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M. Wöhr · R.K.W. Schwarting
Experimental and Physiological Psychology, Philipps University of Marburg, Marburg

Rodent ultrasonic communication and its relevance for models of neuropsychiatric disorders

Introduction

The effectiveness of animal models is dependent on their validity. In particular, animal models should incorporate strong analogies to the human syndrome (face validity). It is of further relevance whether the same biological dysfunction that causes the human disorder is causing related symptoms in the model organism (construct validity) and whether analogous responses can be seen in the model organism when treated in ways known to prevent or reverse symptoms in the human disorder (predictive validity). Particularly in the case of neuropsychiatric disorders, however, already the incorporation of face validity is often difficult. In fact, there are a number of symptoms that can hardly be modeled in animal models, ranging from impairments in affect regulation to deficits in social behaviour and communication. How does one measure low mood and the inability to experience pleasure, typical symptoms of a major depression, in mice and rats? Or symptoms of neurodevelopmental disorders such as autism, including the deficits in social information processing and delay in language acquisition? One might also think about the negative symptoms of schizophrenia: flat or inappropriate affect, poverty of speech and lack of desire to form social relationships.

The incorporation of such symptoms in animal models appears to be restricted to visible and hence observable behaviours. Thus, impairment in affect regulation is most commonly measured by approach and avoidance behaviour. Similarly, the only approach used to determine deficits in social behaviour and communication is in most cases the observation of social interactions. Here, we will show that the hitherto "one-dimensional" approach that is restricted to observable behaviour can be extended by a second "dimension".

Types of ultrasonic vocalizations—mouse and rat

Mice and rats produce and perceive calls in the ultrasonic range (so-called ultrasonic vocalizations, USV). Various USV types can be differentiated on the basis of distinct acoustic features. Their occurrence is dependent on stage of development, affective state and social context.

Mouse: In 1956 Hanna-Maria Zippelius and Wolfgang M. Schleidt reported in *Naturwissenschaften* for the first time that infant mice emit USV during the first days of life when isolated from mother and littermates ("isolation-induced USV"; 60-kHz USV; **Fig. 1a**) [52]. Already in this very first study, they suggested that such USV reflect a negative affective state and named them "*Pfeifen des Verlassenseins*" (whistles of loneliness) according to Konrad Lorenz. They further suggested that pup USV serve communicative purposes, since they observed that mothers leave the nest to retrieve vocalizing pups which were scattered outside the nest, while no search and retrieval behaviour was seen in response to non-vocalizing pups.

It was recently found that juvenile mice also produce USV. As reported by Jules B. Panksepp et al. (2007) in *PloS One* [23], juvenile mice emit USV during social investigation of conspecifics ("interaction-induced USV"; 70-kHz USV; **Fig. 1b**). Gilian D. Sewell discovered already in 1967 [32] that adult mice emit USV. She reported in *Nature* that USV occur during mating behaviour [32]. USV occurring during mating behaviour are emitted by the male and it was shown shortly later that such USV occur also when a male is exposed to female urine. It was therefore believed that such USV induce receptive behaviour in the female ("female-induced USV"; 70-kHz USV; **Fig. 1c**). The important difference between USV emitted by juvenile mice during social investigation of conspecifics and USV emitted by males exposed to females is a lack of the whistle-like character in the latter. Female-induced USV appear rough and noisy.

In view of the recently discovered interaction-induced USV in juvenile mice, female-induced USV appear to be a subtype of interaction-induced USV. Interaction-induced USV are emitted by juvenile mice of both sexes. In males, however, their emission declines rapidly with sexual maturity. They begin to emit the rough and noisy female-induced USV. In females, however, interaction-induced USV persist into adulthood and are still detectable during social investigation of conspecifics in adult females. In fact, interaction-induced USV in adult females have been known about for several years, but they were not taken into account, since the production of USV in adult mice was studied almost exclusively during mating behaviour. A common feature of both call types, interaction-induced USV in general as well as its subtype, female-induced USV, is their affiliative, i.e. social contact-inducing, communicative function.

Rat: Three USV types can also be distinguished in the rat. As shown by Eliane Noirot (1968) in *Animal Behaviour* [19],

