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Do juvenile rats use specific ultrasonic calls to coordinate their social play?

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Play fighting in juvenile rats is associated with a high occurrence of 50 kHz vocalizations. These calls are varied in form, ranging from long, flat calls to short, frequency-modulated ones. We hypothesize that at least some types of calls serve as play signals to facilitate play. In the present study, pairs of juvenile male rats that were unfamiliar with one another were paired in a neutral test enclosure to which they had been habituated. Video and audio records were made of the encounters. Pairs were of two types: both pairs could vocalize or only one partner could do so. There were some differences between the play of pairs containing a devocalized partner, but overall, the pattern of play, the frequency and types of calls were similar between the two types of pairs. We used a Monte Carlo shuffling technique to analyse the correlations between the playful actions performed and the types and frequencies of various 50 kHz calls that were produced. The analyses revealed that there were strong associations between types of calls and types of social contact: an approach followed by playful nape contact was associated with calls, but an approach followed by nonplayful contact (e.g. anogenital sniffing) was not. Similarly, different calls were associated with different actions, such as nape contact, evade and wrestling, with most of these calls being uttered by the initiator of the action, not the recipient. However, coordinating calls reciprocally with complementary calls uttered by participants as they engaged in complementary actions (e.g. attacking, being attacked) appeared to be a way in which calls could potentially be used as play signals to influence the ongoing cooperation needed to sustain play fights.

In rats, rough-and-tumble play, or play fighting, involves attack and defence of the nape of the neck, which is nuzzled by the snout if contacted (Pellis & Pellis, 1987; Siviy & Panksepp, 1987). Although such play can be distinguished from serious fighting, which involves biting attacks directed at the face and lower flanks and dorsum (Blanchard, Blanchard, Takahashi, & Kelley, 1977; Pellis & Pellis, 1987), in some situations, play fighting can be quite rough and escalate to serious fighting (Smith, Fantella, & Pellis, 1999; Takahashi, 1986; Takahashi & Lore, 1983). A widely held view is that many species use play signals to inform the recipient that the upcoming contact is playful (Bateson, 1956; Bekoff, 1975). Several play signals, such as the canine play bow (Bekoff, 1995), head shaking in spider monkeys (Eisenberg & Kuehn, 1966) and the open mouth play face of catarrhine monkeys (van Hoof, 1967) and some carnivores (Poole, 1978), have been shown to promote engagement in play (Palagi et al., 2016). Hops (Pellis & Pellis, 1983) and open-mouth facial gesturing (Panksepp & Burgdorf, 2003) have been identified as possible play signals in the play fighting of rats, but given that play fighting in this species can occur in darkness or in low light conditions (Himmler, Pellis, & Pellis, 2013), it is unlikely that visual signals are critical in signalling play. However, rats produce many ultrasonic vocalizations during play (Burgdorf et al., 2008) and it is possible that some of these may be used as play signals (Himmler, Kisko, Euston, Kolb, & Pellis, 2014). Indeed, vocalizations that facilitate play have been identified in Barbary macaques, *Macaca sylvanus* (Kipper & Todt, 2002) and keas, *Nestor notabilis*, a New Zealand parrot (Schwing et al., 2016).

Two broad categories of ultrasonic vocalizations (USVs) exist in juvenile and adult rats. Rats utter 22 kHz calls in threatening or aversive situations (Knutson, Burgdorf, & Panksepp, 2002), such as in the presence of predator odour (Blanchard, Blanchard, Agullana, & Weiss, 1991), delivery of foot shock (Tonoue, Ashida, Makino, & Hata, 1986), when undergoing fear conditioning (Antoniadis & McDonald, 1999; Kikusui, Nishizawa, Takeuchi, & Mori, 2003; Wöhr & Schwarting, 2008), in the presence of a dominant

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conspecific (Panksepp, Burgdorf, Beinfeld, Kroes, & Moskal, 2004; Sales, 1972; Thomas, Takahashi, & Barfield, 1983), following acoustic startle (Kaltwasser, 1990), when handled by an unfamiliar experimenter (Blanchard, Flannelly, & Blanchard, 1986; Brudzynski & Ociepa, 1992) and following social defeat (Panksepp et al., 2004; Thomas et al., 1983). Calls that occur at a frequency of 33 kHz and higher are typically regarded as 50 kHz calls (Wöhr, van Galeen, & Schwarting, 2015), and these are associated with positive affective states, induced in circumstances such as when anticipating the delivery of an addictive drug (amphetamine or methamphetamine) (Knutson, Burgdorf, & Panksepp, 1999) or the stimulation of brain reward pathways (Burgdorf, Knutson, & Panksepp, 2000), during sexual encounters (Bialy, Rydz, & Kaczmarek, 2000), social play with peers (Burgdorf et al., 2008) and when 'tickled' by human handlers (Panksepp & Burgdorf, 2000). The 50 kHz category can be further divided into flat-type calls and frequency-modulated (FM) calls (Burgdorf et al., 2008). Frequency-modulated calls, in turn, have been further divided into as many as 12 different subtypes (Wright, Gourdon, & Clarke, 2010). Given that many of these varieties of calls are produced during play (Himmler et al., 2014), there are many possible ways in which different calls could be used to facilitate play.

