Cell Tissue Res (2013) 354:81–97
DOI 10.1007/s00441-013-1607-9

REVIEW

Affective communication in rodents: ultrasonic vocalizations as a tool for research on emotion and motivation

Markus Wöhr · Rainer K. W. Schwarting

Received: 26 January 2013 / Accepted: 28 February 2013 / Published online: 11 April 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Mice and rats emit and perceive calls in the ultrasonic range, i.e., above the human hearing threshold of about 20 kHz: so-called ultrasonic vocalizations (USV). Juvenile and adult rats emit 22-kHz USV in aversive situations, such as predator exposure and fighting or during drug withdrawal, whereas 50-kHz USV occur in appetitive situations, such as rough-and-tumble play and mating or in response to drugs of abuse, e.g., amphetamine. Aversive 22-kHz USV and appetitive 50-kHz USV serve distinct communicative functions. Whereas 22-kHz USV induce freezing behavior in the receiver, 50-kHz USV lead to social approach behavior. These opposite behavioral responses are paralleled by distinct patterns of brain activation. Freezing behavior in response to 22-kHz USV is paralleled by increased neuronal activity in brain areas regulating fear and anxiety, such as the amygdala and periaqueductal gray, whereas social approach behavior elicited by 50-kHz USV is accompanied by reduced activity levels in the amygdala but enhanced activity in the nucleus accumbens, a brain area implicated in reward processing. These opposing behavioral responses, together with distinct patterns of brain activation, particularly the bidirectional tonic activation or deactivation of the amygdala elicited by 22-kHz and 50-kHz USV, respectively, concur with a wealth of behavioral and neuroimaging studies in humans involving emotionally salient stimuli, such as fearful and happy facial expressions. Affective ultrasonic communication therefore offers a translational tool for studying the neurobiology underlying socio-affective communication. This is particularly relevant for rodent models of neurodevelopmental disorders characterized by social and communication deficits, such as autism and schizophrenia.

Keywords Anxiety · Fear · Autism · Social behavior · Preparedness

Introduction

Mice and rats emit and perceive calls in the ultrasonic range, i.e., above the human hearing threshold of about 20 kHz: so-called ultrasonic vocalizations (USV). This has important implications for preclinical research, ranging from practical considerations, e.g., ambient ultrasonic noise that might affect the well-being of the animal, to new approaches for the assessment of psychological states and functions in rodents that are not readily accessible with the standard paradigms in use today, e.g., USV as a measure for communication deficits in rodent models of human neuropsychiatric disorders, such as autism.

High rates of USV occur throughout the life span in a variety of socially relevant situations. They are emitted by pups during the first days of life when removed from the nest, in juveniles engaging in social play behavior, in adult females during social investigation and in adult males when exposed to females or during aggression. Moreover, we know that various USV categories exist with distinct acoustic features. Some USV categories are present in both mice and rats, whereas others are solely emitted by one species.

Mouse In mice, typically, three USV categories are differentiated: (1) USV emitted by pups during social isolation after being separated from their mother and littermates (Fig. 1a; isolation-induced USV); (2) USV emitted by juvenile males and females and by adult females during social interactions (Fig. 1b; interaction-induced USV); (3) USV emitted by adult

M.W. is supported by the Deutsche Forschungsgemeinschaft (DFG WO 1732/1-1).

M. Wöhr (✉) · R. K. W. Schwarting
Behavioral Neuroscience, Experimental and Physiological Psychology, Philipps University of Marburg, Gutenbergstrasse 18, 35032 Marburg, Germany
e-mail: markus.woehr@staff.uni-marburg.de

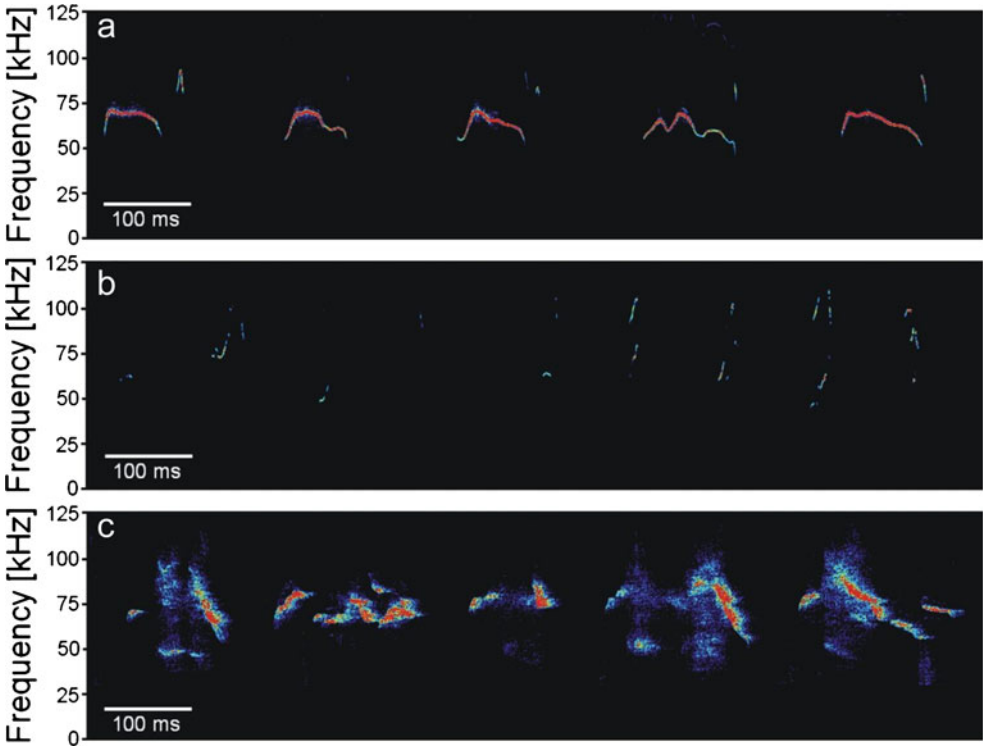

Fig. 1 Types of ultrasonic vocalizations (USV) in mouse. **a** Isolation-induced USV emitted by an 8-day-old male C57BL/6J mouse during isolation from its mother and littermates. **b** Interaction-induced USV emitted by a 25-day-old male C57BL/6J mouse during social interaction with a C57BL/6J mouse of the same age. **c** Female-induced USV emitted by a 3-month-old male C57BL/6J mouse exposed to female urine. For all recordings, an UltraSoundGate Condenser Microphone CM16/CMPA (sampling rate: 250 kHz; 16 bit) was used. Spectrograms were generated with Avisoft SASLab Pro (Fast Fourier Transform; time resolution: 0.427 ms; frequency resolution: 0.586 kHz). Equipment and software: Avisoft Bioacoustics, Berlin, Germany

male mice when exposed to females or female urinary cues (Fig. 1c; female-induced USV; however, little is known about the ontogenic profiles of the last two and female-induced USV might simply be a subtype of interaction-induced USV). The first observation of USV in mice was made by Zippelius and Schleidt in 1956. They found that mouse pups emit USV when taken out from the nest and isolated. As they identified social isolation as the crucial factor for pup USV, they named them as "Pfeifen des Verlassenseins" ("whistles of loneliness") following Konrad Lorenz and suggested that isolation-induced USV reflect a negative affective state. Zippelius and Schleidt (1956) further suggested that isolation-induced USV serve a communicative function, namely to induce maternal search and retrieval behavior. They showed that mothers leave the nest in response to pups that were scattered outside the nest and that were able to vocalize but not in response to dead or anesthetized pups unable to vocalize. The important communicative function of isolation-induced USV was later confirmed by means of playback experiments (Sewell 1970). Sewell (1970) presented isolation-induced USV to lactating mothers through an ultrasonic loudspeaker placed on one or the other side of a T partition away from the nest. She found that mothers left the nest in approximately 90% of presentations, typically within 5–30 s after the onset of playback, whereas mothers did not respond to background noise or artificial ultrasonic pulses. These two pioneering studies were the starting point of the two main tracks in research on USV: affective state and communicative function. Today, USV are used to assess the efficacy of novel potential treatments for anxiety disorders in preclinical studies (e.g., Fish et al. 2004; Takahashi et al. 2009) and serve as the most commonly used measure for communication deficits in rodent models of neurodevelopmental disorders and speech impairments (e.g., Fujita et al. 2008; Wöhr et al. 2011b; for reviews on mouse USV, see Ehret 2005; Scattoni et al. 2009).

Rat As in mice, typically, three USV categories are differentiated in rats: (1) USV emitted by pups during social isolation after being separated from their mother and littermates (Fig. 2a; isolation-induced USV); (2) USV emitted by juvenile and adult rats in aversive situations, such as predator exposure and fighting or during drug withdrawal (Fig. 2b; aversive 22-kHz USV); (3) USV emitted by juvenile and adult rats in appetitive situations, such as rough-and-tumble play and mating or in response to drugs of abuse, e.g., amphetamine (Fig. 2c; appetitive 50-kHz USV). Here, we will focus on appetitive and aversive USV emitted by juvenile and adult rats and describe examples that support the assumption that they reflect positive and negative affective states, respectively and that provide evidence for their important communicative functions (for reviews on rat pup USV, see Hofer and Shair 1993; Schwarting and Wöhr 2012).

Affective ultrasonic communication in rats

Aversive 22-kHz USV

Affective state The 22-kHz USV are widely believed to reflect a negative affective state akin to anxiety and fear.

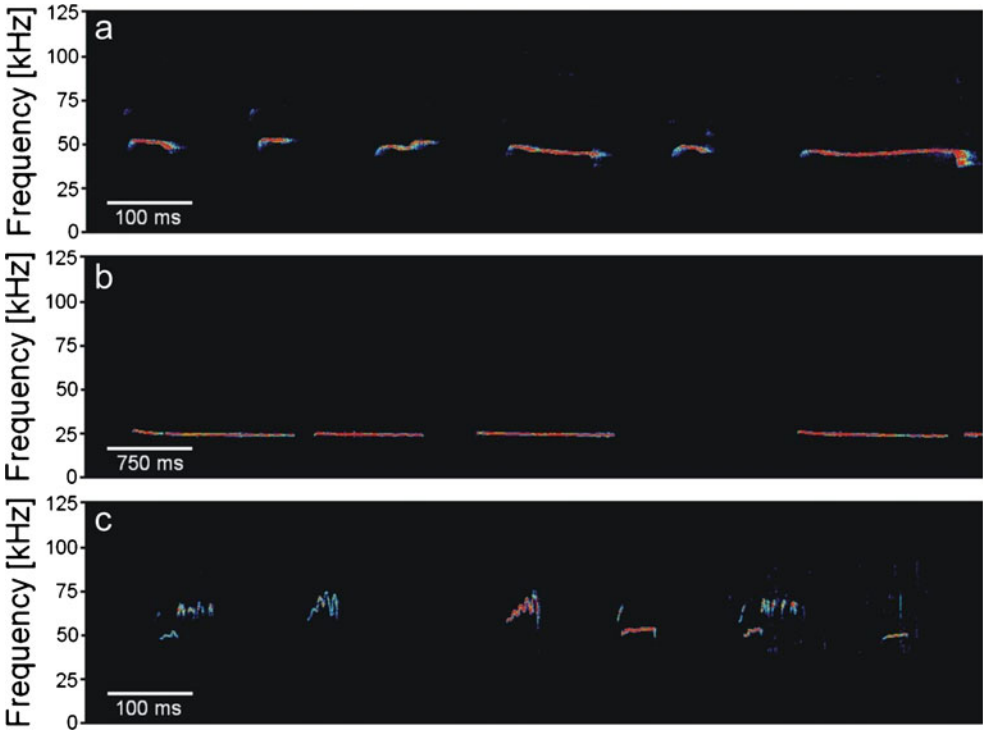

Fig. 2 Types of ultrasonic vocalizations (USV) in rat. **a** Isolation-induced USV emitted by an 11-day-old male Wistar rat during isolation from its mother and littermates. **b** Aversive 22-kHz USV emitted by a 3-month-old male Wistar rat during fear conditioning. **c** Appetitive 50-kHz USV emitted by a 3-month-old male Wistar rat searching for conspecifics. Note the difference in time resolution in **b** as compared with **a**, **c**. For details of recording equipment and software analysis, see legend to Fig. 1