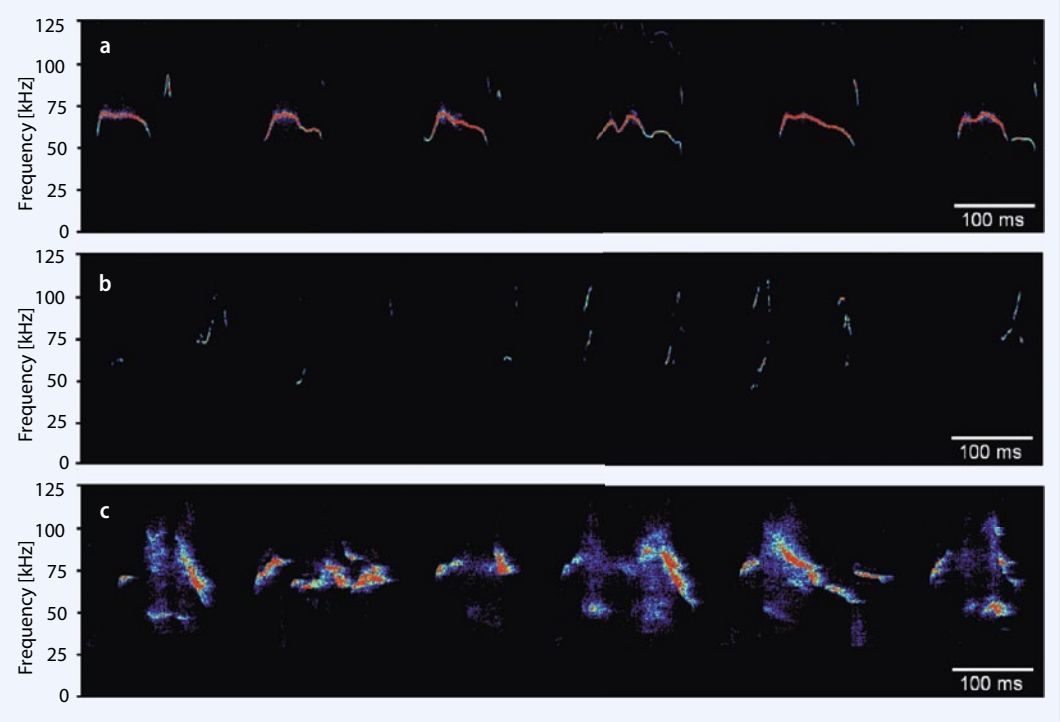

Fig. 1 ▲ Types of ultrasonic vocalizations (USV): mouse. **a** Isolation-induced USV (60-kHz USV) emitted by an 8-day-old male C57BL/6J mouse during isolation from mother and littermates. **b** Interaction-induced USV (70-kHz USV, juvenile) emitted by a 25-day-old male C57BL/6J mouse during social interaction with a C57BL/6J mouse of the same age. **c** Female-induced USV (70-kHz USV, adult) emitted by a 3-month-old male C57BL/6J mouse exposed to female urine. All recordings were made using an UltraSoundGate Condenser Microphone CM16/CMPA (sampling rate: 250 kHz; 16 bit). Spectrograms were generated with Avisoft SASLab Pro by conducting a fast Fourier transform (time resolution: 0.427 ms; frequency resolution: 0.586 kHz). Equipment and software: Avisoft Bioacoustics, Berlin, Germany

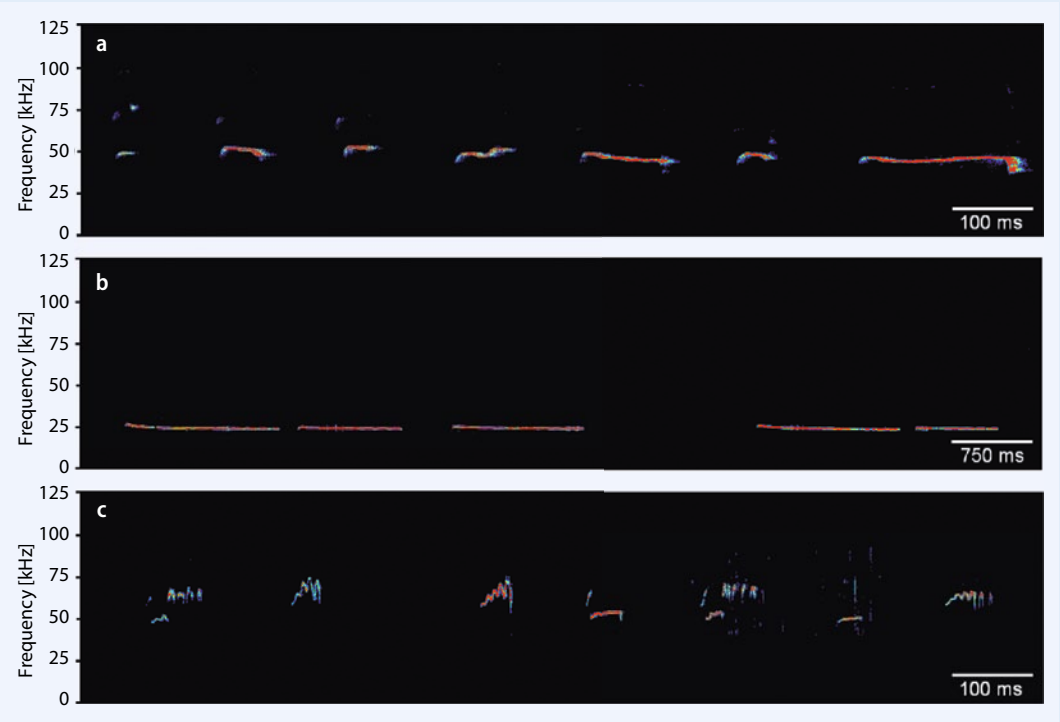

Fig. 2 ◀ Types of ultrasonic vocalizations (USV): rat. **a** Isolation-induced USV (40-kHz USV) emitted by an 11-day-old male Wistar rat during isolation from mother and littermates. **b** Fear-induced USV (22-kHz USV) emitted by a 3-month-old male Wistar rat during fear conditioning. **c** Interaction-induced USV (50-kHz USV) emitted by a 3-month-old male Wistar rat searching for conspecifics. Please note the change in time resolution in **b** as compared to **a** and **c**. For details on recording and spectrogram: see legend to **Fig. 1**

infant rats emit USV when separated from their mother and littermates like mice, indicating a similar function ("isolation-induced USV"; 40-kHz USV; ◘ **Fig. 2a**).

In juvenile and adult rats, two USV types are known. Their occurrence is dependent on the emotional valence of the context. While low-frequency USV occur in potentially dangerous situations, such as predator exposure, submission during intermale fighting or in response to painful stimuli like foot-shocks ("fear-induced USV"; 22-kHz USV; ◘ **Fig. 2b**), high-frequency USV occur in presumably appetitive situations such as rough-and-tumble play ("interaction-induced USV "; 50-kHz USV; ◘ **Fig. 2c**). The low-frequency 22-kHZ USV are characterized by high amplitudes and long durations. In contrast to the other USV types known in mice and rats, which typically last up to 300 ms at maximum, 22-kHz USV last around 1000 ms on average. Probably because of these acoustic characteristics, which make detection relatively easy, 22-kHz USV were the first USV discovered in rodents: John W. Anderson reported in *Science* (1954) [1] that rats which have been singly housed, i.e. socially isolated, emit 22-kHz USV. He assumed that rats use 22-kHz USV for orientation in a manner comparable to the process of echolocation employed by bats. Today, however, one would interpret the occurrence of 22-kHz USV during social isolation as the result of a negative affective state induced by isolation and it is believed that 22-kHz USV serve as alarm calls. A USV type similar to rat 22-kHz USV is not known in mice.

High-frequency 50-kHz USV that occur in presumably appetitive situations resemble 70-kHz USV in mice. Indeed, both were detected for the first time by Gilian D. Sewell [32] during mating behaviour. Recent studies, however, have shown that the production of 50-kHz USV is not restricted to the sexual context. Rather, 50-kHz USV occur during rough-and-tumble play and when it is mimicked by a human experimenter through so-called tickling. Furthermore, 50-kHz USV can be elicited by administering drugs of abuse such as amphetamine and cocaine. Jaak Panksepp postulated in *Science* (2005) [20] that 50-kHz USV reflect the rat's positive affective state and that they resemble human laughter. As for human laughter, it is believed that 50-kHz USV serve an affiliative communicative function.

