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Isolation induced changes in Guinea Pig *Cavia porcellus* pup distress whistles

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ABSTRACT

Guinea Pig *Cavia porcellus* pups emit high-pitched distress whistles when separated from their mother. In order to assess the influence of the duration of a brief isolation period on whistle acoustic structure, we recorded the distress whistles of six 8-day old pups separated for 15 min from their group in a novel environment and compared the mean values of the first and last 30 whistle notes. Acoustic analysis revealed, throughout the session, a significant decrease in whistle duration, an increase in mean frequency and a tendency for a decrease in number of harmonics in the first part of the note. Results demonstrate that, throughout a brief isolation period, the vocal response of Guinea Pig pups to isolation undergoes structural changes possibly related to time-dependent changes in motivational state.

Key words: acoustic communication, isolation calls, Guinea Pig.

INTRODUCTION

In socially-bonding species, separation from familiar attachment figures stimulates physiological and behavioral stress responses. Guinea Pig *Cavia porcellus* pups exhibit high rates of whistling and increases in plasma concentrations of cortisol when separated from their mothers and from other members of the group (Hennessy 2003, Ritchey and Hennessy 1987, Tokumaru 1995). Isolated pup's distress whistles are high-pitched and are composed of repeated harmonic notes with marked frequency modulation (Berryman 1976, Pettijohn 1979, Tokumaru 2000).

Variation in the intensity and/or structure of vocalizations throughout a separation period has been interpreted as produced by motivational changes (Marler et al. 1992). Newman and Goedeking (1992) found that the mean and peak frequency of the vocalizations of Common Marmoset *Callithrix jacchus* pups increased and duration of the call decreased, from the beginning to the end of a 15 min isolation period. There were also significant stable inter-individual differences in initial frequency, final frequency and pitch variation of the marmoset calls.

Changes in the rate of the emission of whistles of Guinea Pig pups throughout an isolation period have already been described. The rate of whistle notes emitted decreases (Pettijohn 1979), while plasma cortisol levels and time spent in immobilization increase during a 30 min isolation period (Ritchey and Hennessy 1987, Hennessy 1988, Hennessy and Sharp 1990, Sachser et al. 1998, Hennessy et al. 2001).

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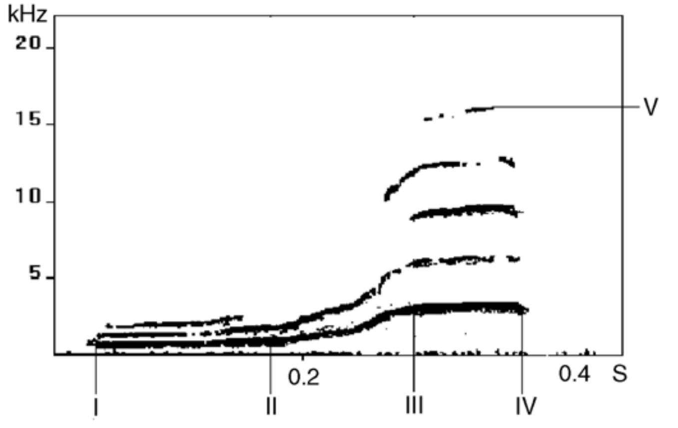

Fig. 1 – Sonogram of a whistle note, showing its harmonic structure (V = fifth harmonic). The note is divided into three segments: A, from I to II; B, from II to III and C, from III to IV.

We here address the question of whether time in isolation induces structural changes in distress whistles. Such changes may be relevant to an understanding of the functional aspects of distress calling in Guinea Pig, and to the time course of behavioral distress reactions.

MATERIALS AND METHODS

Six Guinea Pig pups, from three litters born in the colony of the Department of Experimental Psychology (São Paulo University), descendants of a heterogeneous stock, served in the experiment. Each litter was housed with the mother and the father in $90 \times 60 \times 30$ cm white polypropylene boxes. Water, rabbit chow and fresh vegetables were offered once a day.

On the eighth day of life, each pup was transported from the colony to the test room, put into a $49 \times 47 \times 27$ cm wooden box and left in isolation for 15 minutes. Recordings of vocalizations were obtained with a unidirectional microphone Sennheiser ME88 connected to a Sony DAT TCD-D8 (sampling frequency: 48 kHz, frequency response: 20 Hz to 22.000 Hz $\pm$ 1.0 dB; dynamic range $>$ 87 dB). The microphone was located 50 cm above the center of the box.