First of all, such an association is simply suggested by the finding that rats emit 22-kHz USV in aversive situations, such as predator exposure (e.g., Blanchard et al. 1990, 1991) and fighting (e.g.,Kaltwasser 1990; Sales 1972a), or during withdrawal from drugs, such as alcohol, benzodiazepines, opiates and psychostimulants (e.g., Covington and Miczek 2003; Vivian et al. 1994). In the laboratory, the most common approach to elicit such USV is fear conditioning (see below). During fear conditioning, an aversive stimulus, such as an electric foot shock (unconditioned stimulus, US) is repeatedly paired with a formerly neutral stimulus, such as a light or tone (conditioned stimulus, CS). During this procedure, the CS gains the efficacy to elicit fear-related responses (CRs) in the absence of the US. The most commonly measured CR is freezing behavior, i.e., the lack of all somatic motility except respiratory activity. However, in addition to freezing behavior, the emission of 22-kHz USV is also a prominent part of the CRs. Typically, both CRs, namely freezing behavior and 22-kHz USV emission, are positively correlated during fear conditioning and during fear testing (Fig. 3; Wöhr and Schwarting 2008a). The finding that rats emit 22-kHz USV as part of their CRs during fear testing in response to a tone (CS), previously paired with an electric foot shock (US), shows that a painful stimulus is not needed to elicit the production of 22-kHz USV, as the anticipation of an aversive event is sufficient. This indicates that 22-kHz USV emission in the fear conditioning paradigm is not a pain response but, rather, that it is an emotional response. Pharmacological and neuroanatomical studies support this conclusion. For instance, Jelen et al. (2003) have shown that the emission of 22-kHz USV as CR in a fear conditioning paradigm is blocked by anxiolytic compounds, namely diazepam and buspirone, whereas the anxiogenic compound pentyletetrazole enhances 22-kHz USV production. In agreement with this study, Choi and Brown (2003) have demonstrated that central amygdala lesions block 22-kHz USV emission as a conditional but not an unconditional response.

The production of 22-kHz USV in aversive situations depends on a wide range of factors, including test context, individual dispositions and early environmental factors. In agreement with the assumption that 22-kHz USV reflect a negative affective state, we found that 22-kHz USV rates increase with the aversiveness of the situation (Wöhr et al. 2005). Rats exposed to higher foot shock intensities during fear conditioning are found to display more freezing behavior and vocalize more and louder than rats exposed to lower foot shock intensities. Rats exposed to tones but not foot shocks, do not emit 22-kHz USV. In addition to the aversiveness of the situation, however, individual dispositions to show anxiety-related behavior also play an important role (Borta et al. 2006). We screened a sample of normal laboratory rats for their tendency to show anxiety-related behavior on the elevated plus maze. Based on their open arm avoidance, we split the group into animals with high and low anxiety. Then, all rats underwent exactly the same fear conditioning paradigm. Rats that were characterized as highly anxious, based on their behavior displayed on the elevated plus maze, emitted more 22-kHz USV than less anxious animals during fear conditioning. Importantly, both

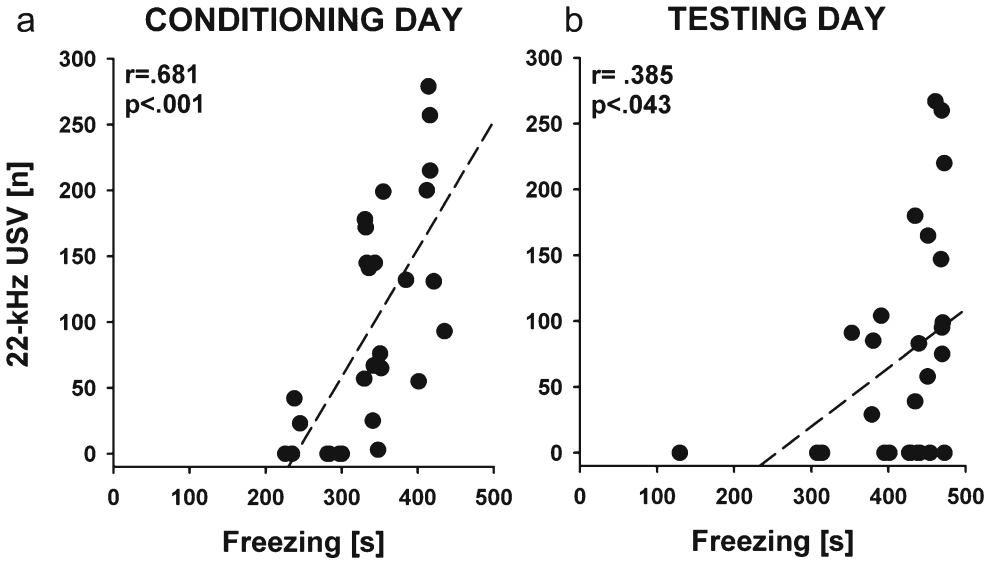

Fig. 3 Freezing behavior and emission of aversive 22-kHz ultrasonic vocalizations (USV) are positively correlated during fear conditioning and testing in adult rats. **a** On the fear conditioning day during which rats were exposed to tone-shock pairings. **b** On the fear testing day during which rats were exposed to the tone previously associated with shock application. Each *circle* represents an individual rat. Based on data from Wöhr and Schwarting (2008a)

groups did not differ in their pain sensitivity as assessed by means of the hot plate test. Amongst others, such individual differences in the emission of 22-kHz USV could be attributable to early environmental factors. For instance, we tested whether juvenile stress exposure affects 22-kHz USV production in adulthood (Yee et al. 2012a). Juvenile exposure to a series of acute and variable stressors, namely forced swimming, exposure to an elevated platform and immobilization stress, is known to cause behavioral, physiological and molecular changes that persist into adulthood (e.g., Tsoory et al. 2007, 2008). Rats exposed to such stressors during the prepubescent period emitted more 22-kHz USV in response to fear conditioning in adulthood than unexposed controls (Fig. 4; Yee et al. 2012b). We further found that maternal immune activation during pregnancy via polyinosinic:polycytidylic acid (poly I:C) affected the production of 22-kHz USV during fear conditioning in adult offspring (Yee et al. 2012b). Poly I:C administration to pregnant mice or rats during specific gestational periods leads to a number of behavioral impairments in the offspring, including deficient sensorimotor gating and reduced levels of social behaviors (e.g., Ehninger et al. 2012; Wolf and Bilkey 2008). In our fear conditioning paradigm, poly I:C exposure during pregnancy caused an increase of 22-kHz USV emission to 300% that of saline controls (Fig. 5; Yee et al. 2012b). In addition to USV rates, acoustic features and temporal patterning were affected. For instance, rats exposed to poly I:C emitted 22-kHz USV with shorter durations. The observed effects were specific for 22-kHz USV, since the production of audible calls acutely emitted in response to painful stimuli did not differ between groups, indicating that vocal pain responses were not affected by poly I:C treatment. Importantly, alterations in the production of 22-kHz USV were observed despite the fact that a detailed analysis of visible behavior did not reveal any group differences. This highlights the importance of assessing 22-

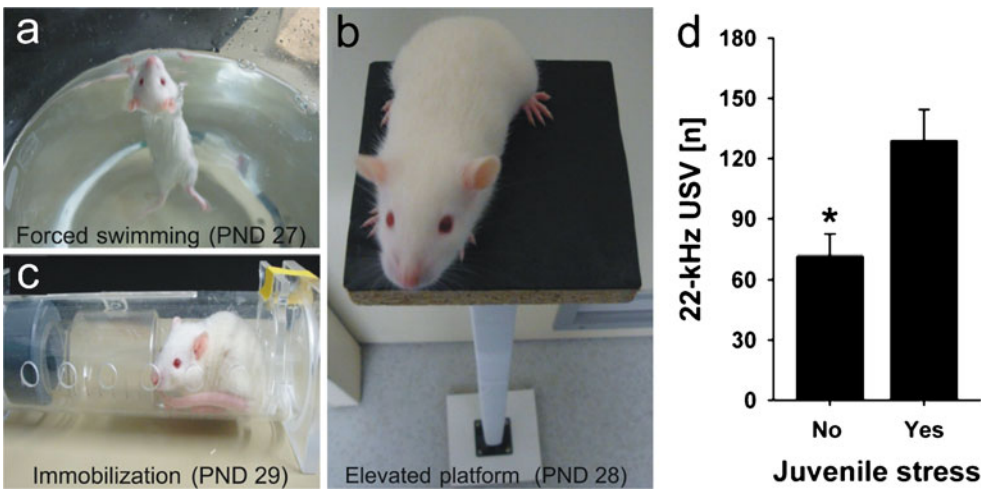

Fig. 4 Juvenile stress potentiates the emission of aversive 22-kHz ultrasonic vocalizations (USV) during fear conditioning and testing in adult rats. **a** Forced swimming on postnatal day 27 (*PND 27*). **b** Elevated platform exposure on postnatal day 28 (*PND 28*). **c** Restraint stress on postnatal day 29 (*PND 29*). **d** Emission of aversive 22-kHz USV in rats exposed (*Yes*) or not exposed (*No*) to juvenile stress. Data are presented as means ± SEM. **P*<0.050 for Yes versus No. Based on data from Yee et al. (2012a)

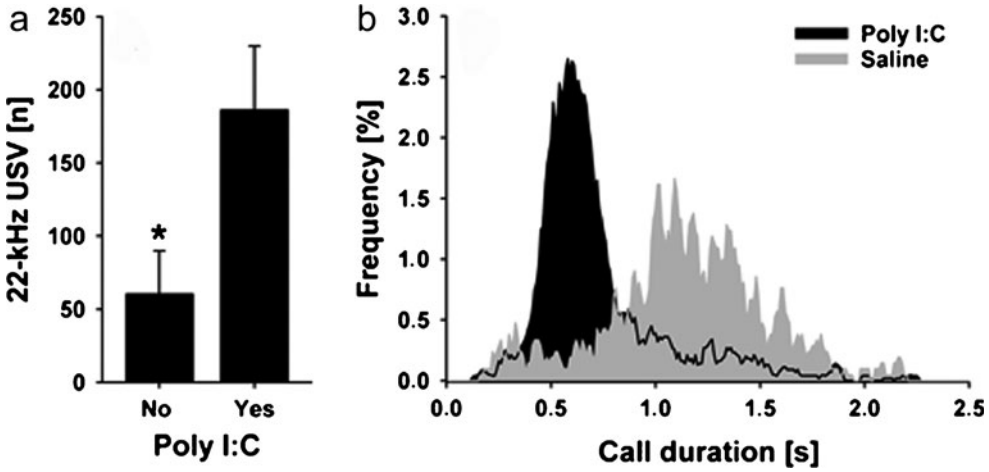

Fig. 5 Maternal immune activation during pregnancy via polyinosinic:polycytidylic acid (*Poly I:C*) potentiates the emission of aversive 22-kHz ultrasonic vocalizations (USV) during fear conditioning and testing in adult rats. **a** Emission of aversive 22-kHz USV in rats exposed (*Yes*) or not exposed (*No*) to Poly I:C. **b** Histogram of the call durations of aversive 22-kHz USV in percentages (moving average). Data are presented as means ± SEM. *P<0.050 for Yes versus No. Based on data from Yee et al. (2012b)

kHz USV as an additional measure of fear, as it might help to detect treatment effects not detectable by conventional behavioral approaches.

