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Research report

Inhibition of the glucocorticoid synthesis reverses stress-induced decrease in rat's 50-kHz ultrasonic vocalizations

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HIGHLIGHTS

- Manual somatosensory stimulation ("tickling") results in a positive affect in rats.
- The positive affect triggers emission of 50-kHz ultrasonic vocalizations (USVs).
- Prolonged restraint stress diminished "tickling"-induced 50-kHz USVs.
- Metyrapone inhibits the synthesis of glucocorticoids.
- Metyrapone prevented stress-induced decrease in expression of positive affect.

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ABSTRACT

The playful, experimenter-administered manual somatosensory stimulation of rats results in a positive affect that triggers emission of ~50-kHz ultrasonic vocalizations (USVs), which have been proposed to index positive emotions akin to human joy and laughter. Our earlier findings showed that restraint stress decreased rat's tendency to emit 50-kHz USVs. Here we investigated whether the effects of stress on "tickling"-induced vocalizations could be alleviated by the glucocorticoid synthesis inhibitor, metyrapone. After the daily tickling sessions carried out until the USV response to tickling has stabilized, the rats were subjected to either handling, handling and metyrapone treatment, restraint stress lasting one week or the restraint stress and metyrapone treatment. Our results confirmed that animals exposed to restraint stress diminish the number of "tickling"-induced vocalizations as compared to the "tickled" but handled conspecifics. Metyrapone treatment prevented this effect in stressed animals having no effects in handled rats. The off-line analysis revealed that the majority (82–88%) of "tickling"-induced USVs were of the 50-kHz frequency modulated type and that the flat USVs appeared much less frequently (8.5–12%) while the 22-kHz alarm calls appeared sporadically (0.3–8%). Moreover, the acoustic parameters of the 50-kHz frequency modulated and flat USVs resembled the calls described earlier in adult rats. The results of the present study offer a way of identifying anti-stress and perhaps anti-depressant action of novel compounds based on the measurement of a positive affect of animals.

1. Introduction

Traditional methods of identifying the therapeutic agents for the treatment of psychiatric conditions like the depressive disorder, appear not to provide the novel, more effective compounds [20]. Among the reasons considered is the lack of innovative methods being able to identify new principles, and the continuous use of the screening tests developed more than three decades ago (e.g. [28]) that apparently "catch" only certain class of compounds of established, yet of limited clinical efficacy. Thus, desired are novel drug identification methods oriented at behavioral phenomena that reflect the emotional status of the animal. At present, the most commonly used index of the animal's affective state relies on the measurements of hedonia (i.e., the ability to perceive pleasure), characterized by the consumption of sucrose solution [30,35] and the assessment of the electrical self-stimulation threshold [21]. While anhedonia belongs to the core symptoms of major depression and schizophrenia, and is observed in stressed animals, it appears to reflect the *sensitivity to the reward* rather than the *positive affect* of animals. The direct measurement of animals' affective state could be proposed as an alternative index of the subjects' well being.

Converging lines of evidence indicate that fifty-kilohertz ultrasonic vocalizations (50-kHz USVs) may index positive affective states in rats accompanying social interactions [6,16,19], the anticipation of food presentation [4], and the action of euphorigenic

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drugs, such as amphetamine [5], as well as targeted electrical brain stimulation [4,9]. These findings demonstrate that the laboratory rat can communicate positive emotions upon exposure to the stimuli that are similarly perceived by man, because, as described by Burgdorf et al. [8], in humans, positive affective states are elicited primarily by rewarding social interactions, food, and exercise and are decreased by negative affective stimuli [11,34].

The discovery of 50-kHz "rat laughter" [25] has introduced a novel strategy to understand animal emotions [23]. When the rats are stimulated ("tickled") in a playful way by humans, they emit similar 50-kHz calls, as these accompanying positive affective states [25]. The calling behavior induced in rats by manual "tickling" was proposed to be homologous to, or at least functionally akin to, human laughter and joy [26], and it has been recently shown to make rats more "optimistic" [31].

Recent data from this laboratory [27], which confirmed the earlier observations of Mällo et al. [18] indicate that prolonged stress diminishes rats' propensity to emit 50-kHz USVs in response to manual "tickling" by an experimenter. The present experiments were designed to investigate whether stress-attenuating effects of the glucocorticoid synthesis inhibitor metyrapone, could also translate into increased positive affect of the animals.

2. Methods

2.1. Ethics statement

The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for Animal Experiments, Institute of Pharmacology.