The first 30 and the last 30 whistle notes emitted during the isolation period were selected for analysis. Recordings were digitized with an 8-bit acquisition card using Avisoft-SASLab Pro 3.2 (Raimund Specht, Berlin, Germany). This software generates sonograms with frequencies between 0 and 24 kHz (sampling frequency, 48 kHz). Settings used for the generation of the sonograms were FFT size: 512; bandwidth: 111 Hz; frequency resolution: 86 Hz; weighting function: Hamming; time resolution: 0.73 ms.

Whistle notes were divided into 3 segments (Fig. 1): (A) an initial segment with little or no frequency modulation; (B) a segment with marked ascending frequency modulation, and (C) a segment with variable (generally descending) frequency modulation (Tokumaru 2000).

The following acoustic parameters were measured: (1) duration of the note; (2) mean interval between consecutive notes; (3) number of harmonics in segment A; (4) number of harmonics in segment C; (5) frequency modulation of the note (difference between starting and ending point of fundamental frequency): (6) dominant frequency, i.e. frequency with the highest intensity; (7) mean frequency. Mean values of the first and last 30 whistle notes of each pup were compared with a nonparametric Wilcoxon test (SPSS 11.0 software).

RESULTS

Mean values and standard deviations of acoustic parameters of the whistle notes of Guinea Pig pups are shown in Table I. Throughout the isolation period, there was a significant decrease in whistle duration ($Z = -2.232,\ p < 0.05$), an increase in mean frequency ($Z = -1.992,\ p < 0.05$) and a tendency for a decrease in number of harmonics in A ($Z = -1.782,\ p = 0.075$). The internote interval increased, throughout the isolation period, in 4 pups and the number of harmonics in C decreased in 5 out of 6 pups, at the end of the period (Table I).

DISCUSSION

Our data are the first demonstration of time-dependent changes in the acoustic structure of Guinea Pigs distress whistles. Whistle notes were shortened, mean frequency increased and there was a small but significant increase in number of harmonics in A, at the end of a 15 min isolation period. Such temporal changes are analogous to those observed by Newman and Goedeking (1992) in the calls of Common Marmoset pups: duration of isolation correlated negatively with call duration and positively with mean frequency and frequency peaks of calls. Frequency and structure of vocal emissions of Squirrel Monkey *Saimiri sciureus* pups vary as a function of different kinds of isolation contexts. Total separation from the mother is associated with a smaller number of vocalizations, higher frequency peaks and higher plasma cortisol levels than a partial (with visual contact) separation (Wiener et al. 1990). Wiener et al. (1990) interpret the calls Squirrel Monkey pups emit in isolation as stimuli that elicit recovery behaviors in the mother.

Vocalizations of isolated Guinea Pigs, as other separation calls, function as signals which elicit approach of the mother. Immediately after separation, as the mother is likely not to be very far, it may be advantageous for the pup to keep moving and to emit fast repetitive sequences of notes, maximizing the probability of the vocalization to be heard and responded to. As time passes in isolation, probability of recovery by the mother decreases and less conspicuous vocal signals and immobility may represent the best trade off between calling and defense against predators. There is indeed evidence that, in Guinea Pig pups, immobility and plasma cortisol levels increase and vocalization rate decreases with time in isolation (Hennessy et al. 1991, 1997). The long-note, high rate whistling recorded at the start of an isolation period may be interpreted as the pup's investment in short-term recovery; the short-note, low rate whistles recorded at the end of the isolation period, as a compromise between attention getting and defensive tendencies.

The whistle, characterized by short, high pitched pulses, with a broadband frequency spectrum uttered in fast repetitive sequences, is similar in structure to other distress or isolation calls (Maestrepieri and Call 1996) and can be interpreted, according to Morton's (1977) structural-motivational rules, as indicating that the sender is fearful and not hostile.

In adult Barbary Macaques *Macaca sylvanus*, duration and internote interval of disturbance calls decrease with time from the onset of a disturbing situation, but increase in infant-crying episodes (Fischer et al. 1995). Thus, infants seem to be more distressed than adults as time from disturbance onset increases. In Guinea Pigs, isolation vocalization, which is similarly restricted to pups, can reflect time-dependent changes in motivation related to attachment and fear.

