 50-kHz USV serve as play signals, Kisko et al. (2015b) hypothesized that rough-and-tumble play behavior is disrupted in their absence in pairs of devocalized rats. Consistent with this hypothesis, Kisko et al. (2015b) observed clearly reduced levels of rough-and-tumble play behavior in pairs of devocalized rats, with devocalized rats launching fewer playful attacks. They also found more startle responses when contacted by the play partner and that devocalized rats are more likely to defend themselves in response to a playful attack, as compared to control rats exposed to sham surgery. Social investigation and allogrooming, in contrast, were not affected by surgical devocalization. As shown in a subsequent study (Kisko et al. 2017), rough-and-tumble play behavior is not only reduced in devocalized pairs but also in intact pairs that were housed with devocalized cage mates before testing. It thus appears possible that there is a critical period to learn about the contextual uses of 50-kHz USV during the rough-and-tumble play period and that an intact rat playing with a devocalized cage mate during this critical period may not receive the necessary feedback from hearing 50-kHz USV.

In dyads with one devocalized and one intact rat, Kisko et al. (2015a, b) repeatedly observed alterations in specific components of the rough-and-tumble play behavioral repertoire, such as defensive responses. By this means, Kisko et al. (2017) further provided evidence that the rat that is pinning is emitting appetitive 50-kHz USV, since 50-kHz USV emission rates were found to be substantially higher when the intact rat is pinning than when it is pinned by the devocalized partner. Moreover, in line with the study by Himmler et al. (2014), Kisko et al. (2015b) found that appetitive 50-kHz USV are more frequent before playful contact is made than when such contact is terminated, yet this temporal pattern was not affected by devocalization of one of the play partners. In fact, it was found that pre-contact 50-kHz USV are just as frequent when the devocalized play partner initiated the playful interaction as when an intact one was. As indicated by the fact that 50-kHz USV were emitted by the initiator of the playful interaction, they still may be used to signal playful attack, but given that they were just as likely to be emitted by the receiving partner, they may also function as enticements by one rat to solicit playful attack from another (Kisko et al. 2015b). Kisko et al. (2015b) therefore tested whether rats prefer to engage in rough-and-tumble play with an intact play partner over a devocalized play partner when both partners are simultaneously available. Contrary to the findings obtained by Panksepp et al. (2002, 2003), they found “that the non-vocal rats were just as attractive as ones that can vocalize” (Kisko et al. 2015b). In their subsequent study, Kisko et al. (2015a) replicated this finding using unfamiliar rather than familiar play partners. However, they also found that in dyads more playful attacks are launched against mute partners and that mute partners respond to playful attacks more often than intact partners. This response pattern was specifically seen in juveniles. In adulthood, devocalization did not affect the number of playful attacks, yet agonistic attacks were occurring more often in pairs containing a devocalized partner. The increase in agonistic attacks was found to be paralleled by more aggression-related behaviors, such as tail rattling, piloerection, and lateral display. This suggests that in pairs of adult rats, where one rat is unable to vocalize, the risk that a playful interaction escalates to serious fighting is higher. Together, the series of devocalization studies performed by Kisko et al. (2015a, b, 2017) indicate that appetitive 50-kHz USV are necessary to facilitate rough-and-tumble play in juvenile rats, perhaps by promoting a positive affective state in the play partners or by establishing and maintaining social proximity. Surgical devocalization increases agonistic attacks in adulthood.

Experimental evidence: playback studies Another experimental, yet noninvasive approach to study the communicative functions of appetitive 50-kHz USV under standardized conditions is playback. In playback studies, precisely defined acoustic stimuli can be presented and between-subject variance is easily minimized, with all subjects being exposed to the exact same stimulus. For instance, Burgdorf et al. (2008) showed that rats engage in more nose-poking behavior in an instrumental task to elicit playback of frequency-modulated 50-kHz USV, indicating that the presentation of frequency-modulated 50-kHz USV is rewarding. Playback of flat 50-kHz USV had no effect, while playback of aversive 22-kHz USV led to a reduction in nose-poking behavior, indicating that rats avoid them.