Functions of isolation-induced USV

Affective state: The idea that isolation-induced USV reflect the animal's affective state was already included in the interpretative term "whistles of loneliness" chosen by Zippelius and Schleidt [52]. To date, however, there is an at times fierce scientific debate as to whether such USV reflect a negative affective state or whether they are simply the byproduct of a thermoregulation process [3]. There is a wealth of evidence, however, that argues against the interpretation that USV are simply physiological artifacts.

As part of a comprehensive selective breeding study, Brunelli and Hofer [5] demonstrated that it is possible to breed rats dependent on their level of isolation-induced USV and that such selective breeding affects anxiety-related and depression-related behaviour in adulthood. Adult rats from the breeding line characterized by high levels of pup isolation-induced USV display enhanced levels of neophobia and helplessness as compared to rats bred for low levels of isolation-induced USV. The former also have an elevated stress response. Remarkably, selective breeding for adult anxiety-related behaviour also affects the emission of isolation-induced USV in rat [38] and mouse pups [16].

The affective hypothesis is further supported by the fact that the production of isolation-induced USV can be modulated by pharmacological agents that are known for their potency in affecting anxiety in humans. Gardner [12] and Insel et al. [13] showed that anxiolytic compounds such as the benzodiazepines diazepam and chlordiazepoxide inhibit calling behaviour without impairing motor behaviour, while an increase in calling behaviour was found when anxiogenic compounds such as pentylenetetrazole were administered. Also, selective serotonin reuptake inhibitors (SSRI) such as paroxetine and citalopram inhibit the emission of isolation-induced USV [39].

Abstract

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Abstract
Mice and rats produce and perceive calls in the ultrasonic range (so-called ultrasonic vocalizations, USV). Various USV types can be differentiated on the basis of distinct acoustic features. Their occurrence is dependent on stage of development, affective state and social context. When separated from nest and littermates, young mice and rats emit isolation-induced USV, which induce maternal search and retrieval behaviour. Isolation-induced USV are used as an early marker of anxiety. Adult rats emit fear-induced USV in aversive situations such as predator exposure. They fulfil an alarm function and induce anxiety-related behaviour in conspecifics. Fear-induced USV are also used in the field of anxiety research. Finally, juvenile and adult mice and rats emit interaction-induced USV in presumably appetitive situations such as rough-and-tumble play or social investigation. As they can also be elicited by drugs of abuse, they are used in the field of addiction and depression research. They have an affiliative communicative function and induce social approach behaviour in the recipient. Focusing on the communicative function of interaction-induced USV, they serve as a measure for deficits in social behaviour and communication and hence are increasingly used in animal models for neurodevelopmental disorders such as autism.

Keywords
Behavioural neuroscience · Behavioural phenotyping · Communication · Emotion · Social behaviour

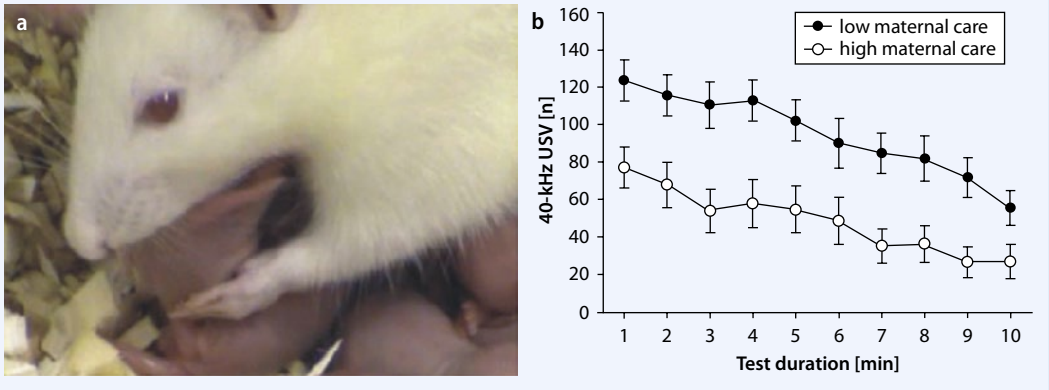

Fig. 3 ▲ Affective state, 40-kHz ultrasonic vocalizations (USV). **a** Anogenital licking behaviour. **b** Emission of 40-kHz USV in 11-day-old male Wistar rats during isolation from mother and littermates dependent on the level of maternal caregiving behaviour (maternal licking behaviour) received on postnatal days 3–6: low levels of maternal care (*black circles*, *n*=10), high level of maternal care (*white circles*, *n*=8). Based on data from [48]

Further evidence in support of the idea that isolation-induced USV reflect a negative affective state comes from studies on the effects of early environmental factors on early development. It is known that high levels of maternal care received during the very first days of life reduce the occurrence of anxiety-related behaviour in adulthood [8]. In line herewith, we showed that rat pups that received low levels of maternal care emit very high numbers of USV in isolation, while the ones that received high levels of maternal care displayed reduced calling behaviour (**Fig. 3a,b**, [48]). In mice, we found similar effects. By conducting an embryo-transfer study, we showed that the isolation-induced calling behaviour in infant mice is highly dependent on early environmental factors such as maternal care [41]. We found that even the closely related mouse strains C57BL/6JOla and C57BL/6N differ in USV production in isolation. Infant C57BL/6JOla mice vocalized more than C57BL/6N mouse pups, irrespective of sex. However, C57BL/6JOla pups that were delivered and raised by C57BL/6N mothers displayed markedly reduced calling behaviour that was even lower than the one seen in C57BL/6N pups. Certain call parameters such as call frequency, however, were dependent on genotype only. One important difference between the two mouse strains C57BL/6JOla and C57BL/6N is the lack of several genes on chromosome 6 in C57BL/6JOla due to a spontaneous deletion. Among them is the *Snca* gene for α-synuclein. Mutations in the *SNCA* gene are implicated in the pathogenesis of Parkinson's disease and dementia with Lewy bodies. In order to test whether the lack of α-synuclein causes changes in USV production in mouse pups, we compared *Snca* knockout mice with wildtype littermate controls [17]. In line with the difference between the two mouse strains C57BL/6JOla and C57BL/6N, we found that the lack of the *Snca* gene causes an increase in the number of isolation-induced USV. In addition to changes in the production of USV, we found a change in the expression of the *Foxp* gene family in *Snca* knockout mice. This is remarkable as the *FOXP* gene family was repeatedly found to be associated with USV production in rodents, song-learning in birds and language acquisition in humans [34].