Communicative function: behavioral responses The most widely accepted hypothesis is that 22-kHz USV serve as alarm calls to warn conspecifics about external danger. This hypothesis was born out of a series of studies conducted by Blanchard et al. (1991). Using groups of rats living in a naturally established colony in a visible burrow system, Blanchard et al. (1991) found that the production of 22-kHz USV in response to a predator, namely a cat, was facilitated by the presence of an audience, i.e., an identified group of familiar listeners, indicating that 22-kHz USV production is not only dependent on specific eliciting stimuli but also on the social context of the sender. In agreement with a putative alarming function, Blanchard et al. (1991) further showed that 22-kHz USV emission in response to cat exposure led to a profound and long-lasting set of defensive behaviors in conspecifics that had not seen the cat themselves, including USV emission in juvenile rats. Blanchard et al. (1990) therefore suggested that the production of USV was socially contagious. However, in a more recent study, we did not obtain evidence for an audience effect on the emission of 22-kHz USV (Wöhr and Schwarting 2008b). In this study, rats underwent a conventional fear conditioning paradigm and were trained and tested either alone, with an anesthetized familiar conspecific, or with an awake familiar conspecific in an adjacent chamber. Only experimental rats but not conspecifics, were exposed to foot shocks. In all three experimental groups, emission of 22-kHz USV occurred during fear conditioning and testing and was not potentiated by the close presence of a familiar conspecific. If anything, the presence of a familiar conspecific might even have had a mild attenuating effect on 22-kHz USV emission, possibly because of a phenomenon called social buffering (Kiyokawa et al. 2004). Nevertheless, we should point out that the lack of an audience effect in our study (Wöhr and Schwarting 2008b) does not rule out the possibility of potentiated 22-kHz USV emission in the presence of an audience under different experimental conditions. For instance, a dominant alpha male living in a naturally established rat colony, as in the study by Blanchard et al. (1991), might be crucial for the appearance of an audience effect. Importantly, however, even in the absence of an audience effect, we still found some evidence in favor of the alarm function of 22-kHz USV, namely a high positive correlation between 22-kHz USV emitted by the experimental rat that underwent fear conditioning and the level of freezing behavior shown by the observer in the adjacent chamber (Fig. 6; Wöhr and Schwarting 2008b).

Further support for an alarm function of 22-kHz USV was obtained in playback experiments. A number of studies showed that playback of natural 22-kHz USV or 20 kHz sine wave tones activate the fight/flight/freeze system of rats. The studies carried out so far indicate that the behavioral responses elicited by such ultrasonic stimuli are strain-dependent. Specifically, in response to playback, Wistar rats (Brudzynski and Chiu 1995; Burman et al. 2007; Commissaris et al. 2000; Neophytou et al. 2000; Nobre and Brandão 2004; Sales 1991; Wöhr and Schwarting 2007) and Sprague Dawley rats (Endres et al. 2007) have been found to show a reduction in locomotor activity and a limited freezing response (behavioral inhibition), whereas Lister hooded rats display bursts of running and jumping (behavioral excitation), which is characteristic of an active defensive response (Beckett et al. 1996, 1997; Commissaris et al. 1998, 2000; Finn et al. 2004; Neophytou et al. 2000; Nicolas et al. 2007; Voits et al. 1999).

However, we should emphasize that evidence in favor of strong behavioral changes occurring specifically in response to 22-kHz USV is weak. Indeed, clear behavioral responses are only seen in studies involving loud and artificial continuous sine wave tones (Beckett et al. 1996, 1997; Commissaris et al. 1998, 2000; Finn et al. 2004; Nicolas et al. 2007; Neophytou et al. 2000; Nobre and Brandão 2004; Voits et al. 1999). Conversely, mostly weak effects have

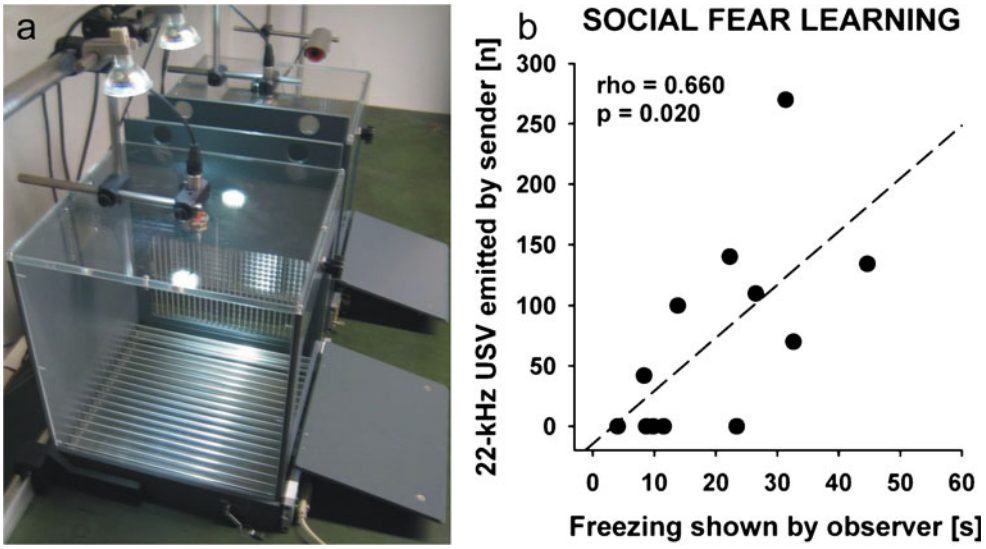

Fig. 6 Emission of aversive 22-kHz ultrasonic vocalizations (USV) by the sender and freezing behavior shown by the observer are positively correlated during social fear learning. **a** Set-up used for conducting social fear learning experiments. **b** Correlation between the emission of aversive 22-kHz USV by the sender exposed to tone-shock pairings and freezing behavior shown by the observer not exposed to shock application. Each *circle* represents a sender-observer pair of rats. Based on data from Wöhr and Schwarting (2008b)

been observed in studies in which natural stimuli have been used (Brudzynski and Chiu 1995; Burman et al. 2007; Endres et al. 2007; Sales 1991; Wöhr and Schwarting 2007). As early as the first study with natural 22-kHz USV as playback stimuli, Sales (1991) reported only modest locomotor inhibition. Specifically, rats entered floor squares less often during 22-kHz USV playback (ca. 55 times) than when noise was presented (ca. 70 times). However, the level of locomotor inhibition reported in response to 22-kHz USV was similar to that seen when rats were exposed to an artificial 38 kHz stimulus. Moreover, Brudzynski and Chiu (1995), who performed a similar experiment some few years later, observed no acute effects during playback (with ca. 500 activity counts per observation before and during playback). They only reported a slight but significant, decrease in locomotor activity after playback (with ca. 350 activity counts). Similarly, three more recent studies also observed only weak behavioral effects of 22-kHz USV playback. In a study performed by Burman et al. (2007), just one out of two natural 22-kHz stimuli was effective in increasing the latency to emerge from the test box; the other was without any effect. Moreover, Endres et al. (2007) observed only a non-significant and modest increase in the time rats spent freezing when exposed to 22-kHz USV (ca. 25% versus ca. 8% during silence). As in the study by Sales (1991), however, the observed response was not specific for 22-kHz USV. Endres et al. (2007) compared the behavioral change induced by playback of 22-kHz USV with those seen in response to various other stimuli, namely 50-kHz USV, 22-kHz sine wave tones, 22-kHz USV shifted to about 45 kHz and white noise in the range from 17 to 27 kHz. Comparisons between responses to 22-kHz USV and all the other stimuli demonstrated no specific response of naive rats to 22-kHz USV playback. Only when the various stimuli with acoustic characteristics close to 22-kHz were pooled together was a moderate increase in freezing observed during and after stimulus presentation. Such an induction of freezing was not seen in the other pooled groups. Similarly, we observed only a non-significant and modest decrease in the distance travelled during playback of 22-kHz USV (ca. 10 cm/min) as compared with silence (ca. 40 cm/min; Wöhr and Schwarting 2007). Finally, some studies did not detect any behavioral response to playback of natural 22-kHz USV (Bang et al. 2008; Lindquist et al. 2004; Parsana et al. 2012a, 2012b; Sadananda et al. 2008).

Moreover, in playback studies with artificial stimuli, rats were found to show a stronger behavioral response to 7 kHz or 12 kHz sine wave tones than to 20 kHz sine wave tones (Commissaris et al. 2000), indicating that the behavioral effects were clearly not related to the communicative value of the 22-kHz USV. More likely, these effects were caused by the high sound pressure levels (SPL) used in the experiments. In some studies, artificial stimuli have been presented with more than 100 dB SPL (Commissaris et al. 2000; Voits et al. 1999), which is much higher than the usual SPL of natural 22-kHz USV, with approximately 60–80 dB SPL from a distance of 20–30 cm (Wöhr et al. 2005; Wöhr and Schwarting 2008a, 2008b). A remarkable exception, however, is a study by Nobre and Brandão (2004) in which ultrasonic sine wave tones with 75 dB SPL were used and freezing behavior was specifically seen in response to tones in the range of 20 to 25 kHz but not below or above.

As only weak (Brudzynski and Chiu 1995; Burman et al. 2007; Endres et al. 2007; Sales 1991; Wöhr and Schwarting 2007) or no behavioral responses (Bang et al. 2008; Lindquist et al. 2004; Parsana et al. 2012a, 2012b; Sadananda et al. 2008) were elicited by presenting natural 22-kHz USV as US, fear conditioning studies were performed, in which 22-kHz USV served as CS (Bang et al. 2008; Endres et al. 2007). Endres et al. (2007) addressed

the questions of whether the recognition of 22-kHz USV as alarm calls can be learned and whether this learning is facilitated by a preparedness to acquire defensive behavioral patterns in response to such stimuli. They showed that rats quickly learn to associate an aversive event with 22-kHz USV, retain this information longer in their memory and are more reluctant to extinguish this memory than in the case of the association of aversive events with other types of ultrasonic stimuli, such as artificial 22-kHz sine wave tones. This indicates that "rats are predisposed to acquire adaptive defensive behavior in response to alarm calls" and that "better encoding of such learning in rats leads to a stable memory which better resists extinction" (Endres et al. 2007).

Evidence in support of a predisposition to associate 22-kHz USV with aversive events was also obtained by Bang et al. (2008) using a differential fear conditioning paradigm. Here, 22-kHz USV, 50-kHz USV and various artificial stimuli created to deconstruct the 22-kHz USV into simpler acoustic features, such as frequency changes, frequency and amplitude modulation and temporal patterning, were tested. During differential fear conditioning, one of these stimuli (CS+) always co-terminated with a foot-shock (US), whereas another (CS−) was explicitly unpaired with the US. As in the study by Endres et al. (2007), 22-kHz USV did not differ from the other ultrasonic stimuli in terms of the unconditional elicitation of freezing behavior but after pairing 22-kHz USV and foot shocks, 22-kHz USV induced freezing behavior, suggesting that freezing in response to 22-kHz USV is not innate but instead emerges as a consequence of associative learning. In contrast to the study by Endres et al. (2007), however, Bang et al. (2008) found that 22-kHz USV serving as the CS+ were no more effective than 50-kHz USV or the artificial stimuli for conditional freezing. However, in favor of a biological preparedness to associate 22-kHz USV with aversive events, an asymmetrical stimulus generalization was discovered. Specifically, when 22-kHz USV served as the CS+, less generalization of fear to the CS− was seen than when 22-kHz USV served as the CS−. Under the latter circumstance, rats failed to discriminate between CS+ and CS−. By contrast, they clearly did discriminate when 22-kHz USV served as the CS+. Thus, the amount of stimulus generalization (from CS+ to CS−) depended on which stimuli served as CS+ or CS−. Despite the differences between the findings reported by Endres et al. (2007) and Bang et al. (2008), both studies support the notion that the behavioral response to 22-kHz USV is not innate but rather emerges through associative learning, which, in turn, is facilitated by predisposition. The existence of biological preparedness to associate certain stimuli over others has previously been demonstrated, e.g., in taste aversion learning (Garcia and Koelling 1966; for review see Seligman 1970).