Around the same time, Wöhr and Schwarting (2007; for review, see: Seffer et al. 2014; for methodological details, see: Wöhr et al. 2016) developed a radial maze playback paradigm to study the communicative functions of appetitive 50-kHz USV by assessing behavioral responses in recipient rats. In this paradigm, a given rat is exposed to playback of appetitive 50-kHz USV and appropriate acoustic control stimuli in a counter-balanced manner on an elevated radial eight arm maze. Social approach behavior is defined by a higher number of entries into and more time spent on proximal arms, i.e., the three arms close to the active ultrasonic speaker used for playback of appetitive 50-kHz USV, as compared to the three distal ones away from the speaker. By means of this paradigm, it was repeatedly shown that playback of appetitive 50-kHz USV leads to social approach behavior in juvenile and adult male (Wöhr and Schwarting 2007, 2009, 2012; Willuhn et al. 2014; Seffer et al. 2015; Brenes et al. 2016; Engelhardt et al. 2017a) as well as female (Willadsen et al. 2014) rats, supporting the notion that they serve an affiliative function as social contact calls irrespective of the sex of the recipients. A number of related observations support this view. For instance, 50-kHz USV occur in anticipation of social contact. Specifically, in rats exploring an environment, the emission of 50-kHz USV was found to be driven by potential social contact and elicited by the odor of conspecifics, with 50-kHz USV emission rates being proportional to the number of rats that left their odor in the environment (Brudzynski and Pniak 2002). Moreover, 50-kHz USV occur after separation from a conspecific. Separation results in a transient increase in 50-kHz USV emission, most notably in flat calls, probably to reestablish social proximity (Wöhr et al. 2008). Beyond that, 50-kHz USV might further orchestrate complex social interactions, such as cooperative actions to obtain food rewards, as indicated by a positive covariation between cooperative behavior and 50-kHz USV emission (Łopuch and Popik 2011).

By means of the 50-kHz USV radial maze playback paradigm, it was further found that the behavioral response pattern evoked in the recipients depends on certain acoustic features of appetitive 50-kHz USV. By comparing the response patterns evoked by appetitive 50-kHz USV with the ones elicited by other acoustic stimuli, it was demonstrated that social approach behavior specifically occurs in rats exposed to 50-kHz USV and, to a lower extent, in response to 50-kHz USV sine wave tones. No evidence for social approach behavior was obtained in response to 22-kHz USV, time- and amplitude-matched white noise, and background noise. While the appetitive 50-kHz USV presented were mostly frequency-modulated, the 50-kHz sine wave tones were identical to the 50-kHz USV with respect to peak frequency, call length, and temporal patterning, yet were lacking frequency modulation (Wöhr and Schwarting 2007). The fact that playback of 50-kHz USV sine wave tones is efficient in inducing social approach behavior, although at a lower level, indicates that frequency modulation of 50-kHz USV is not a necessary component of appetitive 50-kHz USV for eliciting social approach behavior. This is consistent with the idea that primarily the flat 50-kHz USV subtype functions as social contact call, as suggested by the transient increase in mostly flat 50-kHz USV typically seen following separation from a conspecific (Wöhr et al. 2008).

Besides stimulus type, age and sex play an important role. Specifically, juvenile rats were found to display strong social approach behavior in response to appetitive 50-kHz USV (Wöhr and Schwarting 2007, 2009, 2012; Seffer et al. 2015; Engelhardt et al. 2017a). In adulthood, however, the strength of the response appears to be sex-dependent. While in adult male rats comparatively weak responses occurred and only moderate social approach behavior was evoked by appetitive 50-kHz USV (Wöhr and Schwarting 2007, 2009; Willuhn et al. 2014; Seffer et al. 2015; Brenes et al. 2016), strong social approach behavior was seen in adult female rats (Willadsen et al. 2014). However, there is a study by Snoeren and Ågmo (2014) on female rats where no evidence for social approach behavior in response to playback of appetitive 50-kHz USV was obtained. An important difference between the two studies is that Willadsen et al. (2014) presented 50-kHz USV only once, whereas Snoeren and Ågmo (2014) repeatedly exposed females to 50-kHz USV.