Communicative function: The observation by Zippelius and Schleidt [52] that mothers display search and retrieval behaviour only in response to pups scattered outside the nest when they emit USV, while ignoring anesthetized and hence non-vocalizing pups, indicates a communicative function of USV. However, it appears possible that other stimuli associated with the pup such as olfactory signals also cause maternal search and retrieval behaviour. The first experimental demonstration that isolation-induced USV indeed induce maternal search and retrieval behaviour was conducted by Sewell [33]. She showed that lactating females leave the nest and start to explore the environment when tape-recorded isolation-induced USV are presented. Exploration behaviour was typically directed to the loudspeaker. As control signals such as an artificial 45-kHz tone were found not to be efficient, it can be concluded that maternal exploration behaviour is not simply due to curiosity, but instead to brood care. Further playback studies with artificial stimuli were conducted to determine the call characteristics that are necessary to elicit maternal search and retrieval behaviour. Ehret und Haack [11] showed that all signals higher than 35 kHz in frequency and 20 ms in duration with peak amplitudes lower than 20 dB in neighboring frequencies induce maternal search and retrieval behaviour.

The communicative function of isolation-induced USV is further highlighted by the fact that their emission is dependent on social stimuli. In general, all social stimuli associated with maternal contact such as nest odour reduce calling behaviour [18]. Also, the presence of an anesthetized pup inhibits the emission of isolation-induced USV. The fact that the temperature of the anesthetized pup can be as low as room temperature without losing its potency in reducing calling behaviour again argues against the idea that isolation-induced USV are simply a byproduct of a thermoregulation process [7]. Pharmacological studies showed that quieting in the presence of an anesthetized pup is due to the release of endogenous

opioids as it can be blocked by administering the opioid antagonist naloxone [7]. In line with this, opioid agonists such as morphine on the other hand are potent inhibitors of calling behaviour. Further evidence for the involvement of μ-opioid receptors in the modulation of isolation-induced USV by the social context was provided by studies on μ-opioid receptor knockout mice. Mice lacking μ-opioid receptors can produce USV, for instance when exposed to non-social stressors such as a considerable decrease in body temperature. However, social stressors such as isolation from mother and littermates do not induce the production of USV and no modulation by social context was detected, indicating a deficit in social information processing [18].

Potential applications: Two fields of application exist for isolation-induced USV: Firstly, the huge and well-established field of anxiety research, based on the assumption that isolation-induced USV reflect a negative affective state; secondly, the developing field of neurodevelopmental disorders, focusing on communicative functions in relevant animal models. One reason why isolation-induced USV are interesting for both fields is that they can be determined very early during development. Thus, it is possible to measure the anxiety level already at a time point at which standard measures for anxiety cannot be applied due to the pup's immaturity. This makes a prediction of anxiety-related behaviour in adulthood possible and hence provides the opportunity to test the efficacy of treatments that are applied early during development. The same is true for the field of neurodevelopmental disorders since it is crucial to determine communication deficits as early as possible. First promising studies indeed found deficits in the production of isolation-induced USV in mouse models of human neurodevelopmental disorders [30]. In this context, it would also be interesting to use isolation-induced USV as an independent variable, namely as an elicitor of maternal behaviours. For more details on isolation-induced USV see [45].

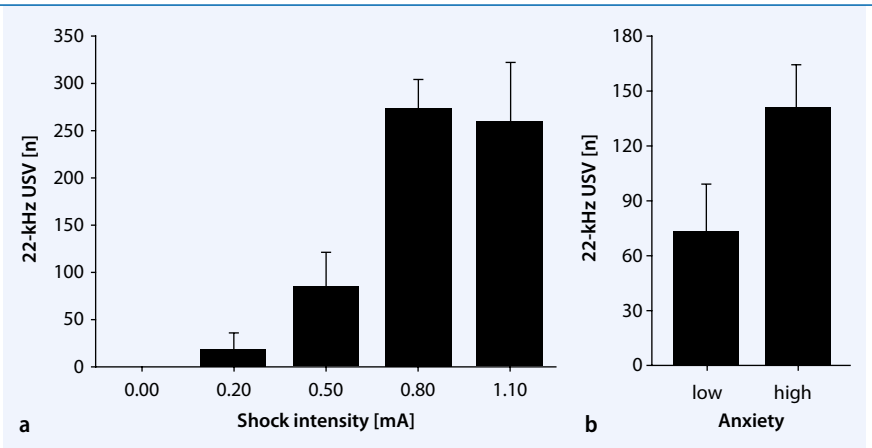

Fig. 4 ▲ Affective state, 22-kHz ultrasonic vocalizations (USV). **a** Emission of 22-kHz USV in 3-month-old male Wistar rats during fear conditioning dependent on shock intensity: 0.00 mA (*n*=7); 0.20 mA (*n*=7), 0.50 mA (*n*=8), 0.80 mA (*n*=8), 1.10 mA (*n*=6). Based on data from [40], with extensions. **b** Emission of 22-kHz USV in 3-month-old male Wistar rats during fear conditioning dependent on the individual disposition to show anxiety-related behaviour as measured on the elevated plus maze: low (*n*=10), high (*n*=10). Based on data from [4]

Functions of fear-induced USV

Affective state: The idea that 22-kHz USV reflect a negative affective state akin to anxiety and fear is based on the fact that such USV occur in a number of aversive situations such as predator exposure, submission during intermale fighting or in response to painful stimuli like foot shocks. It is further supported by observations made during fear conditioning. When the application of an aversive stimulus such as an electric shock (unconditioned stimulus, US) is repeatedly paired with a formerly neutral stimulus such as a tone (conditioned stimulus, CS), the CS gains the efficacy to elicit fear-related conditional responses (CRs) in the absence of the US. Part of the CRs, the fear-related conditional responses, is not only freezing behaviour that is typically determined, but also the emission of 22-kHz USV. This means that 22-kHz USV can occur in the absence of painful stimuli, namely in response to danger-predicting signals, the CS. Both CRs, freezing and the emission of 22-kHz USV, are strongly dependent on the aversiveness of the situation. In line with the affective hypothesis, we found that rats exposed to higher shock intensities display more freezing behaviour and vocalize more and louder than rats exposed to low shock intensities (**Fig. 4a**, [40]). The affective hypothesis is further supported by two additional observations. Firstly, not all rats emit 22-kHz USV. Secondly, we observed that the time spent freezing and the number of 22-kHz USV was positively correlated in individual rats. Rats that displayed high levels of freezing behaviour vocalized more than those that displayed low levels of freezing behaviour [48]. Both observations indicate that not only situational factors, for instance the aversiveness of the context, affect the emission of 22-kHz USV. Rather, the individual dispositions of the rat itself appear to also play a role. Interestingly, it has long been known that rats differ in their individual disposition to show anxiety-related behaviour [24]. In fact, we demonstrated that individual differences in calling behaviour are associated with individual differences in anxiety-related behaviour. Thus, rats that were characterized as anxious based on their anxiety-related behaviour displayed on the elevated plus maze emitted more 22-kHz USV during fear conditioning than less anxious rats—an effect that was not simply due to differences in pain sensitivity (**Fig. 4b**, [4]). Finally, pharmacological and neuroanatomical studies are also in line with the affective hypothesis. For instance, the emission of 22-kHz USV in response to the CS can be inhibited by anxiolytic compounds such as diazepam and buspirone [14] and no 22-kHz USV can be detected after amygdala lesion [9].