A different approach to testing the communicative function of 22-kHz USV was applied by Kim et al. (2010). They compared the following three groups of pair-housed rats to see whether the emission of 22-kHz USV by the sender induces freezing behavior in the receiver: (1) the sender underwent conventional fear conditioning, while the partner stayed in the home cage; (2) the sender underwent conventional fear conditioning, while the partner was exposed to an aversive stimulation, namely a series of foot shocks but not fear conditioning, i.e., no tone-shock pairings were applied; (3) the sender stayed in the home cage, while the partner was exposed to the aversive stimulation. The next day, the rat pairs were placed in a novel environment and the presentation of the tone used for fear conditioning elicited the emission of 22-kHz USV by the conditioned senders. The production of 22-kHz USV by the senders led to freezing behavior in the partners exposed to the series of foot shocks the day before but not in naive partners. Importantly, partner rats exposed to the aversive stimulation but tested with naive senders not emitting 22-kHz USV in response to the tone did not show freezing behavior, indicating that 22-kHz USV emission causes freezing behavior in experienced partner rats, rather than sensitization. This conclusion is further supported by the finding that the disruption of the primary auditory pathway by lesions of the medial geniculate nucleus of the thalamus effectively blocks the freezing response in the partner when being tested with a sender emitting 22-kHz USV. The lack of freezing behavior in the partners with medial geniculate nucleus lesions shows that the emission of 22-kHz USV by the sender and not olfactory cues or visible behavioral changes elicits the freezing response in the partner. However, probably the most important finding of the study by Kim et al. (2010) is that an aversive stimulation, i.e., a fear experience, prior to 22-kHz USV exposure, is necessary for inducing freezing behavior through 22-kHz USV. The fear experience appears to prime rats to show a freezing response to 22-kHz USV. Indeed, the onset latencies of the 22-kHz USV of the partner during fear experience correlated with their freezing responses when exposed to the 22-kHz USV emitted by the sender. This suggests auto-conditioning as the underlying mechanism. To test the auto-conditioning hypothesis, Kim et al. (2010) inactivated the medial geniculate nucleus of partner rats during their exposure to the aversive stimulation but not during testing with senders. Intriguingly, partner rats did not display freezing behavior in response to 22-kHz USV emitted by the sender, meaning that they behaved as naive rats, i.e., as if not exposed to the aversive stimulation. The auto-conditioning hypothesis was recently confirmed by Parsana et al. (2012b). Using the playback approach, they demonstrated that rats that underwent an aversive experience before displayed freezing behavior in response to 22-kHz USV, whereas no freezing response was observed in rats that did not undergo such an aversive experience. Effects of aversive experience were reported to be specific for 22-kHz

USV, as experienced rats were unresponsive to 50-kHz USV. Again, 22-kHz USV production during the aversive stimulation was predictive of subsequent freezing to 22-kHz USV. Parsana et al. (2012b) therefore suggest that auto-conditioning "is sufficiently rapid, reliable, and stimulus-specific to serve an adaptive defensive function in rats". Importantly, these last two studies by Kim et al. (2010) and Parsana et al. (2012b) probably explain the inconsistencies in the literature regarding the effects of 22-kHz USV playback on the behavioral responses of the recipients and highlight the relevance of controlling for the affective experiences of the rats in studies on the communicative functions of 22-kHz USV.

Communicative function: neuronal responses Behavioral responses elicited by playback of natural 22-kHz USV or artificial 20-kHz sine wave tones are paralleled by the activation of brain areas regulating anxiety- and fear-related behaviors. In one of the earliest studies, Brandão et al. (2001) showed that 22-kHz sine wave tones and other fear-evoking stimulations, such as a light previously associated with foot shock application, produced an increase in the amplitude of auditory-evoked potentials in the inferior colliculus, indicating that 22-kHz USV can enhance auditory processing in aversive situations. Activation of the inferior colliculus is known to cause freezing behavior (Brandão et al. 2001).

Beckett et al. (1997) performed the first comprehensive study on brain activity patterns elicited by playback of artificial 20-kHz sine wave tones. By means of immunohistochemical assessment of the immediate early gene c-fos, a marker for neuronal activity, they demonstrated that locomotor hyperactivity in Lister hooded rats in response to artificial 20-kHz sine wave tones was associated with increased neuronal activity in the periaqueductal gray (PAG), amygdala, hypothalamus, and thalamus. Specifically, the dorsal but not ventral, part of the rostrocaudal PAG was activated. Within the amygdala, the medial, basolateral, central and lateral nuclei showed an increase in neuronal activity. Within the hypothalamus, activation was observed in the dorsomedial nucleus but not the anterior and ventromedial nuclei. Finally, the stria terminalis and the paraventricular nucleus of the thalamus were also activated. In a subsequent study that addressed the question of whether strain differences in behavioral responses to artificial 20-kHz sine wave tones were paralleled by differences in brain activity, the general pattern of neuronal activation was confirmed for Lister hooded rats (Neophytou et al. 2000). Compared with them, however, playback of 20-kHz sine wave tones led to relatively weak neuronal changes in Wistar rats. Increased activity was observed in the basolateral amygdala, stria terminalis and entorhinal cortex. Most importantly, the PAG activity pattern differed from that of Lister hooded rats and paralleled substantial behavioral differences. In Lister hooded rats, playback induced locomotor hyperactivity, which was associated with neuronal activity preferentially in the dorsal region of the rostral and caudal PAG, whereas in Wistar rats, locomotor hypoactivity and freezing responses were observed, accompanied by activation in the ventral region of the caudal PAG. The PAG presumably represents the final common pathway in the behavioral expression of aversive states (Vianna and Brandão 2003) and electrical or chemical stimulation of the dorsal part of the PAG has been shown to elicit fleeing, whereas stimulation of the ventral parts of the PAG produces freezing (Depaulis et al. 1994; Morgan et al. 1998). Accordingly, inactivation of the dorsal parts of the PAG increase fear-induced freezing, whereas inactivation of ventral parts disrupts this behavior (De Oca et al. 1998). In addition to the up-regulation of activity in brain areas implicated in the regulation of fear and anxiety, a reduction in activity has been observed in the paraventricular nucleus of the thalamus and the raphé nuclei in Wistar rats.

Using a similar experimental approach, we have examined the expression of the immediate early gene c-fos after exposure to natural 22-kHz USV (Sadananda et al. 2008). In our study, the overall immunohistochemical staining pattern was similar to that obtained in studies with artificial stimuli (Beckett et al. 1997; Neophytou et al. 2000). Specifically, an increase in neuronal activity induced by 22-kHz USV is observed in the PAG, with the highest activity levels in its rostral part (Sadananda et al. 2008). In addition to PAG, increased activation is seen in the amygdala, particularly in the lateral/basolateral part (Sadananda et al. 2008). Increased amygdala activity during playback of 22-kHz USV has also been reported in a study by Parsana et al. (2012a). By means of single unit recordings in freely behaving rats, Parsana et al. (2012a) have addressed the question of whether neurons in the amygdala are tuned to respond to 22-kHz USV as an ethologically important natural stimulus and have compared firing rates and patterns between 22-kHz USV and various controls, including 22-kHz sine wave tones. They have found that approximately 40% of lateral/basolateral amygdala neurons respond to 22-kHz USV. Two attributes of the firing patterns, namely whether playback causes an increase (+) or decrease (-) in firing rates and whether the induced change is transient (phasic) or sustained (tonic) have been differentiated, resulting in four elemental firing patterns: phasic onset (+), phasic offset (-) tonic (+), and tonic (-). Most firing responses elicited by 22-kHz USV are tonic (+) responses, meaning that 22-kHz USV leads to a sustained increase in firing. Changes in firing rates have also been observed in response to 22-kHz sine wave tones. As for 22-kHz USV, the most common change is a sustained increase in firing, a tonic (+) response. Latency analysis has shown that changes in firing occur after 10–20 ms, consistent with activation via the short subcortical pathway, as opposed to the long cortical pathway (Parsana et al. 2012a). The amygdala is a key structure in affective information processing and fear has been the function most closely

associated with it (Fendt and Fanselow 1999; LeDoux 2000; Maren and Quirk 2004). The findings that 22-kHz USV lead to an increase in amygdala activation add 22-kHz USV to the group of motivationally relevant and negatively valenced stimuli capable of increasing amygdala activity, such as foot shock and restrain (Duncan et al. 1996; Kovács 1998). The finding that 22-kHz USV yields an increase in the lateral/basolateral but not in the central amygdala points to the functional importance of intra-amygdaloid circuits (Pitkänen et al. 1997). The lateral/basolateral part is generally considered as the sensory gateway into the amygdala, receiving inputs from all sensory systems, whereas the central amygdala is viewed as the output region. Convergent evidence from lesion and electrophysiological studies has been accumulated in support of the hypothesis that the lateral/basolateral amygdala is critically involved in fear conditioning, i.e., in the acquisition of fear-related CRs (Fendt and Fanselow 1999; LeDoux 2000; Maren and Quirk 2004). Typically, incoming novel stimuli elicit increased firing in the amygdala, which rapidly habituates as long as they are not accompanied by biologically significant stimuli. However, pairing an initially neutral stimulus (later becoming a CS) with a biologically significant stimulus (US) leads to changes in synaptic plasticity in the lateral/basolateral amygdala. Such plasticity changes result in modifications of the intra-amygdaloid circuits that allow the CS alone to flow through its lateral/basolateral part and to activate the central amygdala. The central amygdala then orchestrates responses in order to cope with the detected biologically significant event. In the case of threat, for instance, its output connections to the PAG induce freezing behavior. The lack of an increase in neuronal activity in the central amygdala in response to 22-kHz USV (Sadananda et al. 2008) is therefore in accordance with the observation that 22-kHz USV induce only a subtle amount of freezing (Brudzynski and Chiu 1995; Burman et al. 2007; Endres et al. 2007; Sales 1991; Wöhr and Schwarting 2007) or no freezing at all (Bang et al. 2008; Lindquist et al. 2004; Parsana et al. 2012a; Sadananda et al. 2008) in rats lacking aversive experience (Kim et al. 2010; Parsana et al. 2012b). However, the clear increase in neuronal activity in the lateral/basolateral part of the amygdala as assessed by c-fos (Sadananda et al. 2008) and single unit recordings (Parsana et al. 2012a) might reflect the initiation of the synaptic changes that underlie learning processes or provide prerequisites for it. This assumption is supported by recent findings showing that the infusion of the γ-aminobutyric acid agonist muscimol into the basolateral amygdala prior to fear conditioning impairs the acquisition of fear to 22-kHz USV (Allen et al. 2008).