Introducing adult male rats that are unfamiliar with one another in a neutral arena leads to a rougher version of play fighting that may occasionally escalate to aggression (Smith et al., 1999). If one of the partners is devocalized, the risk of escalation from play to aggression is greatly increased (Kisko, Himmler, Himmler, Euston, & Pellis, 2015); this appears to be due to a failure of the partners to coordinate their ultrasonic vocalizations (Burke, Kisko, Pellis, & Euston, 2017). However, deafening (Siviy & Panksepp, 1987) or devocalizing (Kisko, Euston, & Pellis, 2015; Kisko, Himmler et al., 2015) juvenile rats does not prevent play, nor do they increase the risk of escalation to aggression. On the other hand, when both juvenile partners are devocalized, the frequency of play is diminished, as are the cooperative actions that lead to prolonged bouts of play (Kisko, Euston, et al., 2015). Furthermore, when exposed to an intact partner, not only does the intact rat produce calls at twice the baseline rate of pairs when both rats can vocalize, but the frequency of initiating playful attacks by the nonvocal partner is also doubled (Kisko, Himmler et al., 2015), suggesting that USVs, while not necessary for play to occur among juvenile peers, can facilitate playfulness (Kisko, Wöhr, Pellis, & Pellis, 2017).

USVs may act as general motivators of play and may also be important to negotiate particular actions with one's partner. Particular types of 50 kHz calls are associated with particular actions when moving about an enclosure in which a rat is trained to anticipate the arrival of a play partner (Burke, Kisko, Swiftwolfe, Pellis, & Euston, 2017). During actual play fighting, calls are most likely to be uttered immediately prior to contact (Himmler et al., 2014; Kisko, Euston, et al., 2015), and some types of calls appear to be associated with particular defensive actions during play fights (Himmler et al., 2014). Such calling may thus help to coordinate the actions of the partners.

A key distinguishing feature of play fighting is that to sustain such encounters as playful, animals have to follow rules that ensure some degree of reciprocity (Pellis & Pellis, 2017). In juvenile rats, following these rules leads to a 30% likelihood that a play fight will involve a role reversal, whereby the original attacker becomes the defender (Himmler, Himmler, Pellis, & Pellis, 2016). When both partners are devocalized, the rate of role reversals is halved (Kisko, Euston, et al., 2015). Therefore, while USVs may not be necessary for play to occur, they may facilitate its occurrence and help partners negotiate cooperative actions.

Because both different calls and different behavioural actions are produced with markedly different frequencies, it is difficult using standard statistical approaches to detect significant associations (Himmler et al., 2014). Therefore, we developed a scoring scheme that samples both actions and calls throughout the test session and then uses a Monte Carlo shuffling technique to analyse the associations. This method has revealed robust associations between particular calls and actions in both solitary and social situations (Burke, Kisko, Pellis, et al., 2017; Burke, Kisko, Swiftwolfe et al., 2017). To test our hypothesis that juvenile rats use specific USVs to coordinate their play, we applied our new method to analyse the correlations between calls and behaviour in pairs of juvenile rats in which both could vocalize and pairs in which one of the rats was devocalized. The use of a devocalized animal allows for the assignment of vocalizations to a particular individual when engaged in specific behaviours.

METHODS

Subjects and Experimental Procedures

We obtained video and audio files of juvenile male Long–Evans rats playing in a neutral arena from our library of data that had been collected in a previous study (Kisko, Himmler et al., 2015). The testing and data collecting procedures followed have been detailed elsewhere (Burke, Kisko, Swiftwolfe et al., 2017; Himmler et al., 2013, 2014; Kisko, Euston, et al., 2015; Kisko, Himmler et al., 2015). In summary, 24 juvenile rats, age 30–40 days, were housed in quads after weaning at 24 days. Between 28 and 30 days, two members from each of the three quads were devocalized by bilateral transections of the recurrent laryngeal nerves (see Snoeren & Agmo, 2013) and were then maintained in the same quads until testing. Prior to testing, each animal was habituated to the testing enclosure for 30 min per day for three consecutive days. Following this habituation, the animals were isolated for 24 h. Then, using animals from different quads, rats unfamiliar with one another were tested in pairs for 10 min in a 50 × 50 × 50 cm Plexiglas box, lined with approximately 1–2 cm of bedding (Carefresh, HealthyPet, Ferndale, WA, U.S.A.). Altogether, there were six pairs tested in which both partners could vocalize and six pairs in which one partner could not vocalize.

For testing, unfamiliar pair mates were placed in the test enclosure and videotaped in the dark using cameras with night-shot capability for 12 min (for further procedural details, see Himmler et al., 2013). One rat was introduced first and allowed to roam freely for 2 min while awaiting the arrival of his partner. Ultrasonic vocalizations were collected using a high-frequency microphone (Model 4939, Brüel Kjaer, Denmark), with sensitivity to frequencies ranging from 4 Hz to 100 kHz. The microphone was located in the ceiling of the chamber, approximately 65 cm above the play surface. The microphone was connected to a Soundconnect™ amplifier (Listen, Inc., Boston, MA, U.S.A.) and recordings were digitized via a multifunction processor (model RX6, Tucker-Davis Technologies, Alachua, CA, U.S.A.) using a self-developed MATLAB acquisition program. All files were then converted to .wav files and were analysed using the Raven Pro system (Bioacoustics Research Program, Cornell Lab of Ornithology, Ithaca, NY, U.S.A.). To synchronize video and audio recordings, a device emitting simultaneous light and sound cues was used prior to play (Himmler et al., 2014; Kisko, Euston, et al., 2015).

Ethics

All procedures were in accordance with the Animal Research Ethics Board of the University of Lethbridge and the recommendations and guidelines of the Canadian Council on Animal Care.