Communicative function: Several hypotheses have been proposed regarding the possible function of 22-kHz USV,

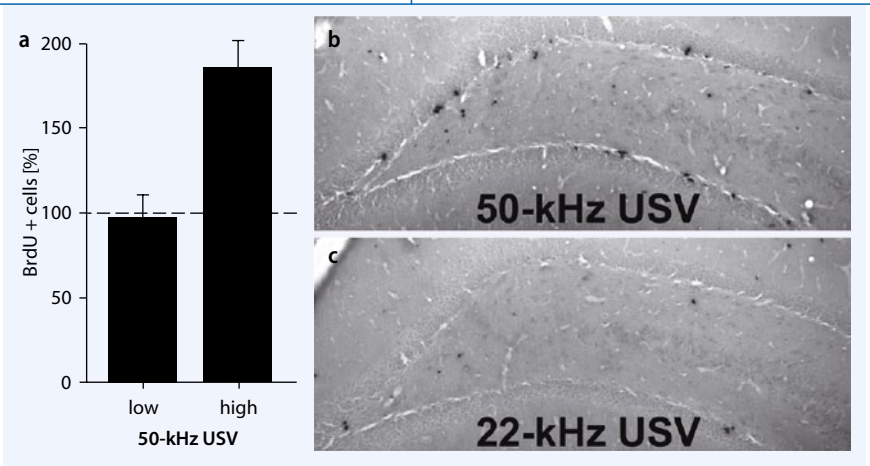

Fig. 5 ▲ Affective state, 50-kHz ultrasonic vocalizations (USV). **a** Hippocampal cell proliferation in the dentate gyrus of 2-month-old male Wistar rats after mimicking rough-and-tumble play by a human experimenter ("tickling") dependent on the emission of 50-kHz USV: *low* (*n*=4), *high* (*n*=4). Hippocampal cell proliferation was determined on the basis of BrdU-labeled cells (*BrdU+cells*) and is shown as a percentage of "non-tickled" controls (*dashed line*). **b** Representative example of a brain slice showing the dentate gyrus of a rat that emitted 50-kHz USV during tickling. **c** Representative example of a brain slice showing the dentate gyrus of a rat that did not emit 50-kHz USV but 22-kHz USV during tickling. Based on data from [43]

ranging from echolocation to signal submission during intermale fighting. However, most of what is currently known is in line with the hypothesis that 22-kHz USV serve an alarm function. Blanchard et al. [2] observed that the production of 22-kHz USV in response to a predator, a cat, is dependent on the presence of conspecifics, i.e. on the presence of an audience. No 22-kHz USV were observed when a rat was exposed to a cat in the absence of conspecifics. In response to 22-kHz USV, conspecifics typically retreated immediately, hiding in a sheltered burrow system. Despite the fact that we were not able to replicate the so-called audience effect under highly standardized laboratory conditions [49], we also found that conspecifics display anxiety-related behaviour such as freezing in response to 22-kHz USV. In order to test whether indeed 22-kHz USV caused the change in behaviour in the recipient, we conducted playback experiments as we did to determine the communicative function of isolation-induced USV. In these studies, we found that 22-kHz USV can induce a strong and long-lasting behavioural inhibition. Even after terminating 22-kHz playback, behavioural inhibition was still evident for several minutes (◘ **Fig. 4a**, [47]). By using immunohistochemical visualization of the immediate early gene c-fos, a neuronal activity marker, in subsequent studies, we showed that the induction of behavioural inhibition by 22-kHz USV is paralleled by an increase in neuronal activity in brain regions implicated in the regulation of anxiety and fear such as basolateral amygdala (◘ **Fig. 4b**) and periaqueductal grey (◘ **Fig. 4c**, [27]). Despite inconsistent audience effect findings, the results obtained in playback studies strongly support the notion that 22-kHz USV serve as alarm calls. It can be further assumed that even when the sender is not actively emitting 22-kHz USV to warn conspecifics about danger, they are still recognized as alarm calls by the recipients. It is unknown, however, whether the recognition of 22-kHz USV as alarm calls is innate or learned. Recent findings indicate that rats are predisposed to associate aversive stimuli and 22-kHz USV. Enhanced encoding of such an association leads to a stable memory, resisting extinction better [10].

Potential applications: In the field of anxiety research, 22-kHz USV are typically used as a dependent variable to measure the rat's negative affective state in addition to freezing behaviour. Occasionally, they are also used in the field of addiction research, here again as a dependent variable to measure the rat's negative affective state that can be expected to occur during drug withdrawal. However, little account has been taken of the fact that 22-kHz USV elicit anxiety-related behaviour in the recipient. The use of 22-kHz USV as an independent variable would not only allow the induction of anxiety-related behaviour by an ethologically valid stimulus, but would also provide an opportunity for studying social learning processes in the acquisition of fear. For more details on fear-induced USV see [51].

