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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

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A B S T R A C T

Rats emit distinct types of ultrasonic vocalizations (USVs), which serve as situation-dependent affective signals. In appetitive situations, such as rough-and-tumble-play, high-frequency 50-kHz USVs occur, whereas low-frequency 22-kHz USVs can be observed in aversive situations, such as social defeat. USVs serve distinct communicative functions and induce call-specific behavioral responses in the receiver. While aversive 22-kHz USVs serve as alarm calls and induce behavioral inhibition, appetitive 50-kHz USVs have a pro-social communicative function and elicit social approach behavior, supporting the notion that they serve as social contact calls to (re)establish or maintain contact among conspecifics. The aim of the present study was to use the rat's ability to communicate in the ultrasonic range via high-frequency 50-kHz USVs in order to develop a test for social acoustic memory in rats with relevance for human verbal memory. Verbal learning and memory is among the seven cognitive domains identified as commonly deficient in human schizophrenia patients, but particularly difficult to model. We therefore tested whether the induction of social approach behavior by playback of appetitive 50-kHz USVs is dependent on (1) acoustic stimulus configuration and (2) social long-term memory, and whether (3) social long-term memory effects can be blocked by the administration of scopolamine, a muscarinic acetylcholine antagonist producing amnesia. Results show that social approach behavior in response to playback of natural 50-kHz USVs depends on acoustic stimulus configuration and occurs only when sound energy is concentrated to a critical frequency band in the ultrasonic range. Social approach behavior was detected during the first exposure to playback of 50-kHz USVs, whereas no such response was observed during the second exposure 1 week later, indicating a stable memory trace. In contrast, when memory formation was blocked by i.p. administration of scopolamine (0.5 mg/kg or 1.5 mg/kg) immediately after the first exposure, rats displayed social approach behavior during the second exposure as well. Induction of social approach behavior in response to repeated playback of natural 50-kHz USVs may therefore provide a new and rather unique approach for testing social acoustic memory in rats with relevance to human verbal memory.

1. Introduction

Rats emit distinct types of ultrasonic vocalizations (USVs), which serve as situation-dependent affective signals (for reviews see: Costantini & D'Amato, 2006; Knutson, Burgdorf, & Panksepp, 2002; Portfors, 2007; Wöhr & Schwarting, 2010). In appetitive situations, juvenile and adult rats produce high-frequency 50-kHz USVs. They are elicited most robustly during social interactions and occur in juvenile rats particularly during rough-and-tumble-play or when being tickled playfully (Brunelli et al., 2006; Burgdorf et al., 2008; Knutson, Burgdorf, & Panksepp, 1998; Panksepp & Burgdorf, 2000, 2003; Schwarting, Jegan, & Wöhr, 2007; Wöhr et al., 2009), while in adult rats highest rates of 50-kHz USVs are observed during mating (Burgdorf et al., 2008; McIntosh, Barfield, & Geyer, 1978; Sales, 1972a). In aversive situations, on the other hand, juvenile and adult rats emit low-frequency 22-kHz USVs. Typically, they are emitted during predator exposure (Blanchard, Agullana, McGee, Weiss, & Blanchard, 1992; Blanchard, Blanchard, Agullana, & Weiss, 1991; Blanchard, Blanchard, Rodgers, & Weiss, 1990), social defeat (Burgdorf et al., 2008; Sales, 1972b), or fear learning (Borta, Wöhr, & Schwarting, 2006; Choi & Brown, 2003; Jelen, Soltysik, & Zagrodzka, 2003; Kim, Kim, Covey, & Kim, 2010; Schwarting et al., 2007; Wöhr, Borta, & Schwarting, 2005; Wöhr & Schwarting, 2008a,b; Yee, Schwarting, Fuchs, & Wöhr, in press). Panksepp and Burgdorf (2003) suggested that appetitive 50-kHz USVs might provide an archaic form of human laughter ("rat

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laughter"), whereas aversive 22-kHz USVs might reflect an anxious or depressed state.

Low-frequency 22-kHz USVs and high-frequency 50-kHz USVs serve distinct communicative functions and induce call-specific behavioral responses in the receiver. Thus, 22-kHz USVs emitted in aversive situations probably serve as alarm calls to warn conspecifics about external danger (Blanchard et al., 1991). In line with an alarm function, it was shown that rats are predisposed to associate the occurrence of 22-kHz USVs with aversive events (Endres, Widmann, & Fendt, 2007), that these signals can elicit behavioral inhibition in the recipient (Blanchard et al., 1991; Brudzynski & Chiu, 1995; Burman, Ilyat, Jones, & Mendl, 2007; Sales, 1991; Wöhr & Schwarting, 2007), and that they are the main factor for social transmission of fear (Kim et al., 2010; Wöhr, Houx, Schwarting, & Spruijt, 2008b). In contrast, 50-kHz USVs appear to serve a pro-social communicative function. By performing playback studies, we have recently shown that appetitive 50-kHz USVs, but not aversive 22-kHz USVs, can induce social approach behavior, supporting the notion that they serve as social contact calls (Wöhr & Schwarting, 2007, 2009). Apart from social approach, playback of 50-kHz USVs also induced ultrasonic calling in the recipient with substantial call rates in juveniles (Wöhr & Schwarting 2009) and lower rates in adults (Sadananda, Wöhr, & Schwarting, 2008; Wöhr & Schwarting, 2007, 2009). The opposed behavioral responses elicited by aversive 22-kHz USVs and appetitive 50-kHz USVs are paralleled by distinct patterns of brain activation. While aversive 22-kHz USVs induce activation in brain areas implicated in fear processing such as amygdala and central gray (Parsana, Li, & Brown, 2012; Sadananda et al., 2008), appetitive 50-kHz USVs are followed by activation in brain areas implicated in appetitive processing such as the nucleus accumbens (Sadananda et al., 2008) and an inhibition of the amygdala (Parsana et al., 2012). In line with a pro-social communicative function of 50-kHz USVs, it was found that deafening or devocalizing rats affects rough-and-tumble-play (Siviy & Panksepp, 1987). Also, rats spent more time with conspecifics, which vocalize a lot, than with ones, which display less calling behavior (Panksepp, Gordon, & Burgdorf, 2002).