In fact, an interesting phenomenon is the fast extinction of the social approach response elicited by playback of appetitive 50-kHz USV. Typically, recipient rats display an immediate orientation response and strong social approach behavior towards the sound source within a couple of seconds when exposed to 50-kHz USV. However, this response rarely lasts longer than 2 or 3 min (Wöhr and Schwarting 2012; Willuhn et al., 2014; Seffer et al. 2015). Moreover, rats repeatedly exposed to playback of 50-kHz USV do not display a preference for the sound source during the second presentation. Lack of preference is seen even after a long delay of 1 week between the two stimulus presentations (Wöhr and Schwarting 2012). Through pharmacological manipulations, evidence was provided that the underlying mechanism is acetylcholine-dependent memory consolidation. Specifically, rats treated with the amnestic agent scopolamine, a muscarinic acetylcholine receptor antagonist blocking memory consolidation, immediately after the first presentation of 50-kHz USV displayed social approach behavior during the second presentation, while vehicle-treated controls did not (Wöhr and Schwarting 2012). More recent studies further showed that the strength of the social approach response elicited by appetitive 50-kHz USV depends on social experiences of the recipient rat, such as post-weaning social isolation (Seffer et al. 2015) and social versus physical environmental enrichment (Brenes et al. 2016; for review, see: Wöhr et al. 2017). Finally, inter-individual differences in trait-like variations in the emission of 50-kHz USV play an important role as well (Engelhardt et al. 2017b). Of note, Saito et al. (2016) showed that playback of appetitive 50-kHz USV affects decision-making, with rats exposed to 50-kHz USV making more "optimistic" decisions in an instrumental task where rats had to press a lever in order to avoid foot shock or to receive a food reward in response to tone presentations.

The 50-kHz USV radial maze playback paradigm and its modification were recently also used to study neurobiological processes involved in rodent ultrasonic communication. One research line focused on brain activation patterns evoked in recipient rats. Through electrophysiological recordings and immunohistochemistry, it was shown that playback of appetitive 50-kHz USV results in decreased firing rates and reduced c-fos expression in the amygdala, whereas playback of aversive 22-kHz USV leads to enhanced firing (Sadananda et al. 2008; Parsana et al. 2012; Ouda et al. 2016; Kagawa et al. 2017). The amygdala is orchestrating anxiety and fear responses and is strongly involved in aversive learning processes (Fendt and Fanselow 1999; Maren and Quirk 2004). The deactivation of the amygdala in response to appetitive 50-kHz USV was found to be paralleled by an activation of the nucleus accumbens, where immediate early gene expression was increased following 50-kHz USV playback (Sadananda et al. 2008; Pultorak et al. 2016), but also in the anterior cingulate cortex (Saito and Okanoya 2017). The nucleus accumbens is a key brain area implicated in reward processing, with dopamine signaling being strongly involved (Schultz 2007; Salamone and Correa 2012). Consistently, fast-scan cyclic voltammetry recordings showed that playback of 50-kHz USV leads to phasic dopamine release in the nucleus accumbens within a couple of seconds, while no phasic dopamine release was evident in response to multiple acoustic control stimuli. Interestingly, phasic dopamine release levels were positively correlated with social approach behavior and characterized by a similarly fast extinction as social approach when rats were repeatedly exposed to playback of 50-kHz USV (Willuhn et al. 2014).

Another related research line focused on neurotransmitters and neuropeptides by systematically manipulating relevant characteristics of the recipient. In line with the findings obtained by fast-scan cyclic voltammetry recordings (Willuhn et al. 2014), Engelhardt et al. (2017a) found that the systemic administration of moderate doses of amphetamine, which leads to a massive increase in monoamines, including dopamine, in the synaptic cleft, results in increased social approach behavior in response to playback of appetitive 50-kHz USV. Besides dopamine, the opioid system also appears to play an important role, as it was found that systemic application of low doses of the μ-opioid agonist morphine leads to enhanced social approach behavior, while the μ-opioid antagonist naloxone had opposite effects (Wöhr and Schwarting 2009).

Recently, for the first time, 50-kHz USV from a genetic rat model were applied as stimulus in the 50-kHz USV playback paradigm and it was found that appetitive 50-kHz USV recorded from the genetic Parkinson's disease model elicited reduced social approach behavior in recipients (Pultorak et al. 2016). Together, the playback studies (Wöhr and Schwarting 2007, 2009, 2012; Burgdorf et al. 2008; Sadananda et al. 2008; Parsana et al. 2012; Snoeren and Ågmo 2014; Willadsen et al. 2014; Willuhn et al. 2014; Seffer et al. 2015; Brenes et al. 2016; Ouda et al. 2016; Pultorak et al. 2016; Saito et al. 2016; Saito and Okanoya 2017; Engelhardt et al. 2017a, b; Kagawa et al. 2017) show that appetitive 50-kHz USV induce social approach behavior in recipients, with the strength of responding being dependent on a number of factors, such as age, sex, and trait-like inter-individual differences as well as certain acoustic features of appetitive 50-kHz USV.

Conclusion

In conclusion, evidence from selective breeding, devocalization, and playback studies strongly suggests that appetitive 50-kHz USV serve as situation-dependent socio-affective signals with important communicative functions in rats, for instance as play signals and/or social contact calls.

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Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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