Functions of interaction-induced USV

Affective state: It was long believed that also 50-kHz USV reflect a negative affective state. This was because of the fact that rats do not only emit 22-kHz USV but also 50-kHz USV during intermale fighting [28]. This idea was challenged, however, when Knuston et al. [15] showed that juvenile rats emit 50-kHz USV during rough-and-tumble play. On the basis of this observation Panksepp and Burgdorf [21] started to mimic rough-and-tumble play in rats using a human experimenter—a method they termed "tickling". As described in sensational studies that received a great deal of attention, they were able to induce 50-kHz USV when a rat was "tickled" by a human experimenter [21]. Panksepp and Burgdorf [22] also showed that those rats in particular that emitted high numbers of 50-kHz USV experienced the "tickling" procedure as appetitive, as indicated by short latencies to approach the hand of the human experimenter. Panksepp [20] therefore came up with the provocative idea that 50-kHz USV do not only reflect a positive affective state, but that they also have similarities to human laughter—evolutionary antecedents of human joy. They were able to find evidence in a number of studies that at least partially supports the idea. For instance, they found that aversive stimuli such as cat odour or bright light reduce the emission of 50-kHz USV [22], while the administration of drugs of abuse such as amphetamine and cocaine elicit 50-kHz USV—especially when directly administered to the nucleus accumbens, a brain region implicated in reward processing [36].

What has been neglected is the fact that the production of 50-kHz USV is characterized by a huge level of inter-individual differences. In "tickling" experiments, we

found that not all rats emit 50-kHz USV—some rats do not emit 50-kHz USV at all, while others even emit 22-kHz USV [31]. We asked whether inter-individual differences in the production of 50-kHz USV are correlated with the level of hippocampal cell proliferation as hippocampal cell proliferation has been repeatedly associated with affect regulation. Thus, it is known that aversive stimuli such as submission during intermale fighting reduce hippocampal cell proliferation and Santarelli et al. [29] demonstrated that hippocampal cell proliferation is necessary for the antidepressant effects of SSRI. We found that the emission of 50-kHz USV during "tickling" are highly positively correlated with hippocampal cell proliferation, while a highly negative correlation between the emission of 22-kHz USV and hippocampal cell proliferation was evident. Remarkably, we further found that rats that experienced "tickling" presumably as appetitive, as indicated by high numbers of 50-kHz USV, had clearly elevated hippocampal cell proliferation levels as compared to "non-tickled" controls. The fact that rats that emitted only very few 50-kHz USV during "tickling" had hippocampal cell proliferation levels comparable to "non-tickled" controls argues in favour of the induction of hippocampal cell proliferation by "tickling" in those rats that experienced "tickling" presumably as appetitive (**Fig. 5a**, [43]).

In light of these findings, the fact that rats emit 50-kHz USV during intermale fighting was reinterpreted—not as expression of a negative affective state elicited by fighting, but instead as expression of a positive affective state of the dominant rat [6]. Rat 50-kHz USV and mouse 70-kHz USV elicited by mating behaviour are now also viewed as expression of a hereby elicited positive affective state and hence incorporated into the affective hypothesis "reversed" from negative to positive.

Communicative function: However, rats also emit 50-kHz USV in situations that are probably not appetitive. As we demonstrated, 50-kHz USV occur in response to separation from conspecifics [42]. When removing one of two rats from a cage, the removed rat starts to vocalize when isolated in an empty cage. 50-kHz USV occur predominately during the very first few minutes—fading out rapidly. Interestingly, 50-kHz USV are also emitted by the rat remaining alone in the home cage after removal of its cage mate. This shows that separation from conspecifics rather than exposure to a novel environment causes the production of 50-kHz USV. We hypothesized, therefore, that 50-kHz USV reflect the need for social contact and that they serve as social contact calls. We conducted a playback experiment in order to test our hypothesis. We found that 50-kHz USV induce social exploratory behaviour (**Fig. 6a**) directed towards the sound source in recipients (**Fig. 7a,b**, [47]). The induction of social approach behaviour by 50-kHz USV is in line with their affiliative communicative function.

The strength of social approach behaviour is dependent on the level of social motivation in the recipient. This was shown in a study where we manipulated the level of social motivation pharmacologically. It is known that low doses of μ-opioid agonists such as morphine increase rough-and-tumble play and hence the emission of 50-kHz USV in juvenile rats, while μ-opioid antagonists such as naloxone have inhibitory effects [37]. By using this approach, we demonstrated that morphine enhances social approach behaviour in response to playback of 50-kHz USV, while naloxone reduces it [50], supporting the notion that the opioid system is implicated in the regulation of social approach behaviour. This raises the question of whether μ-opioid receptor knockout mice with social information processing deficits and a lack of isolation-induced USV in infancy [18] display social exploratory and approach behaviour in response to playback of USV as adults. In order to test this, we

