

SHORT COMMUNICATIONS

The endocrine stress response and alarm vocalizations in rhesus macaques

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Darwin (1872) reasoned that because 'the pitch of the voice bears some relation to certain states of feeling . . . vocal utterances express emotion'. Stressful situations directly influence the acoustic morphology of speech utterances (Laver 1980; Scherer & Kappas 1988). Our study examined the relationship between alarm vocalizations and the endocrine stress response in rhesus macaques, *Macaca mulatta*. Although a number of investigations in both avian and anuran species have documented a connection between endocrine state and vocal behaviour (Wingfield et al. 1994), such a relationship in non-human primates is largely unknown (see Newman 1988a). We show that suppressed cortisol concentrations were associated with reduced intensity and rate of alarm call vocalizations.

Subjects were unrelated multiparous rhesus macaques residing in social groups at the Sabana Seca Field Station, Puerto Rico. Mother–infant dyads were removed from their social groups and brought to a holding area adjacent to the test cage (21 × 39 × 35 m). To minimize the number of animals subjected to stress, we used only 12 females. All infants were old enough (≥6 months) to be quasi-independent. We calculated maternal baseline cortisol concentrations from analysis of blood samples obtained from unanaesthetized individuals immediately after separation from their infant. Although elevated glucocorticoids can occur in non-stressful situations, cortisol is probably the most frequently analysed hormone in research involving the stress response in non-human primates (e.g. Sapolsky 1987, 1993; Clarke 1991).

Following separation from infants and blood collection, females received an intravenous injection of either vehicle (5% dextrose; Baxter

Healthcare, Deerfield, Illinois) or metyrapone (ca 30 mg/kg; Sigma, St Louis, Missouri) dissolved in vehicle. Metyrapone is an adrenal steroidogenic inhibitor that decreases 11 β -hydroxylation in the adrenal cortex and functions primarily to block glucocorticoid output. Plasma cortisol concentrations in savanna baboons, *Papio cynocephalus anubis*, decline by about 30% within 30 min of administration of metyrapone (Sapolsky & Krey 1988). The rapidity of metyrapone's pharmacological mode of action allowed us to keep the duration of separation to a minimum (≤ 45 min), but was long enough to register changes in systemic hormone levels. All experiments were conducted between 0915 and 1115 hours to control for diurnal rhythmicity of cortisol. Because our study involved a directional hypothesis, comparisons between conditions were analysed with one-tailed statistical tests.

After administration of either vehicle or metyrapone, caretakers placed each female in an individual cage (62 × 48 × 64 cm) directly across from the cage housing her own infant. Release into the test cage and exposure to three different low-intensity threats occurred 0.5 h later. Threats used to elicit alarm vocalizations were (1) simulated capture by personnel trotting around the perimeter of the test cage with a capture net, (2) personnel holding and staring directly at the test subject's own infant outside of the cage, but in full view of the subject, and (3) personnel holding and staring directly at an infant unrelated to the test subject in a fashion identical with (2). The same animal caretaker participated in all three trials for each subject. The test subject was exposed to each stimulus for approximately 30 s.