Furthermore, neuronal activation is also evident in the perirhinal cortex (Sadananda et al. 2008), which is adjacent to and reciprocally connected with the amygdala (Pitkänen et al. 1997). Preliminary evidence that the perirhinal cortex is implicated in the processing of 22-kHz USV was obtained in a lesion study (Lindquist et al. 2004). Perirhinal lesions, performed prior to training, were found to severely impair delay fear conditioning to a 22-kHz USV or artificial 22-kHz USV-like stimuli such as CS, whereas such lesions were ineffective when the CS was a continuous tone of the same or a lower frequency (Kholodar-Smith et al. 2008a; Lindquist et al. 2004). Based on these findings, the discontinuous nature of 22-kHz USV, i.e., its "bout" structure, has been suggested to be at least part of the reason that normal fear conditioning to 22-kHz USV requires cortical processing, whereas cortical processing is not necessary for conditioning to continuous tones: "Cortical processing may be required to integrate these discontinuous auditory stimuli across time, in order for normal fear conditioning to occur" (Allen et al. 2007). In this context, we need to note that the perirhinal cortex is required for trace fear conditioning but not delay fear conditioning (Kholodar-Smith et al. 2008b). The difference between both paradigms is that, in the delay fear conditioning, the US is presented at the end of the CS, whereas in the trace fear conditioning, the CS is followed by a trace interval, which is terminated by the US. This indicates that the role of the perirhinal cortex in trace fear conditioning is distinct from its more perceptual functions in delay fear conditioning.

As in Parsana et al. (2012a) for the amygdala, single unit recordings were used to test whether neurons in the perirhinal cortex were also tuned to respond to 22-kHz USV (Allen et al. 2007). Firing patterns were assessed in response to 22-kHz USV and to several acoustic control stimuli, namely frequency and temporally matched discontinuous tones and continuous tones with the same or lower frequencies. A comparison of the number of neurons responding to the auditory stimuli revealed no difference between 22-kHz USV and control stimuli. Overall, approximately 40% of the neurons responded to one or more of the auditory stimulus types used, out of which 69% responded to 22-kHz USV. Most of the elicited firing patterns were phasic onset (+) responses. Indeed, discontinuous tones, among them natural 22-kHz USV, sometimes elicited temporally matched firing patterns, which consisted in a transient increase in the firing frequency that was triggered by the onset or, less often, by the offset of each of the successive tones or USV within a bout. Such temporally matched firing patterns occurred more often in the perirhinal cortex (Allen et al. 2007) than in the amygdala in which tonic (+) responses were most common (Parsana et al. 2012a). In line with the observed brain region-specific differences in elicited firing patterns, Parsana et al. (2012a) suggested that phasic (+) responses indicated the detection of an event, whereas tonic (+ or -) responses reflected its valence, i.e., aversive versus appetitive.

In a subsequent study, Furtak et al. (2007) used a classic fear conditioning paradigm in which 22-kHz USV or a

continuous 22-kHz sine wave tone served as CS and examined fear-conditioning-induced changes in single unit firing elicited in the perirhinal cortex. Firing changes were observed in approximately 70% of the recorded units in response to 22-kHz USV or a continuous 22-kHz sine wave tone after the stimuli had been paired with a foot shock (US). Conditioning caused widespread changes in neuronal firing regardless of whether 22-kHz USV or a 22-kHz sine wave tone served as a cue. Remarkably, approximately 30% of units that were initially CS-unresponsive became CS-responsive after conditioning. Despite these general changes, however, two differences between single unit responses elicited by the 22-kHz USV and those elicited by the 22-kHz sine wave tone were evident. First, approximately 10% of the units recorded from the rat group, which was conditioned to 22-kHz USV, displayed a precisely timed increase in firing rate during the interval in which the US occurred during conditioning. This response pattern was unique to this group and was not seen in rats conditioned to a 22-kHz sine wave tone. Second, before conditioning, the neurons started firing to both CS after circa 55 ms. Following conditioning, however, neurons started firing in response to the 22-kHz sine wave tone as early as circa 25 ms, whereas conditioning to 22-kHz USV had no effect on the firing latency. Based on these findings, the authors suggested that firing in response to both CS was mediated by cortical rather than subcortical pathways to the perirhinal cortex before conditioning but that subcortical pathways gained the control of firing through conditioning to the 22-kHz sine wave tone but not to the 22-kHz USV (Furtak et al. 2007).

Overall, strong evidence has therefore been presented that 22-kHz USV and 22-kHz USV-like stimuli activate brain regions regulating anxiety- and fear-related behaviors, most notably the perirhinal cortex, amygdala and PAG. In line with the neuronal activation pattern, behavioral responses elicited by 20-kHz USV-like stimuli have been demonstrated to be efficiently blocked by anxiolytic compounds, including muscimol and midazolam (Beckett et al. 1996; Nicolas et al. 2007; Nobre and Brandão 2004). Neuronal response studies are further in agreement with the assumption of Endres et al. (2007) who have suggested a "neural template" that better encodes the USV of conspecifics than other auditory stimuli. This conclusion was based on their behavioral study in which rats quickly learned to associate an aversive event with 22-kHz USV, retained this information longer in their memory and were more reluctant to extinguish this memory than in the case of other types of ultrasonic stimuli. Thus, perirhinal cortex and amygdala probably at least part of this "neural template", which is responsible for the predisposition observed when 22-kHz USV are used as CS.

Appetitive 50-kHz USV

Affective state Because 50-kHz USV occur in appetitive situations, such as rough-and-tumble play (e.g., Knutson et al. 1998; Webber et al. 2012) and mating (e.g., Sales 1972b; Thomas and Barfield 1985) or in response to drugs of abuse, e.g., amphetamine (e.g., Burgdorf et al. 2001; Thompson et al. 2006), they have been suggested to reflect a positive affective state akin to joy and happiness. This view is supported by a series of experiments conducted by Panksepp and Burgdorf (2000). Inspired by the observation that rats emit 50-kHz USV during rough-and-tumble play, they decided to mimic rough-and-tumble play in rats through a human experimenter by tickling and showed that it is possible to induce 50-kHz USV by hetero-specific play (Panksepp and Burgdorf 2000). Similar to rough-and-tumble play behavior, the rate of tickling-induced 50-kHz USV is enhanced by a short period of social isolation, indicating that high levels of social motivation are associated with high levels of 50-kHz USV (Panksepp and Burgdorf 2000, 2003). Indeed, Panksepp and Burgdorf (2003) further showed that, in particular, those rats that emit high numbers of 50-kHz USV experience the tickling procedure as appetitive, as indicated by short latencies to approach the hand of the human experimenter. In addition to the tickling itself, even the presentation of cues associated with it, such as the experimenter's hand, are effective in eliciting 50-kHz USV (Panksepp and Burgdorf 2000, 2003). In contrast, aversive stimuli, such as cat odour or bright light, reduce 50-kHz USV emission (Panksepp and Burgdorf 2003). Panksepp (2005) therefore considers 50-kHz USV as a rat homolog or antecedent of human laughter.

Like 22-kHz USV emission, the production of 50-kHz USV is characterized by huge inter-individual differences, reflecting individual dispositions (Mällo et al. 2007; Schwarting et al. 2007). For instance, in our tickling experiments, we typically see a huge proportion of rats emitting 50-kHz USV, whereas others do not emit 50-kHz USV and some even emit 22-kHz USV (Schwarting et al. 2007). Similar findings have been reported by Mällo et al. (2007). They tickled rats repeatedly during a period of 45 days and found correlation coefficients between days mainly ranging between 0.6 and 0.9, indicating a highly stable trait. Surprisingly, however, the inter-individual differences in this trait were only weakly and often inconsistently associated with individual dispositions to show anxiety- and depression-like behavior, as assessed in standard paradigms, such as the elevated plus maze and forced swimming (Mällo et al. 2007; Schwarting et al. 2007). Nevertheless, some remarkable exceptions have been reported. For instance, Rygula et al. (2012) trained rats in an operant conditioning paradigm to press a lever in response to a tone to receive a reward, namely sucrose solution and to press another lever when a different tone was presented to avoid punishment by administration of a foot shock. After training, rats were tickled and split into two groups: (1) rats that produced high rates of 50-kHz USV during tickling and (2) rats that did not

emit many tickling-induced 50-kHz USV. Then, both groups of rats were exposed to an ambiguous tone with a frequency intermediate between the two tones used during training and measurements were made regarding how often the rats pressed the food-rewarding lever versus the one that they learned to press to avoid foot shocks. Rygula et al. (2012) found that rats emitting many 50-kHz USV displayed an "optimistic" bias towards the food-rewarding lever in response to the ambiguous tone. They pressed the food-rewarding lever more often than rats that produced only a few tickling-induced 50-kHz USV.

However, little is known about biological factors underlying the observed individual disposition in 50-kHz USV emission. Out of the many factors potentially involved, we decided to study hippocampal cell proliferation, as the level of neurogenesis in the dentate gyrus of the hippocampus has been repeatedly associated with affect regulation. Thus, aversive stimuli, such as submission during intermale fighting, is known to reduce hippocampal cell proliferation (Czéh et al. 2007) and Santarelli et al. (2003) have demonstrated that hippocampal cell proliferation is necessary for the antidepressant effects of selective serotonin reuptake inhibitors. We therefore tickled rats and correlated tickling-induced 50-kHz USV with hippocampal cell proliferation. In agreement with its role in affect regulation, we found that the emission of 50-kHz USV was highly positively correlated with hippocampal cell proliferation, whereas a highly negative correlation between the emission of 22-kHz USV and hippocampal cell proliferation was evident. Remarkably, we further found that hippocampal cell proliferation was strongly elevated in rats that experienced the tickling procedure presumably as appetitive, as indicated by the high numbers of 50-kHz USV. In contrast, rats with low tickling-induced 50-kHz USV rates had levels comparable with those of non-tickled controls. This suggests that tickling induces hippocampal cell proliferation in those rats that experience tickling presumably as appetitive (Wöhr and Schwarting 2009). This finding has been replicated in a more recent study by Yamamuro et al. (2010), who have also shown that the effects are long-lasting and still detectable after a 3-week survival period.

Burgdorf et al. (2005) used a different approach to unravel underlying biological factors. They selectively bred rats emitting high or low rates of 50-kHz USV during tickling. When comparing the resulting lines, they mainly found differences in social motivation and social behavior, such as rough-and-tumble play in juveniles, with the low-rate line showing deficits (Harmon et al. 2008; Webber et al. 2012). Remarkably, such deficits were found to be associated with changes in gene expression patterns in autism candidate genes (Moskal et al. 2011).

Communicative function: behavioral responses The emission of 50-kHz USV is a prominent part of mating behavior in rats and, hence, their functional role has been extensively studied. Behavioral observations, devocalization studies and playback experiments, all indicate that 50-kHz USV play an important role in establishing and maintaining close social contact (Geyer and Barfield 1978; McIntosh et al. 1978; Sales 1972b; Thomas and Barfield 1985; Thomas et al. 1981, 1982; White and Barfield 1987, 1989, 1990). More recent studies, however, have shown that 50-kHz USV also serve important communicative functions in the non-sexual context. Panksepp et al. (2002) have found that rats spend more time with conspecifics that emit high levels of 50-kHz USV than with others producing fewer 50-kHz USV. This again indicates that 50-kHz USV serve as social contact calls to establish and maintain contact among conspecifics. Consistent with this view, social context and social stimuli have been reported to modulate the production of 50-kHz USV. For instance, the number of 50-kHz USV emitted by rats exposed to a test environment containing the odor of conspecifics is positively correlated with the number of rats leaving their odor in this environment, suggesting that 50 kHz USV emission is driven by potential social contact (Brudzynski and Pniak 2002). However, rats were found to emit 50-kHz USV not only in anticipation of social contact but also in response to social separation, with emission being the highest immediately after separation (Schwarting et al. 2007; Wöhr et al. 2008), again in agreement with the idea that 50-kHz USV are emitted to maintain social contact.

