The Critical Point at which Post-weaning Individual Housing Conditions Affect the Emission of 22-kHz Calls in Male Rats

Hideaki INAGAKI 1) and Yuji MORI 1)

1) Laboratory of Veterinary Ethology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Correspondence should be sent to: Hideaki Inagaki, PhD, DVM
Laboratory of Veterinary Ethology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Tel: +81-3-5841-7577
Fax: +81-3-5841-8190
E-mail: ahin@mail.ecc.u-tokyo.ac.jp

Running head: POST-WEANING CONDITIONS AND 22-kHz CALLS
ABSTRACT. It is known that long-term post-weaning individual housing significantly reduces emissions of 22-kHz calls in male rats. In this study, we assessed post-weaning successive changes in 22-kHz calls emitted by male rats under two different types of post-weaning housing conditions (individually and socially). In addition, we evaluated the critical point at which a significant reduction in 22-kHz calls could be observed in male rats housed individually after weaning. Significantly fewer 22-kHz calls were emitted by individually housed rats compared to socially housed rats at 16 weeks of age, indicating that weeks after weaning may be a critical point for the reduction of 22-kHz calls caused by post-weaning individual housing.

KEY WORDS: early social isolation, indicator of stress, ultrasonic vocalization.
When adult rats are exposed to potentially harmful or life-threatening situations, they emit long (0.3–3.0 s) ultrasonic bouts of 20–30 kHz within a narrow bandwidth of 1–4 kHz, which are referred to as “22-kHz calls” [1–3, 5, 10]. These calls act as ethologically important social alarm signals [3, 5, 10]. A previous study investigating the effect of post-weaning social conditions on emissions of 22-kHz calls in male rats demonstrated that long-term post-weaning individual housing drastically reduced the total duration of tactile stimuli-induced 22-kHz calls in male rats compared to 22-kHz calls emitted by male rats housed socially after weaning [7]. These results demonstrated that social interactions after weaning are necessary for usual emissions of 22-kHz calls in male rats. In the previous study, although subjects were housed individually or socially for 6 months after weaning before being used in the experiment, the amount of time truly required to observe differences in emissions of 22-kHz calls caused by different social conditions after weaning remains unknown. Thus, we assessed post-weaning successive changes in 22-kHz calls emitted by male rats under different two types of post-weaning housing conditions (individually and socially), and examined the critical point at which a significant reduction in 22-kHz calls could be observed in male rats housed individually after weaning.

A total of 22 male Wistar rats (Clea Japan, Tokyo, Japan) were used in this experiment. The animals were provided with water and food ad libitum and maintained on a 12-hr light–dark cycle with lights off at 20:00. The vivarium was maintained at a constant temperature (24 ± 1°C) and humidity (40–45%). All subjects were weaned at 21 days of age. After weaning, each subject was randomly assigned to one of the
following two groups. In the individually housed group, 10 subjects were kept
individually in wire-topped, transparent cages (400 x 125 x 200 mm) with wood
shavings for bedding. These animals were deprived of physical contacts, while still
having olfactory, auditory, and visual contacts with other rats, and having contacts with
humans who handled them during weekly changes of bedding. The 12 rats in the other
group were housed socially (six pairs) in wire-topped, transparent cages (400 x 250 x
200 mm) with wood shavings for bedding.