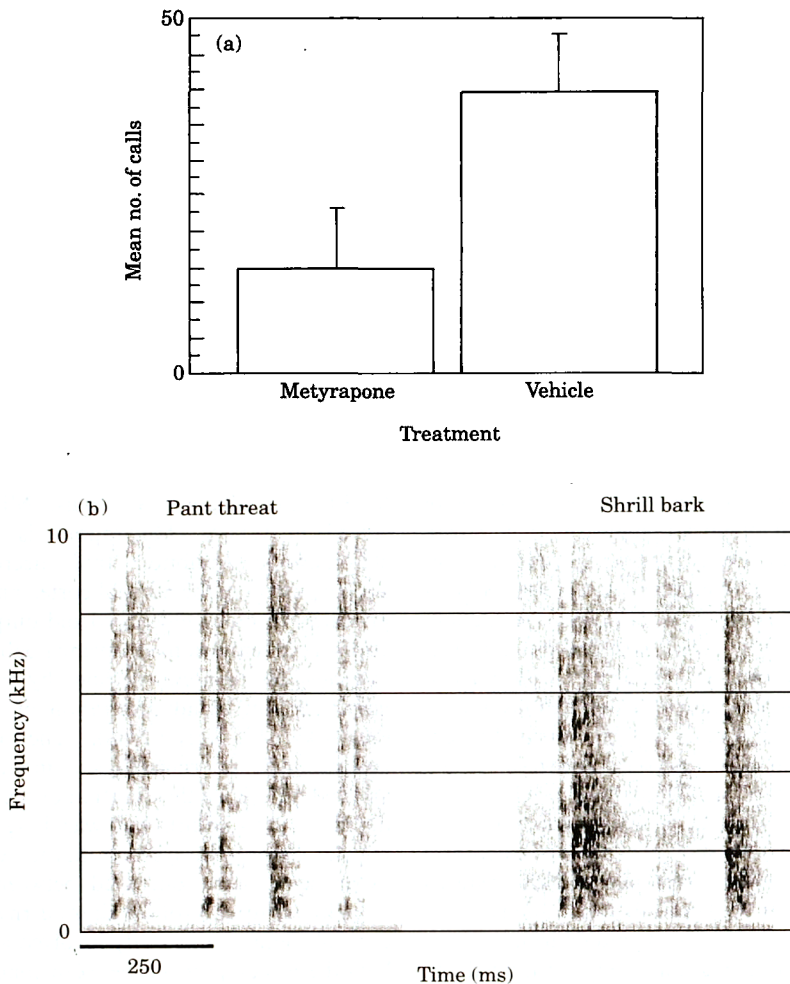


Figure 1. (a) The mean (+SEM) number of alarm vocalizations uttered by females who called during the test session when their own infant was threatened. (b) Two types of alarm vocalizations, pant-threat and shrill bark, uttered by female E290 when the stimulus condition was the threat to her own infant.

Immediately after completion of the three tests, we obtained a second blood sample from each subject. The interval between collection of baseline and post-test blood samples was approximately 35–45 min. We centrifuged blood samples immediately after collection and froze the plasma at -20°C . The Wisconsin Regional Primate Research Center measured cortisol levels in duplicate using a commercially available radioimmunoassay kit (INCSTAR; Stillwater, Minnesota) in a single assay with an intra-assay coefficient of variation of less than 1%.

We recorded vocalizations using a Marantz PMD430 cassette recorder and a Sony directional microphone. We determined call rate and structure using SIGNAL sound analysis software (Beeman 1992). Alarm vocalizations were identified based upon prior classification (Hauser & Marler 1993).

In the original experimental design we intended to use each female as her own control by testing all subjects with either vehicle or metyrapone after a 48-h lapse. Females appeared to habituate rapidly to the test paradigm, however; the second set of experiments yielded only two alarm

vocalizations across all conditions, so it was discarded from analyses.

In the six subjects receiving vehicle, cortisol concentrations underwent a non-significant elevation (paired $t=1.685$, $df=5$, $P>0.10$; $\bar{X} \pm SD=61.92 \pm 10.84$ $\mu\text{g/dl}$ versus 68.02 ± 7.53 $\mu\text{g/dl}$). In contrast, subjects that received metyrapone showed a statistically significant decline in cortisol concentrations (47.44 ± 10.45 $\mu\text{g/dl}$ versus 35.46 ± 7.29 $\mu\text{g/dl}$; paired $t=5.656$, $df=5$, $P=0.002$).

The six subjects uttered no alarm vocalizations under metyrapone treatment when exposed to threats by animal caretakers, whereas three of six of the control subjects gave alarm calls when tested with the same stimulus (Fisher's exact $P=0.09$). When the stimulus was a threat to their own infant, vehicle-treated subjects that gave alarm calls uttered vocalizations at a significantly higher rate than did metyrapone-treated subjects (Mann-Whitney $U=11$, $N_1=4$, $N_2=3$, $P=0.04$; Fig. 1a).

The type of alarm vocalization differed according to treatment. In rhesus macaques, two different alarm vocalizations have been characterized: a low-intensity pant-threat and a high-intensity shrill bark (Fig. 1b; Hauser & Marler 1993). None of the metyrapone-treated subjects uttered shrill barks, whereas all three of the vehicle-treated subjects that produced alarm calls uttered shrill barks when the stimulus was a threat to their own infant.

Our results show that cortisol concentrations and the nature of the external threat can influence the rate and type of alarm vocalization produced by female rhesus macaques. High-intensity alarm vocalizations were only uttered by females when their own infant was threatened, but such calls were not given by females with suppressed cortisol concentrations. None of the six subjects with reduced cortisol concentrations called when confronted with the lowest-intensity threat, although three of the six control females vocalized when exposed to the same stimulus. Finally, when a threat was aimed at their own infant, females with suppressed cortisol concentrations uttered alarm vocalizations at a significantly lower rate than those with slightly elevated cortisol concentrations.

Our findings do not necessarily indicate a direct causal connection between cortisol concentrations and vocal behaviour in rhesus macaques. Metyrapone treatment was related to a dampening of the endocrine stress response and an

alteration in alarm calling behaviour among female rhesus macaques. Attenuating glucocorticoid output was associated with a decrease in the rate of alarm vocalizations and a failure to produce high-intensity shrill barks when a female's own infant was threatened.

Our investigation has at least two implications for the study of animal communication. First, our technique is a useful addition to other types of tests exploring central mechanisms that influence vocal behaviour. The functional distinction between neurotransmitters and hormones is less precise than previously thought (Martin 1985; Norman & Litwack 1987). Glucocorticoids produced in the adrenal cortex occupy receptors in the brain and affect neurotransmitter synthesis and release, sensory perception and behavioural activity (Martin 1985). Opiate and adrenergic mechanisms have been identified as central modulators of distress calls (Kehoe 1988; Newman 1988b), and our findings have identified peripheral correlates of alarm vocalizations. Second, the failure to vocalize under given conditions has often been interpreted in functional terms as withholding information, or deception (Hauser 1992). Our results suggest a complementary explanation: reduced arousal may be the primary mechanism underlying the lack of signal transmission.

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