To test experimentally whether 50-kHz USV indeed serve a pro-social communicative function as social contact calls, we conducted playback experiments and exposed juvenile and adult male rats to natural 50-kHz USV recorded from a male rat while exploring a test environment containing the odor of a familiar male conspecific (Wöhr and Schwarting 2007). Background noise, 22-kHz USV and 50-kHz sine wave tones were used as additional acoustic stimuli. The results of the playback experiment clearly showed that the exposure to 50-kHz USV elicited social approach behavior and USV emission in the recipients. Social approach behavior and USV emission specifically occurred in response to acoustic stimuli within the 50-kHz USV range, namely 50-kHz USV and 50-kHz sine wave tones. No such responses were observed when rats were exposed to background noise or 22-kHz USV. However, the finding that both 50-kHz USV and 50-kHz sine wave tones led to behavioral changes in the recipients indicated that amplitude and frequency modulation carried little or no communicative information, at least under the experimental conditions tested. In contrast to amplitude and frequency modulation, the peak frequency of 50-kHz USV appears to be highly relevant for behavioral changes to occur, as shown in a subsequent study (Wöhr and Schwarting 2012). In this study, social approach behavior was seen in response to 50-kHz USV, whereas locomotor inhibition was observed when rats were exposed to time- and amplitude-matched white noise. The lack of social approach behavior in response to the latter stimulus might have been attributable to the fact

that sound energy was not confined to a critical frequency band in the ultrasonic range as in the case for 50-kHz sine wave tones known to elicit social approach behavior (Wöhr and Schwarting 2007), suggesting a categorical perception mechanism. Indeed, behavioral inhibition seen in response to playback of time- and amplitude-matched white noise was probably caused by sound energy within the critical frequency range for 22-kHz USVs.

Apart from acoustic stimulus configuration, social approach behavior in response to the playback of 50-kHz USV is modulated by a number of factors, including the age of the recipients and social memory. Generally, social approach responses are much stronger in juvenile than in adult rats (Wöhr and Schwarting 2007, 2009), a result that is in agreement with the finding that juvenile rats emit more 50-kHz USV than adult rats (Panksepp and Burgdorf 1999). Social memory processes are indicated by the observation that social approach behavior is only evident during the first exposure to the playback of 50-kHz USV but not during repeated exposures (Wöhr and Schwarting 2012). Even with a 1-week interval between exposures, no social approach behavior has been seen during the second exposure, suggesting that social long-term memory processes are involved. Importantly, such social long-term memory effects can be blocked by the administration of scopolamine immediately after the first exposure (Fig. 7; Wöhr and Schwarting 2012). Administration of the muscarinic acetylcholine antagonist scopolamine leads to amnesia and is commonly used to validate rodent models for social memory (e.g., D'Amato and Moles 2001).

In addition to their role in establishing and maintaining social contact, 50-kHz USV might also be involved in regulating complex social behavior. For instance, the deafening or devocalizing of rats has been shown to affect reciprocal social interaction in juveniles (Siviy and Panksepp 1987), during which high rates of 50-kHz USV occur under normal conditions (Knutson et al. 1998; Webber et al. 2012). Moreover, 50-kHz USV are reported to occur during cooperative behavior in rats, with the number of cooperative behaviors and 50 kHz USV being positively correlated (Łopuch and Popik 2011).

Communicative function: neuronal responses Little is known about brain activity patterns induced by 50-kHz USV. Using immunohistochemical labeling of the immediate early gene c-fos, we compared brain activity patterns in rats exposed to natural 50-kHz USV, 22-kHz USV, background noise and silence (Sadananda et al. 2008). As compared with 22-kHz USV, neuronal activity levels were found to be lower in rats hearing 50-kHz USV. In particular, lower activity levels were observed in the perirhinal cortex, lateral/basolateral amygdala and the rostral part of the PAG. This pattern argues for the specificity of the USV-induced changes in activity but is not surprising, as 22-kHz USV activate such brain regions. However, findings from a recent single unit recording study by Parsana et al. (2012a) show that the observed differences in c-fos labeling are not simply attributable to increased activity levels when the rats are exposed to 22-kHz USV. Parsana et al. (2012a) have found that neurons in the lateral/basolateral amygdala not only respond to playback of 22-kHz USV but also change their firing pattern in response to 50-kHz USV. Whereas most neurons display a tonic (+) response to 22-kHz USV, a tonic (-) response is the most common firing pattern when exposed to 50-kHz USV. The opposite firing patterns elicited by the two USV types are in agreement with their distinct communicative functions and with the idea that tonic responses of neurons in the lateral/basolateral part of the amygdala reflect stimulus valence, i.e., aversive versus appetitive. In our study, reductions of activity levels were found in a number of additional brain regions, such as the lateral habenula and the dorsal raphé nuclei, as compared with silence controls (Sadananda et al. 2008). However, the observed reductions were not specific for 50-kHz USV and also occurred in response to 22-kHz USV, indicating that they reflected arousal and attention rather than stimulus valence (Abrams et al. 2004; Geisler and Trimble 2008).

In addition to decreases, exposure to the playback of 50-kHz USV led to increased c-fos labeling in the frontal cortex and the nucleus accumbens (NAcc; Sadananda et al. 2008). The neuronal activation in the frontal cortex was most pronounced in the secondary motor cortex. This activation was specifically seen in rats exposed to 50-kHz USV. As described above, 50-kHz USV but not 22-kHz USV or background noise, induce strong behavioral activation, typically directed towards the sound source, termed social approach behavior (Sadananda et al. 2008; Wöhr and Schwarting 2007, 2009, 2012). Therefore, the stimulus-specific activation seen in the secondary motor cortex is most probably associated with social approach behavior.

As for the activation seen in the frontal cortex, the increased activity of the NAcc was also specific for 50-kHz USV (Sadananda et al. 2008). Again, the activation of the NAcc through 50-kHz USV is in agreement with the social approach response elicited. Indeed, the NAcc is well known for its important role in appetitive behavior and is believed to act as an "interface between motivation and action" (Mogenson et al. 1980). NAcc activity is critically modulated by its dopaminergic input. One is therefore tempted to speculate that dopamine release in the NAcc is necessary for social approach behavior to occur when rats are exposed to 50-kHz USV. Interestingly, the local administration of the catecholaminergic agonist, amphetamine, into the NAcc has been repeatedly shown to elicit the emission of 50-kHz USV but not 22-kHz USV (Burgdorf et al. 2001; Thompson et al. 2006). Hence, the NAcc might function to close a

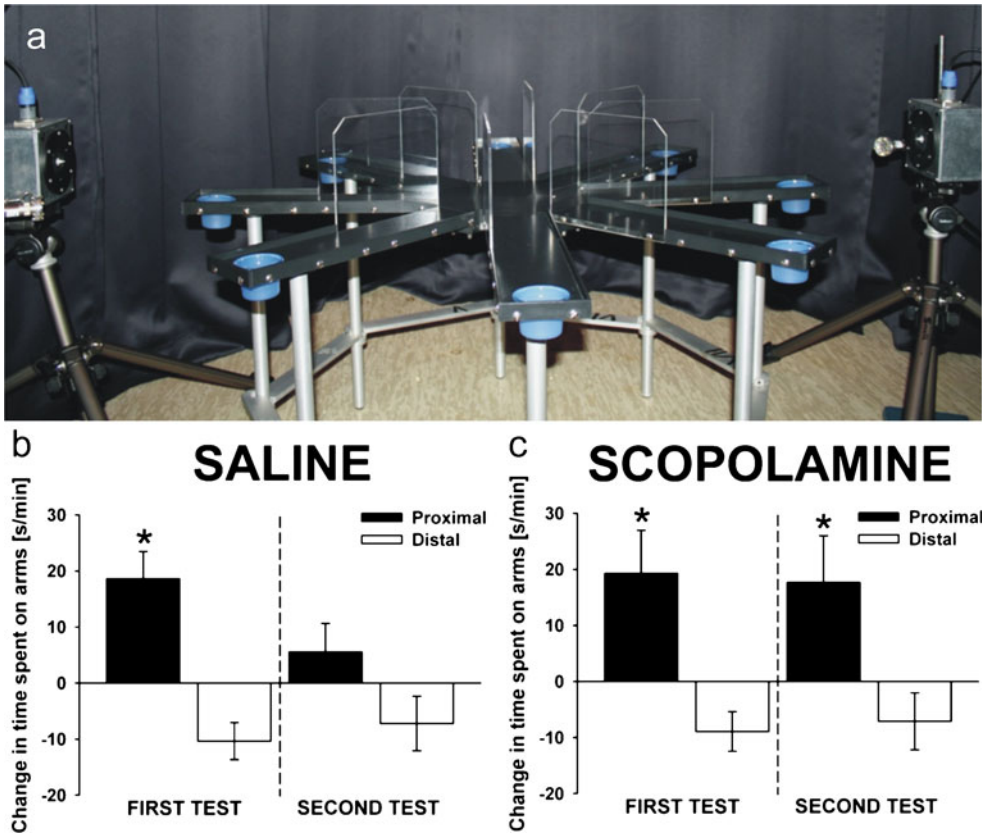

Fig. 7 Social approach behavior in response to appetitive 50-kHz ultrasonic vocalizations (USV) occurs after repeated exposures in rats treated with the amnesia-inducing compound scopolamine but not in saline-treated controls, reflecting social acoustic memory processes. **a** Set-up used for conducting playback experiments to test the communicative functions of USV. **b** Social approach behavior displayed by saline-treated rats during the first exposure (*FIRST TEST*, *left*) and the second exposure one week later (*SECOND TEST*, *right*). **c** Social approach behavior displayed by scopolamine-treated rats (1.5 mg/kg scopolamine) during the first exposure (*FIRST TEST*, *left*) and the second exposure one week later (*SECOND TEST*, *right*). Social approach behavior is given as the playback-induced change (from *baseline*) in the time spent on the arms proximal to (*Proximal*, *black bars*) and distal from (*Distal*, *white bars*) the ultrasonic loud speaker used for the playback of appetitive 50-kHz USV. Drug treatment occurred immediately after the first stimulus exposure. Data are presented as means ± SEM. **P*<0.050, for Proximal versus Distal. Based on data from Wöhr and Schwarting (2012)

perception-and-action loop, linking mechanisms relevant for 50-kHz USV detection and production. Such a loop appears to be particularly relevant for appetitive social and reciprocal communicatory signals. This assumption is further supported by the finding that juvenile rough-and-tumble play, which is typically paralleled by high rates of 50-kHz USV, also leads to increased c-fos labeling in the NAcc (Gordon et al. 2002).

Importantly, the NAcc is also the brain region in which opioids exert at least some of their effects on social behavior (Panksepp and Bishop 1980; Vanderschuren et al. 1995c). The opioid system is well known for its important role in regulating rough-and-tumble play (Beatty and Costello 1982; Panksepp et al. 1985; Vanderschuren et al. 1995a, 1995b). The finding that it also controls social approach behavior induced by playback of 50-kHz USV therefore needs to be highlighted (Wöhr and Schwarting 2009). In agreement with studies of opioidergic effects on rough-and-tumble play, we have shown that the opioid agonist morphine enhances social approach behavior, whereas naloxone, an opioid antagonist, inhibits social approach behavior. An important role of the opioid system in ultrasonic communication is also suggested by findings from mouse studies. Moles et al. (2004) have found that mouse pups lacking the μ-opioid receptor display strongly reduced levels of isolation-induced USVs in response to separation from their mother and littermates. When adult, male mice lacking the μ-opioid receptor do not show behavioral changes in response to the playback of USVs recorded during female-female interactions (Wöhr et al. 2011a).

Concluding remarks

Behavioral studies of aversive 22-kHz USV indicate that the likelihood of emitting 22-kHz USV is largely independent of the social context but might be potentiated by the presence of

conspecifics under certain circumstances, for instance, in established colonies. They further suggest that 22-kHz USV are not innately recognized as alarm calls but that they can obtain an alarm signal value as a consequence of associative learning, such as auto-conditioning, which is facilitated by a biological preparedness to associate 22-kHz USV with aversive events. In addition, neuronal studies indicate that 22-kHz USV activate brain regions implicated in the regulation of fear and anxiety in the receivers, that at least some of these regions are required for fear conditioning to 22-kHz USV and that, among the activated structures, the amygdala and perirhinal cortex might be part of the "neural template" responsible for the biological preparedness to associate 22-kHz USV with aversive events.