The variability here demonstrated in a strongly stereotyped vocal call, such as the distress whistle of Guinea Pig pups, opens a set of interesting questions about motivational and social determinants of communication in this species.

RESUMO

Quando isolados de sua mãe, filhotes de Cobaia-doméstica *Cavia porcellus* emitem assobios agudos. Para avaliar os efeitos do tempo de isolamento sobre a estrutura acústica do assobio, registramos as vocalizações de 6 filhotes de 8 dias de idade, isolados durante 15 minutos, e com-

TABLE I

Mean values and standard deviations of acoustic parameters of six Guinea Pig pups whistle notes. Number of cases for internote interval values is indicated below each value; in all other cases, n = 30.

Parameters	Pup	11a	11b	8b	8d	8e	9c
Note duration (s)	30 first notes	0.20 (±0.03)	0.22 (±0.02)	0.21 (±0.04)	0.19 (±0.02)	0.19 (±0.02)	0.19 (±0.02)
	30 last notes	0.18 (±0.03)	0.16 (±0.04)	0.15 (±0.03)	0.18 (±0.02)	0.17 (±0.03)	0.17 (±0.04)
Internote interval (s)	30 first notes	0.13 (±0.01) (n=4)	0.14 (±0.00) (n=5)	0.124 (±0.02) (n=7)	0.10 (±0.02) (n=30)	0.10 (±0.01) (n=9)	0.10 (±0.02) (n=21)
	30 last notes	0.11 (±0.01) (n=4)	0.24 (±0.22) (n=9)	0.119 (±0.01) (n=6)	0.12 (±0.08) (n=20)	0.14 (±0.04) (n=5)	0.11 (±0.02) (n=23)
Number of harmonics in A	30 first notes	2.27 (±0.45)	2.33 (±0.55)	2.23 (±0.68)	3.00 (±1.11)	2.27 (±0.64)	2.77 (±1.17)
	30 last notes	1.93 (±0.25)	1.27 (±0.45)	1.43 (±0.68)	2.53 (±1.41)	1.13 (±0.35)	3.23 (±1.43)
Number of harmonics in C	30 first notes	4.10 (±0.40)	3.47 (±0.63)	3.73 (±0.69)	4.13 (±0.73)	3.97 (±0.56)	4.10 (±0.80)
	30 last notes	3.97 (±0.18)	3.40 (±0.50)	3.67 (±0.66)	3.80 (±0.81)	4.27 (±0.58)	3.70 (±0.65)
Frequency modulation (kHz)	30 first notes	2.73 (±0.78)	2.89 (±0.19)	2.49 (±0.20)	2.58 (±0.17)	2.61 (±0.16)	2.49 (±0.18)
	30 last notes	2.55 (±0.38)	2.40 (±0.94)	2.56 (±0.34)	2.63 (±0.29)	2.91 (±0.30)	2.59 (±0.44)
Dominant frequency (kHz)	30 first notes	6.31 (±3.02)	3.37 (±2.51)	4.55 (±2.95)	3.30 (±3.31)	4.96 (±3.25)	3.62 (±3.78)
	30 last notes	7.06 (±3.18)	3.73 (±0.12)	6.08 (±3.54)	2.20 (±1.36)	5.24 (±3.03)	2.64 (±1.25)
Mean frequency (kHz)	30 first notes	3.25 (±2.97)	2.40 (±2.07)	2.40 (±3.06)	1.62 (±2.51)	3.25 (±2.66)	2.72 (±3.63)
	30 last notes	5.99 (±3.41)	3.71 (±0.12)	3.70 (±2.88)	1.85 (±1.43)	4.09 (±2.38)	2.57 (±1.35)

paramos os valores médios das 30 primeiras e das 30 últimas notas emitidas. A análise acústica mostrou que, no final do período de separação, as notas de assobio se tornavam mais curtas, com freqüências médias maiores e uma tendência à diminuição do número de harmônicos na parte inicial. Os resultados demonstram que, ao longo de um breve período de isolamento, a resposta vocal de filhotes de cobaias sofre mudanças estruturais possivelmente relacionadas a mudanças no estado motivacional.

Palavras-chave: comunicação acústica, assobio de separação, Cobaia-doméstica.

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