2.2. Animals

Thirty three male Sprague–Dawley rats (Charles River, Germany) weighing ~225 g on arrival were initially used in this study. The animals were group-housed (5 rats/cage) in temperature (21 ± 1 °C) and humidity (40–50%) A/C-controlled colony room under a 12/12-h light/dark cycle (lights on at 06:00 h). Rats were allowed to acclimatize for at least one week before the start of experiments. Behavioral testing was carried out during the light phase of the light/dark cycle. Each experiment was performed on a separate group of rats.

2.3. Procedure and apparatus

The animals were investigated for the propensity to produce 50-kHz calls in response to the somatosensory stimulation. One investigator stimulated the rats and another investigator counted the 50-kHz USVs on-line. The somatosensory body stimulation was carried out as in our previous studies [27,31] and consisted of gentle holding of the rat on its back with the investigator's left hand and rapid right-hand finger movements across the ventral body surface of the animal, followed by its release after 15 s of stimulation. Two 15-sec stimulations, carried out with an interval of a few minutes between them, were averaged for statistical analyses. The USVs were recorded and visualized as spectrograms using Raven Pro 1.4 interactive sound analysis software (Cornell Lab of Ornithology, Bioacoustics Research Program, USA) on a PC computer connected via the A/D converter DAQ device (USB-6251 1.25 MS/s M Series) to the ultrasound microphone (Avisoft Bioacoustics, Germany). This way all high frequency ~50-kHz (but not the 22-kHz alarm) USVs were recorded on-line. However, we also investigated off-line the structure of the calls emitted upon the stimulation, to compare it with the structure described earlier in the literature. The trained investigator manually selected and labeled on the computer screen each of the calls made by 31 animals. Since this procedure was extremely laborious, it was limited only to the set of data consisting of four groups of vehicle-non-stressed, vehicle-stressed, metyrapone-non-stressed and metyrapone-stressed animals, over the 7 daily sessions indicated as "week #2" in Fig. 1. Here, we identified three types of calls: (i) the 22-kHz alarm calls, (ii) the 50-kHz flat calls of a near-constant frequency (i.e., bandwidth < 6 kHz), and (iii) the 50-kHz frequency modulated (bandwidth > 6 kHz) calls containing any frequency changes: increases, decreases, or trills [1,6,36].

The "tickling" procedure was carried out at 12:00, over one week, until the response had stabilized [18,27]. To be included in the analyses, the rat had to meet the arbitrary criterion of 15 or more calls per 15 s during each of the last 5 days preceding the experiment. Ninety five percent of the animals fulfilled this criterion. Following the adaptation to the "tickling" procedure, the animals were handled for a few minutes and "tickled" daily during week #1 and then, they received the vehicle or metyrapone treatment and restraint stress during week #2. This was done because metyrapone produces immediate inhibitory effects on glucocorticoid synthesis [33].

The stress paradigm consisted of 1 h of daily restraint stress for 7 consecutive days [22,27]. Rats were transferred from a housing facility to the stress room, which was separated from the test room. Animals were placed into perforated plastic tubes (6.5 cm inner diameter) of adjustable length. The restraint allowed for normal breathing and limited movements of the head and limbs. The rats were restrained between the hours of 10:00 and 11:00. The non-restrained controls were handled in the housing facility. One hour following the restraint stress or handling, the "tickling"-evoked vocalizations were scored.

2.4. Drugs

Metyrapone (Sigma–Aldrich, Poznan, Poland) was dissolved in polyethyleneglycol (PEG) and then diluted with physiological saline to a final PEG concentration of 40%. The drug and the vehicle were administered intraperitoneally (IP) in a volume of 1 ml/kg of body weight, once daily, 1 h prior to the handling or the restraint stress. Metyrapone, at the doses used in the present study has been shown to block both stress-induced increase in plasma corticosterone and cognitive impairment [12].

2.5. Statistics

The results are presented as the mean + or − SEM number of USVs per 15 s, averaged from the readout of two measurements. The data were analyzed with mixed design ANOVA (the day of test and the block of the days × treatment × stress). The Sidak's test was used as a post hoc test [10] using IBM/SPSS 21 for Windows. The alpha value was set at $P < 0.05$. The homogeneity of variance was measured with Levene's test.

3. Results

Fig. 1 shows that metyrapone treatment protected the animals from the inhibitory effects of stress on tickling-induced USVs and that metyrapone did not affect the number of calls in non-stressed animals. Four-way mixed design ANOVA showed significant effects of stress ($F(1,27) = 4.80$; $P < 0.05$), week of testing ($F(1,27) = 15.91$; $P < 0.001$), week of testing × stress interaction ($F(1,27) = 4.52$; $P < 0.05$), and most importantly, week of testing × treatment × stress interaction ($F(1,27) = 5.79$; $P < 0.05$). There was no interaction of week of testing × day × treatment × stress ($F(6,162) < 1$), suggesting that at the given week of testing, the treatment and stress did not affect the number of calls, depending