Available online at www.sciencedirect.com
SCIENCE DIRECT®

Neurobiology of Learning and Memory 84 (2005) 228–240

Neurobiology of Learning and Memory

www.elsevier.com/locate/ynlme

Overt behavior and ultrasonic vocalization in a fear conditioning paradigm: A dose–response study in the rat

Markus Wöhr, Andreas Borta, Rainer K.W. Schwarting *

Experimental and Physiological Psychology, Philipps-University of Marburg, Gutenbergstr. 18, 35032 Marburg, Germany

Received 3 June 2005; revised 6 July 2005; accepted 7 July 2005
Available online 22 August 2005

Abstract

The behavioral analysis of laboratory rats is usually confined to the level of overt behavior, like locomotion, behavioral inhibition, instrumental responses, and others. Apart from such visible outcome, however, behaviorally relevant information can also be obtained when analyzing the animals' ultrasonic vocalization, which is typically emitted in highly motivational situations, like 22-kHz calls in response to acute or conditioned threat. To further investigate such vocalizations and their relationship with overt behavior, we tested male Wistar rats in a paradigm of Pavlovian fear conditioning, where a tone stimulus (CS) was preceding an aversive foot-shock (US) in a distinct environment. Importantly, the shock dose was varied between groups (0–1.1 mA), and its acute and conditioned outcome were determined. The analysis of visible behavior confirms the usefulness of immobility as a measure of fear conditioning, especially when higher shock doses were used. Rearing and grooming, on the other hand, were more useful to detect conditioned effects with lower shock levels. Ultrasonic vocalization occurred less consistently than changes in overt behavior; however, dose–response relationships were also observed during the phase of conditioning, for example, in latency, call rate and lengths, intervals between calls, and sound amplitude. Furthermore, total calling time (and rate) were highly correlated with overt behavior, namely behavioral inhibition as measured through immobility. These correlations were observed during the phase of fear conditioning, and the subsequent tests. Importantly, conditioned effects in overt behavior were observed, both, to the context and to the CS presented in this context, whereas conditioned vocalization to the context was not observed (except for one rat). In support and extent of previous results, the present data show that a detailed analysis of ultrasonic vocalization can substantially broaden and refine the spectrum of analysis in behavioral work with rats, since it can provide information about situational-, state-, and subject-dependent factors which are partly distinct from what is visible to the experimenter.

Keywords: Immobility; Freezing; Fear; Anxiety; Rearing; Grooming; Ultrasonic vocalization; 22-kHz; Alarm calls; Foot-shock; Associative learning

1. Introduction

Laboratory rats, like many other rodents, can emit vocalizations in the ultrasonic range. Dependent on sound frequency, call lengths, and frequency modulation, different classes of ultrasonic vocalization (USV) can be identified, which are dependent on the subjects' developmental stage, and the appetitiveness or aversiveness of the situation (Knutson, Burgdorf, & Panksepp, 2002). Juvenile and adult rats display calls around 22 and 50 kHz. The latter type is typically observed during play (Knutson, Burgdorf, & Panksepp, 1998), tickling (Panksepp & Burgdorf, 2000), and during sexual interactions (White, Cagiano, Moises, & Barfield, 1990), whereas 22-kHz vocalizations (sometimes termed "low frequency calls") are emitted during confrontation with predators (Blanchard, Blanchard, Agulla, & Weiss, 1991), submissive behavior during inter-male fighting

* Corresponding author. Fax: +6421 282 3610.
E-mail address: schwarti@staff.uni-marburg.de (R.K.W. Schwarting).

1074-7427/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2005.07.004

(Kaltwasser, 1990), or exposure to aversive stimuli, like startling noises (Kaltwasser, 1991), handling and touch (Brudzynski & Ociepa, 1992), air puffs (Knapp & Pohorecky, 1995), or electric shock (De Vry, Benz, Schreiber, & Traber, 1993; Jelen, Soltysik, & Zagrodzka, 2003; van der Poel & Miczek, 1991). Such vocalizations are not only displayed during the actual aversive event (unconditioned stimulus, US), but may also occur in response to stimuli associated with such experiences like the context, or specific conditioned stimuli (CS; Antoniadis & McDonald, 1999; Cuomo, de Salvia, Maselli, Renna, & Racagni, 1988; De Vry et al., 1993; Frysztak & Neafsey, 1991; Jelen et al., 2003; Molewijk, van der Poel, Mos, van der Heyden, & Olivier, 1995; van der Poel & Miczek, 1991; van der Poel, Noach, & Miczeck, 1989). These low frequency calls are considered to be part of the animal's defensive repertoire (Brudzynski, 2001), that is, they are closely associated with the freezing response to actual or potential threat, and might serve as "alarm calls" for conspecifics. Recently, the question was raised which acoustic parameter might convey information reflecting the degree of threat experienced (Brudzynski, 2005). Apart from the number of calls emitted, however, little information is available upon such potential parameters.