Verbal learning and memory is among the seven cognitive domains identified as commonly deficient in schizophrenia (Marder, Fenton, & Youens, 2004). Designing behavioral assays for preclinical research with face validity to the seven cognitive domains affected in human schizophrenia patients is a daunting challenge for behavioral neuroscientists. While reliable tests are available to assess most of the cognitive symptoms, it is particularly difficult to measure verbal learning and memory in rodent models (Young, Powell, Risbrough, Marston, & Geyer, 2009).

The aim of the present study was therefore to use the rat's ability to communicate in the ultrasonic range via high-frequency 50-kHz USVs in order to develop a test for social acoustic memory in rats with relevance for human verbal memory. To date, there is no study available on the role of learning and memory in processing this important social stimulus from the auditory domain. Also, still very little is known about the acoustic features of 50-kHz USVs that are relevant for the induction of social approach behavior. We therefore tested whether the induction of social approach behavior by playback of appetitive 50-kHz USVs is dependent on (1) acoustic stimulus configuration and (2) social long-term memory, and whether (3) social long-term memory effects can be blocked by the administration of scopolamine, a muscarinic acetylcholine antagonist commonly used in memory research due to its amnesia producing effects (D'Amato and Moles, 2001; Di Cara et al., 2007; Klinkenberg & Blokland, 2010; Loiseau, Dekeyne, & Millan, 2008; Millan et al., 2004, 2007, 2008; Perio et al., 1989; Riedel, Kang, Choi, & Platt, 2009; Terranova et al., 1996; Van Kampen et al., 2004; Winslow & Camacho, 1995). To address these questions, two experiments were conducted. In Experiment I, juvenile rats were exposed to playback of natural 50-kHz USVs and time- and amplitude-matched white noise and their behavioral changes were recorded. This test was performed at 4 weeks of age and repeated 1 week later. In Experiment II, an independent cohort of rats underwent the same behavioral testing as in Experiment I, but half of them were treated with scopolamine immediately after the first playback exposure to impair memory formation. Results show that social approach behavior in response to repeated playback of natural 50-kHz USVs depends on social acoustic memory.

2. Materials and methods

2.1. Animals and housing

Juvenile male Wistar rats (HsdCpb:WU, Harlan, Venray, The Netherlands) served as subjects. $N = 68$ rats were used (Experiment I: $N = 12$; Experiment II: $N = 56$). Rats were housed in groups of six on Tapvei peeled aspen bedding (indulab ag, Gams, Switzerland) in polycarbonate Macrolon type IV cages (size: $380 \times 200 \times 590$ mm, plus high stainless steel covers) in an animal room with a 12:12 h light/dark cycle (lights on 8–20 h) where the environmental temperature was maintained between 20–23 °C (humidity: 32–50%). Lab chow (Altromin, Lage, Germany) and water (0.0004% HCl-solution) were available ad libitum. After arrival in the laboratory, all rats were allowed to adjust to housing and light conditions for 1 week and were handled for 3 days in a standardized way for 5 min prior to testing. Body weight was determined on all handling and testing days. All experimental procedures were performed according to legal requirements of Germany and approved by the ethical committee of the local government (Regierungspräsidium Gießen, Germany).

2.2. Experimental setting

Testing for behavioral changes in response to playback of acoustic stimuli was performed on an elevated radial arm maze (for details see: Wöhr & Schwarting, 2007). Acoustic stimuli were presented through an ultrasonic speaker (ScanSpeak, Avisoft Bioacoustics, Berlin, Germany) using an external sound card with a sampling rate of 192 kHz (Fire Wire Audio Capture FA-101, Edirol, London, UK) and a portable ultrasonic power amplifier with a frequency range of 1–125 kHz (Avisoft Bioacoustics; for details see: Wöhr & Schwarting, 2007). The loudspeaker was placed 20 cm away from the end of one arm at a height of 52 cm above the floor. An additional, but inactive ultrasonic speaker was arranged symmetrically opposite to it.

Rats were tested under red light (~10 lux) in a testing room with no other rats present. The experimenter was also not present in the testing room. Stimulus application and animal observation was performed in a separate experimental control room. All behavioral tests were conducted between 8–20 h. Prior to each test, behavioral equipment was cleaned using 0.1% acetic acid solution followed by drying.

2.3. Independent variables

In Experiment I, an experimental design with two independent variables was used, namely acoustic stimulus configuration and social long-term memory. In Experiment II, an experimental design with three independent variables was used, namely acoustic stimulus configuration, social long-term memory, and scopolamine treatment.

2.3.1. Acoustic stimulus configuration

To test for the effects of acoustic stimulus configuration on behavioral changes occurring in response to playback of acoustic