Rats aged 4 weeks participated in the experiment. At the beginning of the
experiment, they were moved to the experimental room and kept in their home cages
for at least 60 min. Each animal was then transferred to a wire-topped transparent
experimental cage (400 x 250 x 200 mm) and habituated to the cage for 5 min. After 5
min, the wire lid was removed and the animal received air puff stimuli, which are
known to reliably induce 22-kHz calls in rats [6, 8]. A total of 30 air puffs at an
interstimulus interval of 2 sec were directed to the nape of the neck of the subject and
delivered from a nozzle (10 mm outer diameter and 2 mm caliber) at a distance of
approximately 50 mm from the subject. The pressure of the air puff was maintained at
0.3 MPa by a pressure valve according to earlier studies [6, 8]. Immediately after the
air puff stimuli, we placed the wire lid on the experimental cage and recorded 22-kHz
calls for 5 min using an ultrasound microphone (Condenser Microphone
CM16/CMPA; Avisoft Bioacoustics, Berlin, Germany) set at a distance of 50 mm from
the top of the wire lid. Data acquisition hardware (UltraSoundGate 116Hbm; Avisoft
Bioacoustics) and recording software (Avisoft-RECORER Version 4.0; Avisoft
Bioacoustics) on a personal computer were also used. Settings included a sampling rate of 100 kHz and a 16-bit format. The abovementioned sequence of air puff stimuli and the subsequent recording of 22-kHz calls were successively repeated three times for each subject. All experimental procedures were conducted between 10:00 and 15:00. All animals were weighed on the day of the experiment. The same experimental protocols were also used when subject rats were 8, 12, and 16 weeks of age. This study was approved by the Animal Care and Use Committee of the Faculty of Agriculture, The University of Tokyo.

For spectrogram generation (Fig.1A), recordings were transferred to Avisoft-SASLab Pro (Version 5.1; Avisoft Bioacoustics), and a fast Fourier transformation (FFT) was conducted. Spectrograms were generated with an FFT-length of 512 points and a time window overlap of 50% (100% Frame, FlatTop window). All calls obtained from each subject were used to sum the total duration of 22-kHz calls, which was measured automatically using Avisoft-SASLab Pro. All data were displayed as the mean ± standard error. Statistical comparisons were performed using a repeated measures analysis of variance (ANOVA) followed by post-hoc Student's t-test to analyze differences in the total duration of emitted 22-kHz calls and body weight gain between post-weaning housing conditions (i.e., individually vs. socially) at each age. The criterion for statistical significance was $P<0.05$ for all comparisons.

Post-weaning housing conditions significantly affected total duration of air puff-induced 22-kHz calls in subjects ($F_{1, 20} = 4.94, P<0.05$). In socially housed male
rats, 22-kHz calls gradually increased with age. In contrast, 22-kHz calls in
individually housed male rats slightly increased and returned to the baseline level.
Differences in the duration of 22-kHz calls between two types of post-weaning social
conditions gradually increased with age. The post-hoc test showed that at 16 weeks of
age, significantly fewer 22-kHz calls were observed in individually housed rats
compared to socially housed rats ($t = 3.52, P<0.01$; Fig. 1B), although duration of
individual 22 kHz calls was comparable across all rats and no significant difference
was found between the two post-weaning conditions (individually: $0.64 \pm 0.15$ s,
socially: $0.77 \pm 0.07$ s; $P = 0.36$). There was no significant difference in body weight
gain between the two post-weaning conditions ($F_{1,20} = 0.05, P = 0.83$; data not
shown).

In the present study, significant effects of post-weaning individual housing status
on emissions of 22-kHz calls in male rats could be observed 13 weeks after weaning
(16 weeks old), which is suggested to be a critical point for the reduction of 22-kHz
calls caused by post-weaning individual housing. Before this critical period, we
observed that the post-weaning individually housed male rats emitted fewer 22-kHz
calls with age, compared to male rats housed socially after weaning. Recent behavioral
and neurobiological studies have shown that a quantitative analysis of 22-kHz calls can
be a useful indicator to evaluate the degree of negative affective states in laboratory
rats [4, 9, 11]. Our results suggest that the effects of long-term individual housing on
22-kHz calls could affect the reliability of using 22-kHz calls as an indicator to assess
stressful conditions in laboratory rats.
ACKNOWLEDGMENT. This study was supported by a Grant-in-Aid for Scientific Research (C) (22500382) from the Japan Society for the Promotion of Science.

FIGURE LEGENDS

Fig. 1. 22-kHz calls recorded in this study. A, Typical spectrogram of air puff-induced 22-kHz calls (3 calls). B, Successive changes in the total duration of air puff-induced 22-kHz calls (summed-up vocalization in seconds) emitted by male rats housed socially (SO, n = 12) and individually (IN, n = 10) after weaning. Each point represents the mean ± standard error, **P<0.01 vs. age-matched male rats housed socially after weaning (Student's t-test).

REFERENCES