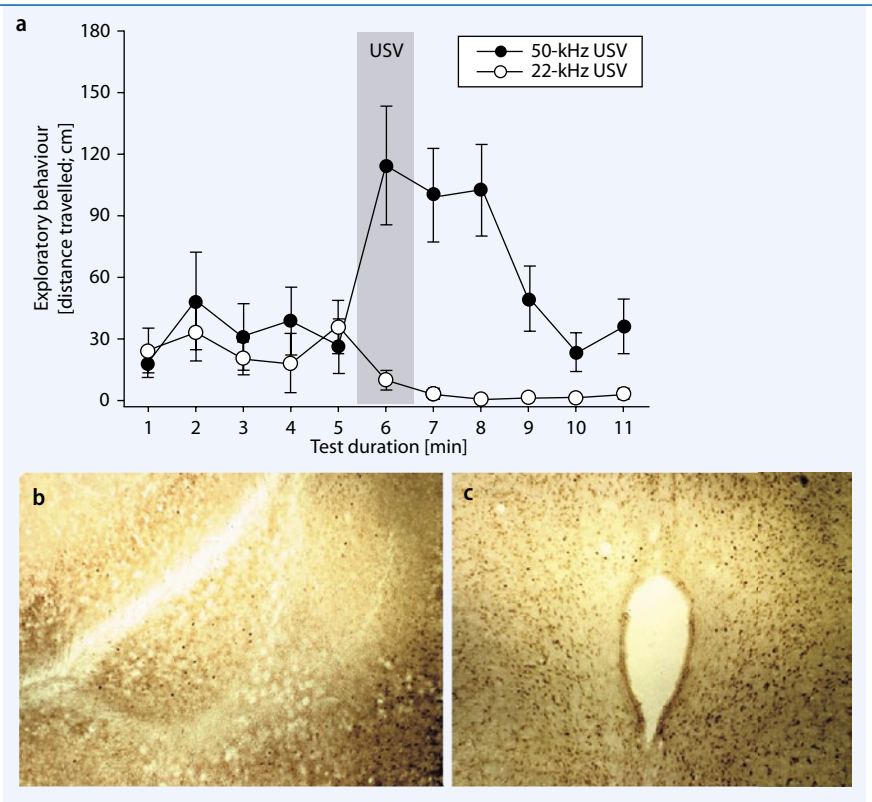

Fig. 6 ▲ Communicative function: behavioural activity and neuronal activation in response to playback of 50-kHz and 22-kHz ultrasonic vocalizations (*USV*). **a** Induction of social approach behaviour by playback of 50-kHz USV (*black circles*, *n*=6) and induction of behavioural inhibition by playback of 22-kHz USV (*white circles*, *n*=6), respectively, shown as distance travelled in centimeters (*cm*). USV were played back in minute (*min*) 6. Playback lasted 1 min (highlighted in *grey*). Behavioural activity before playback of USV in min 1–5 (*white*, *left*) and after playback of USV in min 7–11 (*white*, *right*) is given for the sake of comparison. Male Wistar rats aged 25 days were used. **b** Neuronal activation in the basolateral amygdala in response to playback of 22-kHz USV, visualized by immunohistochemical staining of the immediate early gene c-fos. **c** Neuronal activation in the periaqueductal grey in response to playback of 22-kHz USV, visualized by immunohistochemical staining of the immediate early gene c-fos. Based on data from [47] (with extensions) and [27]

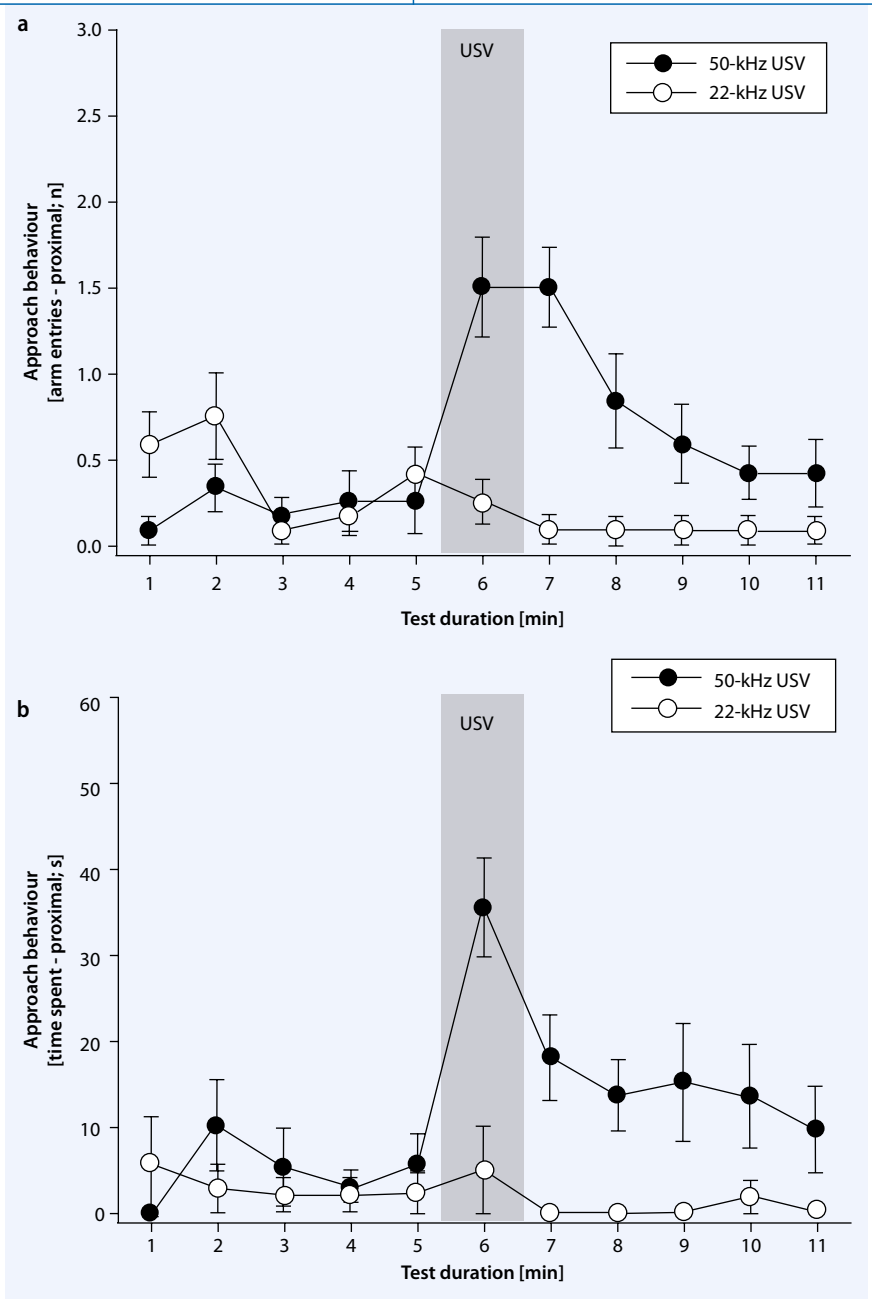

Fig. 7 ▲ Communicative function: stimulus-directed behavioural activity in response to playback of 50-kHz and 22-kHz ultrasonic vocalizations (*USV*). **a** Induction of social approach behaviour by playback of 50-kHz USV (*black circles*, *n*=6) and induction of behavioural inhibition by playback of 22-kHz USV (*white circles*, *n*=6), respectively, shown as the number (*n*) of arm entries into proximal arms, i.e. arms directed towards the stimulus source. **b** Induction of social approach behaviour by playback of 50-kHz USV (*black circles*, *n*=6) and induction of behavioural inhibition by playback of 22-kHz USV (*white circles*, *n*=6), respectively, shown as the time in proximal arms, i.e. arms directed towards the stimulus source, in seconds (*s*). USV were played back in minute (*min*) 6. Playback lasted 1 min (highlighted in *grey*). Stimulus-directed behavioural activity before playback of USV in min 1–5 (*white*, *left*) and after playback of USV in min 7–11 (*white*, *right*) is given for the sake of comparison. Male Wistar rats aged 25 days were used. Based on data from [47] (with extensions)

transferred our rat social approach paradigm to mice. It turned out that wildtype controls show social exploratory behaviour in response to playback of 70-kHz USV, while such a response was not seen in μ-opioid receptor knockout mice [44]. Again, this finding highlights the role of the opioid system for social behaviour. Furthermore, it also shows that the paradigm of playback-induced social approach behaviour can be applied to mice and that it is sensitive enough to detect social deficits in transgenic lines of mice. Finally, these experimental findings indicate that the hypothesis that rat 50-kHz USV and mouse 70-kHz USV foster mating behaviour by attracting the female [25] needs to be generalized, since as a rule, i.e. independent of the sexual context, this USV type appears to serve an affiliative communicative function.