Behavioral studies of appetitive 50-kHz USV indicate that they facilitate mating but also that they have a communicative value in nonsexual contexts in which they appear to serve to establish or maintain contact among conspecifics. They further suggest that such a pro-social communicative function depends on acoustic stimulus configuration, most importantly peak frequency and social acoustic memory. In addition, neuronal studies indicate that 50 kHz USV decrease activation in a large number of brain areas, including the amygdala, whereas two brain regions, namely the frontal cortex, specifically, the secondary motor cortex, and the NAcc, exhibit increased neuronal activity. Neuronal activation in the former region is probably attributable to the finding that 50 kHz USV induce pronounced behavioral activation, whereas activation of the NAcc might be related to the appetitive value of 50 kHz USV. Finally, dopamine and opioids appear to be involved in pro-social ultrasonic communication.

As pointed out by Parsana et al. (2012a), opposite behavioral responses, together with distinct patterns of brain activation, particularly the bidirectional tonic activation or deactivation of the amygdala elicited by 22-kHz and 50-kHz USV, respectively, are in agreement with a wealth of behavioral and neuroimaging studies in humans involving the use of emotionally salient stimuli, such as fearful and happy facial expressions (e.g., Morris et al. 1996; Whalen et al. 1998). Affective ultrasonic communication therefore offers a translational tool for studying the neurobiology underlying socio-affective communication. This is particularly relevant for rodent models of neurodevelopmental disorders. Indeed, socio-affective communication is severely impaired in a number of human neuropsychiatric disorders, including autism and schizophrenia. By means of human neuroimaging studies, such impairments have been repeatedly associated with aberrant amygdala responses during emotion processing, both in schizophrenia (e.g., Gur et al. 2002) and autism (e.g., Critchley et al. 2000), linking disease symptoms, brain activity patterns and genetic variants underlying deficits in socio-affective communication (e.g., Meyer-Lindenberg et al. 2009).

Acknowledgements The authors thank Nicole Yee for the photographs shown in Fig. 4a–c.

References

Abrams JK, Johnson PL, Hollis JH, Lowry CA (2004) Anatomic and functional topography of the dorsal raphe nucleus. Ann N Y Acad Sci 1018:46–57

Allen TA, Furtak SC, Brown TH (2007) Single-unit responses to 22 kHz ultrasonic vocalizations in rat perirhinal cortex. Behav Brain Res 182:327–336

Allen TA, Narayanan NS, Kholodar-Smith DB, Zhao Y, Laubach M, Brown TH (2008) Imaging the spread of reversible brain inactivations using fluorescent muscimol. J Neurosci Methods 171:30–38

Bang SJ, Allen TA, Jones LK, Boguszewski P, Brown TH (2008) Asymmetrical stimulus generalization following differential fear conditioning. Neurobiol Learn Mem 90:200–216

Beatty WW, Costello KB (1982) Naloxone and play fighting in juvenile rats. Pharmacol Biochem Behav 17:905–907

Beckett SRG, Aspley S, Graham M, Marsden CA (1996) Pharmacological manipulation of ultrasound induced defence behaviour in the rat. Psychopharmacology (Berl) 127:384–390

Beckett SRG, Duxon MS, Aspley S, Marsden CA (1997) Central c-fos expression following 20 kHz/ultrasound induced defence behaviour in the rat. Brain Res Bull 42:421–426

Blanchard RJ, Blanchard DC, Rodgers J, Weiss SM (1990) The characterization and modelling of antipredator defensive behavior. Neurosci Biobehav Rev 14:463–472

Blanchard RJ, Blanchard DC, Agullana R, Weiss SM (1991) Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. Physiol Behav 50:967–972

Borta A, Wöhr M, Schwarting RKW (2006) Rat ultrasonic vocalization in aversively motivated situations and the role of individual differences in anxiety-related behavior. Behav Brain Res 166:271–280

Brandão ML, Coimbra NC, Osaki MY (2001) Changes in the auditory-evoked potentials induced by fear-evoking stimulations. Physiol Behav 72:365–372

Brudzynski SM, Chiu EMC (1995) Behavioural responses of laboratory rats to playback of 22 kHz ultrasonic calls. Physiol Behav 57:1039–1044

Brudzynski SM, Pniak A (2002) Social contacts and production of 50-kHz short ultrasonic calls in adult rats. J Comp Psychol 116:73–82

Burgdorf J, Knutson B, Panksepp J, Ikemoto S (2001) Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. Behav Neurosci 115:940–944

Burgdorf J, Panksepp J, Brudzynski SM, Kroes R, Moskal JR (2005) Breeding for 50-kHz positive affective vocalization in rats. Behav Genet 35:67–72

Burman OHP, Ilyat A, Jones G, Mendl M (2007) Ultrasonic vocalizations as indicators of welfare for laboratory rats (*Rattus norvegicus*). Appl Anim Behav Sci 104:116–129

Choi JS, Brown TH (2003) Central amygdala lesions block ultrasonic vocalization and freezing as conditional but not unconditional responses. J Neurosci 23:8713–8721

Commissaris RL, Beckett SRG, Marsden CA (1998) Strychnine effects on ultrasound-elicited behaviours in Lister hooded rats. Psychopharmacology (Berl) 136:162–171

Commissaris RL, Palmer A, Neophytou SI, Graham M, Beckett SRG, Marsden CA (2000) Acoustically elicited behaviours in Lister hooded and Wistar rats. Physiol Behav 68:521–531

Covington HE III, Miczek KA (2003) Vocalizations during withdrawal from opiates and cocaine: possible expressions of affective distress. Eur J Pharmaol 467:1–13

Critchley HD, Daly EM, Bullmore ET, Williams SC, Van Amelsvoort T, Robertson DM, Rowe A, Phillips M, McAlonan G, Howlin P, Murphy DG (2000) The functional neuroanatomy of social behaviour: changes in cerebral blood flow when people with autistic disorder process facial expressions. Brain 123:2203–2212

Czéh B, Müller-Keuker JI, Rygula R, Abumaria N, Hiemke C, Domenici E, Fuchs E (2007) Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. Neuropsychopharmacology 32:1490–1503

D'Amato FR, Moles A (2001) Ultrasonic vocalizations as an index of social memory in female mice. Behav Neurosci 115:834–840

De Oca BM, DeCola JP, Maren S, Fanselow MJ (1998) Distinct regions of the periaqueductal grey are involved in the acquisition and expression of defensive responses. J Neurosci 18:3426–3443

Depaulis A, Keay KA, Bandler R (1994) Longitudinal neuronal organization of defensive reactions in the midbrain periaqueductal gray region of the rat. Exp Brain Res 90:307–318

Duncan GE, Knapp DJ, Breese GR (1996) Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. Brain Res 713:79–91

Ehninger D, Sano Y, de Vries PJ, Dies K, Franz D, Geschwind DH, Kaur M, Lee YS, Li W, Lowe JK, Nakagawa JA, Sahin M, Smith K, Whittemore V, Silva AJ (2012) Gestational immune activation and Tsc2 haploinsufficiency cooperate to disrupt fetal survival and may perturb social behavior in adult mice. Mol Psychiatry 17:62-70

Ehret G (2005) Infant rodent ultrasounds—a gate to the understanding of sound communication. Behav Genet 35:19–22

Endres T, Widmann K, Fendt M (2007) Are rats predisposed to learn 22 kHz calls as danger-predicting signals? Behav Brain Res 185:69–75

Fendt M, Fanselow MS (1999) The neuroanatomical and neurochemical basis of conditioned fear. Neurosci Biobehav Rev 23:743–760

Finn DP, Jhaveri MD, Beckett SRG, Kendall DA, Marsden CA, Chapman V (2004) Cannabinoids modulate ultrasound-induced aversive responses in rats. Psychopharmacology (Berl) 172:41–51

Fish EW, Faccidomo S, Gupta S, Miczek KA (2004) Anxiolytic-like effects of escitalopram, citalopram, and R-citalopram in maternally separated mouse pups. J Pharmacol Ex Ther 308:474–480

Fujita E, Tanabe Y, Shiota A, Ueda M, Suwa K, Momoi MY, Momoi T (2008) Ultrasonic vocalization impairment of Foxp2 (R552H) knockin mice related to speech-language disorder and abnormality of Purkinje cells. Proc Natl Acad Sci USA 105:3117–3122

Furtak SC, Allen TA, Brown TH (2007) Single-unit firing in rat perirhinal cortex caused by fear conditioning to arbitrary and ecological stimuli. J Neurosci 27:12277–12291

Garcia J, Koelling R (1966) Relation of cue to consequence in avoidance learning. Psychon Sci 4:123–124

Geisler S, Trimble M (2008) The lateral habenula: no longer neglected. CNS Spectr 13:484–489

Geyer LA, Barfield RJ (1978) Influence of gonadal hormones and sexual behavior on ultrasonic vocalization in rats. I. Treatment of females. J Comp Physiol Psychol 92:436–446

Gordon NS, Kollack-Walker S, Akil H, Panksepp J (2002) Expression of c-*fos* gene activation during rough and tumble play in juvenile rats. Brain Res Bull 57:651–659

Gur RE, McGrath C, Chan RM, Schroeder L, Turner T, Turetsky BI, Kohler C, Alsop D, Maldjian J, Ragland JD, Gur RC (2002) An fMRI study of facial emotion processing in patients with schizophrenia. Am J Psychiatry 159:1992–1999

Harmon KM, Cromwell HC, Burgdorf J, Moskal JR, Brudzynski SM, Kroes RA, Panksepp J (2008) Rats selectively bred for low levels of 50 kHz ultrasonic vocalizations exhibit alterations in early social motivation. Dev Psychobiol 50:322–331

Hofer MA, Shair HN (1993) Ultrasonic vocalization, laryngeal braking, and thermogenesis in rat pups: a reappraisal. Behav Neurosci 107:354–362

Jelen P, Soltysik S, Zagrodzka J (2003) 22-kHz ultrasonic vocalization in rats as an index of anxiety but not fear: behavioral and pharmacological modulation of affective state. Behav Brain Res 141:63–72

Kaltwasser MT (1990) Acoustic signaling in the black rat (*Rattus rattus*). J Comp Psychol 104:227–232

Kholodar-Smith DB, Allen TA, Brown TH (2008a) Fear conditioning to discontinuous auditory cues requires perirhinal cortical function. Behav Neurosci 122:1178–1185

Kholodar-Smith DB, Boguszewski P, Brown TH (2008b) Auditory trace fear conditioning requires perirhinal cortex. Neurobiol Learn Mem 90:537–543

Kim EJ, Kim ES, Covey E, Kim JJ (2010) Social transmission of fear in rats: the role of 22-kHz ultrasonic distress vocalizations. PLoS One 5:e15077

Kiyokawa Y, Kikusui T, Takeuchi Y, Mori Y (2004) Partner's stress status influences social buffering effects in rats. Behav Neurosci 118:798–804

Knutson B, Burgdorf J, Panksepp J (1998) Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. J Comp Psychol 112:65–73

Kovács KJ (1998) c-fos as a transcription factor: a stressful (re)view from a functional map. Neurochem Int 33:287–297

LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23:155–184

Lindquist DH, Jarrard LE, Brwon TH (2004) Perirhinal cortex supports delay fear conditioning to rat ultrasonic social signals. J Neurosci 24:3610–3617

Łopuch S, Popik P (2011) Cooperative behavior of laboratory rats (*Rattus norvegicus*) in an instrumental task. J Comp Psychol 125:250–253

Mällo T, Matrov D, Herm L, Kõiv K, Eller M, Rinken A, Harro J (2007) Tickling-induced 50-kHz ultrasonic vocalization is individually stable and predicts behaviour in tests of anxiety and depression in rats. Behav Brain Res 184:57–71