Low frequency calls of adult rats usually consist of pure whistles within a frequency range of 18–32 kHz lasting for 300–2000 ms (Blanchard et al., 1991; Brudzynski, Bihari, Ociepa, & Fu, 1993; Brudzynski & Ociepa, 1992; Cuomo et al., 1988; Molewijk et al., 1995; Sales & Pye, 1974; Tonoue, Ashida, Makino, & Hata, 1986; van der Poel et al., 1989; van der Poel & Miczek, 1991). These calls have sound pressure levels as high as 80–85 dB (Barfield & Geyer, 1972, 1975; Brudzynski & Ociepa, 1992), and a narrow bandwidth of 1–6 kHz (Brudzynski et al., 1993; Sales & Pye, 1974). They are emitted either as single pulses or in short bouts. Bouts are separated by inter-bout intervals (>320 ms), whereas calls within bouts are separated by shorter intervals. Most bouts consist of up to five calls (van der Poel et al., 1989). Except for slow downward drifts at the beginning and fast upwards sweeps which terminate vocalization, remarkably little frequency modulation was measured in between (van der Poel & Miczek, 1991). In contrast, large variability between alarm calls was found in call duration (Brudzynski et al., 1993), and it was suggested that this parameter is involved in the expression of signal strength (Brudzynski, 2005).

Here, we investigated the relationship between USV and defensive behavior (usually termed freezing or immobility) in a test of Pavlovian fear conditioning, where a tone stimulus (CS) is preceding an aversive foot-shock (US) in a distinct environment (for review, see Fanselow, 1984). Such tests of fear conditioning are very common in current research, since they are used to study the role of associative and non-associative brain mechanism of learning and memory (Kamprath & Wotjak, 2004), especially at the level of amgydala and hippocampus (for reviews, see Maren & Quirk, 2004; Rudy, Huff, & Matus-Amat, 2004). Their prominent role asks for detailed knowledge of the factors determining the outcome of such procedures. Here, freezing has served most often as the dependent variable, and has provided a rich amount of evidence, including the role of conditioning paradigms (trace, delay, etc.), the effectiveness of CS modalities and context variables, and the relation to US parameters (like shock dose; e.g., Baldi, Lorenzini, & Bucherelli, 2004). In case of USV, on the other hand, there is less behavioral evidence available, especially with respect to dose–response relationships (see Kikusui, Nishizawa, Takeuchi, & Mori, 2003; Molewijk et al., 1995; Nobre & Brandao, 2004; Tonoue et al., 1986; van der Poel & Miczek, 1991). Therefore, we designed an experiment using a short-delayed conditioning procedure where we asked how the aversiveness of the US (i.e., the shock dose) might affect (a) the acute response to it (in terms of immobility and USV), and (b) conditioned responses to the context of conditioning, or the CS presented in this context.

2. Materials and methods

2.1. Animals

We tested 33 male Wistar rats (Harlan Winkelmann, Borchen, Germany) with a mean body weight ranging between 276 and 339 g. They were housed in groups of five in acrylic cages (cage size: 56 × 34 × 35 cm) in an animal room with a 12:12 h light/dark cycle (lights on 07:00–19:00 h) with food and water provided ad libitum. Each animal was handled on three consecutive days (5 min each day) prior to the experiment.

2.2. Fear conditioning

2.2.1. Apparatus

The test of fear conditioning was performed in a shock chamber (33.5 × 35 × 38 cm) made of gray and transparent plastic walls. The roof and one wall were made of transparent plastic to allow video observation during the test. The tone CS was presented using a loudspeaker mounted in one wall 30 cm above the floor. The floor of the shock chamber was made of stainless steel rods (diameter: 5 mm) spaced 1 cm apart. The chamber was placed in a sound attenuating isolation cubicle (51 × 71 × 51 cm; Coulbourn Instruments, USA) equipped with two white-light LED spots (about 40 lux, Conrad Electronic) and a b/w CCD camera (Conrad Electronic) which was connected to a DVD recorder. Ultrasonic vocalization was recorded using a microphone (Electret Ultrasound Microphone, Avisoft