Potential applications: Rat 50-kHz USV and mouse 70-kHz USV can be used for the determination of symptom-related behaviour in animal models of neuropsychiatric disorders for which reliable readouts are currently often missing such as regulation of affect and communication. The finding that 50-kHz USV reflect a positive affective state makes them a valuable tool to study euphoria and dysphoria in the field of depression research. The finding that drugs of abuse elicit 50-kHz USV opens further applications in the field of addiction research. Thus, 50-kHz could be used to determine changes in the affective state across the distinct phases in a drug self-administration paradigm, anticipation ("joyful expectancy"), acquisition ("consumption"), and extinction ("withdrawal").

Rat 50-kHz USV and mouse 70-kHz USV are currently used predominantly as measures for deficits in social behaviour and communication. It was repeatedly demonstrated that several mouse models of autism display severe deficits in USV production, which is in line with their affiliative communicative function. The inbred mouse strain BTBR T+tf/J (BTBR) is characterized by a clearly reduced level of social interactions and an enhancement of repetitive and stereotyped patterns of behaviour throughout development, and is therefore currently viewed as one of the most promising model systems for autism [35]. In light of these autism-like behavioural traits, we expected the autism model BTBR to also display communication deficits. In order to elicit 70-kHz USV, we exposed male BTBR and C57BL/6J mice to female urine of both strains. While social C57BL/6J mice emitted between 400 and 500 70-kHz USV during the 5-min female exposure, BTBR emitted less than 100 70-kHz USV [46]. BTBR mice not on-

ly showed deficits in acoustic communication, but also their olfactory communication was markedly reduced. Specifically, BTBR mice deposited less scent marks in proximity to the female urine [46]. Depending on the biological significance of the social context, acoustic and olfactory communication are typically highly correlated [26].

Currently, the measurement of USV production is almost exclusively used as a measure for communication deficits in animal models of autism, while the behavioural responses seen in recipients exposed to USV are not studied. However, it appears very likely that, in the future, USV will not only be used as a dependent measure, but also as an independent variable to elicit social behaviour. Indeed, the fact that 50-kHz USV induce social approach behaviour in the recipient provides a number of potential applications to study social motivation and communication in animal models. Almost all behavioural test paradigms that are currently used require the presence of a conspecific as the social stimulus. This is associated with a number of methodological problems. When a new conspecific is used in each individual trial, variability seen in the behaviour of the recipients could be due to differences between conspecifics used as social stimuli. However, when the same conspecific is repeatedly used as the social stimulus, it appears likely that its behaviour displayed during the first social interaction differs from that displayed in the tenth social interaction session, which again induces a huge level of variability in the recipients under study. In the case of the induction of social approach behaviour elicited by 50-kHz USV, however, an infinite number of repetitions of an identical social stimulus can be used. This means that all animals can be exposed to exactly the same stimulus. A further advantage is the fact that the appetitive value of the social stimulus, i.e. the strength with which it induces social approach behaviour, can be varied according to experimental needs [47]. For more details on interaction-induced USV see [51].

Conclusion

As communicative signals reflecting the animal's affective state, the distinct USV types provide various potential applications in the field of animal models of neuropsychiatric disorders.

Further reading

Wöhr M, Oddi D, D'Amato FR (2010) Effects of altricial pup ultrasonic vocalization on maternal behavior. In: Brudzynski SM (ed) Handbook of mammalian vocalization. An integrative neuroscience approach. Elsevier
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Corresponding address

Dr. rer. nat. M. Wöhr
Experimental and Physiological Psychology,
Philipps University of Marburg
Gutenbergstr. 18, 35032 Marburg
Germany
markus.woehr@staff.uni-marburg.de

M. Wöhr. Dr. Markus Wöhr studied psychology at the Eberhard-Karls University Tübingen and the Philipps University of Marburg (2000–2006). He received his Ph.D. from the Faculty for Psychology, Philipps University of Marburg (Ph.D. thesis on "Ultrasonic vocalizations in mice and rats – communicative signals of the affective state?"; 2006–2008). During that time, research stays at various European research centers: Division of Medical Pharmacology (Prof. Dr. de Kloet), Leiden/Amsterdam Center for Drug Research & Leiden University Medical Center, The Netherlands; Division of Ethology and Welfare (Prof. Dr. Spruijt), Faculty of Veterinary Medicine, Utrecht University, The Netherlands; Division of Psychobiology and Psychopharmacology (Prof. Dr. D'Amato), CNR Institute of Neuroscience, Rome, Italy. Then, Post-doctoral Visiting Fellow at the Laboratory of Behavioral Neuroscience (Prof. Dr. Crawley), National Institute of Mental Health, National Institutes of Health, Bethesda, USA (2008–2009). Since 2009: Research Associate at the Department for Experimental and Physiological Psychology, Faculty for Psychology, Philipps University of Marburg.

R.K.W. Schwarting
Experimental and Physiological Psychology,
Philipps University of Marburg
Gutenbergstr. 18, 35032 Marburg
Germany

R.K.W. Schwarting. Prof. Dr. Rainer K.W. Schwarting studied psychology in Hamburg und Düsseldorf (1976–1982). 1983: Research stay at the Psychological Research Department, VA Medical Center, Syracuse New York, USA. He received his Ph.D. in 1987 from the University of Düsseldorf. 1993: Habilitation with Venia legendi for psychology in Düsseldorf. 1994–1998: Heisenberg Fellowship at the Institute for Physiological Psychology and Research Group Leader in the Biomedical Research Center of the University of Düsseldorf. 1997–1999: Group Leader of the post-graduate programme "Pathological processes in the nervous system: from genes to behaviour" of the Faculty for Medicine and Natural Sciences, University of Düsseldorf. 1998–1999: Substitute Professorship for Physiological Psychology at the University of Düsseldorf. 1999–2008: W2 Professorship and since 2008 W3 Professorship for Experimental and Physiological Psychology at the University of Marburg. 2001–2003: Dean of the Faculty for Psychology at the University of Marburg. 2004–2010: Group Leader of the interdisciplinary post-graduate programme "Neuronal representation and action control (NeuroAct)" in Marburg and Gießen.

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