Maren S, Quirk GJ (2004) Neuronal signaling of fear memory. Nat Rev Neurosci 5:844–852

McIntosh TK, Barfield RJ, Geyer LA (1978) Ultrasonic vocalisations facilitate sexual behaviour of female rats. Nature 272:163–164

Meyer-Lindenberg A, Kolachana B, Gold B, Olsh A, Nicodemus KK, Mattay V, Dean M, Weinberger DR (2009) Genetic variants in AVPR1A linked to autism predict amygdala activation and personality traits in healthy humans. Mol Psychiatry 14:968–975

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69–97

Moles A, Kieffer BL, D'Amato FR (2004) Deficit in attachment behavior in mice lacking the mu-opioid receptor gene. Science 304:1983–1986

Morgan MM, Whitney PK, Gold MS (1998) Immobility and flight associated with antinociception produced by activation of the ventral and lateral/dorsal regions of the rat periaqueductal grey. Brain Res 804:159–166

Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, Dolan RJ (1996) A differential neural response in the human amygdala to fearful and happy facial expressions. Nature 383:812–815

Moskal JR, Burgdorf J, Kroes RA, Brudzynski SM, Panksepp J (2011) A novel NMDA receptor glycine-site partial agonist, GLYX-13,

has therapeutic potential for the treatment of autism. Neurosci Biobehav Rev 35:1982–1988
Neophytou SI, Graham M, Williams J, Aspley S, Marsden CA, Beckett SRG (2000) Strain differences to the effects of aversive frequency ultrasound on behaviour and brain topography of c-fos expression in the rat. Brain Res 854:158–164
Nicolas LB, Klein S, Prinssen EP (2007) Defensive-like behaviours induced by ultrasound: further pharmacological characterization in Lister-hooded rats. Psychopharmacology (Berl) 194:243–252
Nobre MJ, Brandão ML (2004) Analysis of freezing behavior and ultrasonic vocalization in response to foot-shocks, ultrasound signals and GABAergic inhibition in the inferior colliculus: effects of muscimol and midazolam. Eur Neuropsychopharmacol 14:45–52
Panksepp J (2005) Beyond a joke: from animal laughter to human joy? Science 308:62–63
Panksepp J, Bishop P (1980) An autoradiographic map of [^{3}H] diprenorphine binding in rat brain: effects of social interaction. Brain Res Bull 7:405–410
Panksepp J, Burgdorf J (1999) Laughing rats? Playful tickling arouses high frequency ultrasonic chirping in young rodents. In: Hameroff S, Chalmers C, Kazniak A (eds) Toward a science of consciousness III. MIT Press, Camebridge, Mass., pp 231–244
Panksepp J, Burgdorf J (2000) 50-kHz chirping (laughter?) in response to conditioned and unconditioned tickle-induced reward in rats: effects of social housing and genetic variables. Behav Brain Res 115:25–38
Panksepp J, Burgdorf J (2003) "Laughing" rats and the evolutionary antecedents of human joy? Physiol Behav 79:533–547
Panksepp J, Jalowiec J, DeEskinazi FG, Bishop P (1985) Opiates and play dominance in juvenile rats. Behav Neurosci 99:441–453
Panksepp J, Gordon N, Burgdorf J (2002) Empathy and the action-perception resonances of basic socio-emotional systems of the brain. Behav Brain Sci 25:43–44
Parsana AJ, Li N, Brown TH (2012a) Positive and negative ultrasonic social signals elicit opposing firing patterns in rat amygdala. Behav Brain Res 226:77–86
Parsana AJ, Moran EE, Brown TH (2012b) Rats learn to freeze to 22-kHz ultrasonic vocalizations through autoconditioning. Behav Brain Res 232:395–399
Pitkänen A, Savander V, LeDoux JE (1997) Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci 20:517–523
Rygula R, Pluta H, Popik P (2012) Laughing rats are optimistic. PLoS One 7:e51959
Sadananda M, Wöhr M, Schwarting RKW (2008) Playback of 22-kHz and 50-kHz ultrasonic vocalizations induces differential c-fos expression in rat brain. Neurosci Lett 435:17–23
Sales GD (1972a) Ultrasound and aggressive behaviour in rats and other small mammals. Anim Behav 20:88–100
Sales GD (1972b) Ultrasound and mating behaviour in rodents with some observations on other behavioural situations. J Zool 168:149–164
Sales GD (1991) The effect of 22 kHz calls and artificial 38 kHz signals on activity in rats. Behav Processes 24:83–93
Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809
Scattoni ML, Crawley J, Ricceri L (2009) Ultrasonic vocalizations: a tool for behavioural phenotyping of mouse models of neurodevelopmental disorders. Neurosci Biobehav Rev 33:508–515
Schwarting RKW, Wöhr M (2012) On the relationships between ultrasonic calling and anxiety-related behavior in rats. Braz J Med Biol Res 45:337–348
Schwarting RKW, Jegan N, Wöhr M (2007) Situational factors, conditions and individual variables which can determine ultrasonic vocalizations in male adult Wistar rats. Behav Brain Res 182:208–222
Seligman MEP (1970) On the generality of the laws of learning. Psychol Rev 77:406–418
Sewell GD (1970) Ultrasonic communication in rodents. Nature 227:410
Siviy SM, Panksepp J (1987) Sensory modulation of juvenile play in rats. Dev Psychobiol 20:39–55
Takahashi A, Yap JJ, Bohager DZ, Faccidomo S, Clayton T, Cook JM, Miczek KA (2009) Glutamatergic and GABAergic modulations of ultrasonic vocalizations during maternal separation distress in mouse pups. Psychopharmacology (Berl) 204:61–71
Thomas DA, Barfield RJ (1985) Ultrasonic vocalization of the female rat (*Rattus norvegicus*) during mating. Anim Behav 33:720–725
Thomas DA, Talalas L, Barfield RJ (1981) Effects of devocalization of the male on mating behavior in rats. J Comp Phsysiol Psychol 95:630–637
Thomas DA, Howard SB, Barfield RJ (1982) Male-produced postejaculatory vocalizations and the mating behaviour of estrous female rats. Behav Neural Biol 36:403–410
Thompson B, Leonard KC, Brudzynski SB (2006) Amphetamine-induced 50 kHz calls from rat nucleus accumbens: a quantitative mapping study and acoustic analysis. Behav Brain Res 168:64–73
Tsoory M, Cohen H, Richter-Levin G (2007) Juvenile stress induces a predisposition to either anxiety or depressive-like symptoms following stress in adulthood. Eur Neuropsychopharmacol 17:245–256
Tsoory M, Guterman A, Richter-Levin G (2008) Exposure to stressors during juvenility disrupts development-related alterations in the PSA-NCAM to NCAM expression ratio: potential relevance for mood and anxiety disorders. Neuropsychopharmacology 33:378–393
Vanderschuren LJMJ, Niesink RJM, Spruijt BM, Van Ree JM (1995a) Effects of morphine on different aspects of social play in juvenile rats. Psychopharmacology (Berl) 117:225–231
Vanderschuren LJMJ, Niesink RJM, Spruijt BM, Van Ree JM (1995b) μ- and κ-opioid receptor-mediated opioid effects on social play in juvenile rats. Eur J Pharmacol 276:257–266
Vanderschuren LJMJ, Stein EA, Wiegant VM, Van Ree JM (1995c) Social play alters regional brain opioid receptor binding in juvenile rats. Brain Res 680:148–156
Vianna DML, Brandão ML (2003) Anatomical connections of the periaqueductal gray: specific neural substrates for different kinds of fear. Braz J Med Biol Res 36:557–566
Vivian JA, Farrell WJ, Sapperstein SB, Miczek KA (1994) Diazepam withdrawal: effects of diazepam and gepirone on acoustic startle-induced 22 kHz ultrasonic vocalizations. Psychopharmacology (Berl) 114:101–108
Voits M, Beckett SRG, Marsden CA, Fink H (1999) Role of cholecystokinin type B receptors in ultrasound induced behavior in rats. Peptides 20:383–386
Webber ES, Harmon KM, Beckwith TJ, Peña S, Burgdorf J, Panksepp J, Cromwell HC (2012) Selective breeding for 50 kHz ultrasonic vocalization emission produces alterations in the ontogeny and regulation of rough-and-tumble play. Behav Brain Res 229:138–144
Whalen PJ, Rauch SL, Etcoff NL, McInerney SC, Lee MB, Jenike MA (1998) Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci 18:411–418
White NR, Barfield RJ (1987) Role of the ultrasonic vocalization of the female rat (*Rattus norvegicus*) in sexual behavior. J Comp Psychol 101:73–81
White NR, Barfield RJ (1989) Playback of female rat ultrasonic vocalizations during sexual behavior. Physiol Behav 45:229–233

White NR, Barfield RJ (1990) Effects of male pre-ejaculatory vocalizations on female receptive behavior in the rat (*Rattus norvegicus*). J Comp Psychol 104:140–146

Wöhr M, Schwarting RKW (2007) Ultrasonic communication in rats: can playback of 50-kHz calls induce approach behavior? PLoS One 2:e1365

Wöhr M, Schwarting RKW (2008a) Maternal care, isolation-induced infant ultrasonic calling, and their relations to adult anxiety related behavior in the rat. Behav Neurosci 122:310–330

Wöhr M, Schwarting RKW (2008b) Ultrasonic calling during fear conditioning in the rat: no evidence for an audience effect. Anim Behav 76:749–760

Wöhr M, Schwarting RKW (2009) Ultrasonic communication in rats: effects of morphine and naloxone on vocal and behavioral responses to playback of 50-kHz vocalizations. Pharmacol Biochem Behav 94:285–295

Wöhr M, Schwarting RKW (2012) Testing social acoustic memory in rats: effects of stimulus configuration and long-term memory on the induction of social approach behavior by appetitive 50-kHz ultrasonic vocalizations. Neurobiol Learn Mem 98:154–164

Wöhr M, Borta A, Schwarting RKW (2005) Overt behavior and ultrasonic vocalization in a fear conditioning paradigm—a dose-response study in the rat. Neurobiol Learn Mem 84:228–240

Wöhr M, Houx B, Schwarting RKW, Spruijt B (2008) Effects of experience and context on 50-kHz vocalizations in rats. Physiol Behav 93:766–776

Wöhr M, Moles A, Schwarting RK, D'Amato FR (2011a) Lack of social exploratory activation in male μ-opioid receptor KO mice in response to playback of female ultrasonic vocalizations. Soc Neurosci 6:76–87

Wöhr M, Roullet FI, Hung AY, Sheng M, Crawley JN (2011b) Communication impairments in mice lacking Shank1: reduced levels of ultrasonic vocalizations and scent marking behavior. PLoS One 6:e20631

Wolf AR, Bilkey DK (2008) Immune activation during mid-gestation disrupts sensorimotor gating in rat offspring. Behav Brain Res 190:156–159

Yamamuro T, Senzaki K, Iwamoto S, Nakagawa Y, Hayashi T, Hori M, Sakamoto S, Murakami K, Shiga T, Urayama O (2010) Neurogenesis in the dentate gyrus of the rat hippocampus enhanced by tickling stimulation with positive emotion. Neurosci Res 68:285–289

Yee N, Schwarting RKW, Fuchs E, Wöhr M (2012a) Juvenile stress potentiates aversive 22-kHz ultrasonic vocalizations and freezing during auditory fear conditioning in adult male rats. Stress 15:533–544

Yee N, Schwarting RKW, Fuchs E, Wöhr M (2012b) Increased affective ultrasonic communication during fear learning in adult male rats exposed to maternal immune activation. J Psychiatr Res 46:1199–1205

Zippelius HM, Schleidt WM (1956) Ultraschall-Laute bei jungen Mäusen. Naturwissenschaften 43:502