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Measuring Anxiety in Nonhuman Primates: Effect of Lorazepam on Macaque Scratching

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SCHINO, G., A. TROISI, G. PERRETTA AND V. MONACO. *Measuring anxiety in nonhuman primates: Effect of lorazepam on macaque scratching.* PHARMACOL BIOCHEM BEHAV **38**(4) 889–891, 1991.—Lorazepam (0.2 mg/kg IM) was given to group-living female macaques to assess the effect of anxiolytic treatment on scratching, a behavior pattern referred to as a displacement activity in the primate literature. Lorazepam selectively diminished scratching behavior. The drug effect was status-dependent: especially low-ranking animals showed a marked reduction in scratching. Lorazepam exerted a direct effect on scratching, that is the effect was not due to sedation or mediated by the influence of the drug on other behaviors. These results provide pharmacological validation to the ethological finding that scratching may be a manifestation of anxiety in monkeys. In addition, they suggest to use scratching as a behavioral measure in studies investigating nonhuman primate models of anxiety.

Anxiety Scratching Nonhuman primates Benzodiazepines Displacement activities Animal models Ethopharmacology

MOST animal models relevant to the topic of anxiety have been developed as systems for identifying drugs that may have anxiolytic properties in humans, rather than as attempts to detect emotional states in animals equivalent to the experience of anxiety in humans (7). Such models generally involve experimental paradigms employing highly aversive stimuli unlikely to occur in feral environments (e.g., electric shock). File (4) has argued that these situations of conditioned fear, where a predictable and objectively unpleasant effect is expected, are different from those involving uncertainty, an emotion which may more closely approximate the human experience of anxiety.

It is reasonable to assume that some of the most compelling analogs, if not homologs, to human anxiety can be found among man's closest living relatives, the higher nonhuman primates. Nonhuman primates living in a complex social environment frequently experience situations of impending aggression, danger, conflict, or uncertainty. Ethological studies [e.g., (2,11)] have documented that, under these circumstances, monkeys tend to display certain behaviors that are apparently out of context and consist of different body care activities such as scratching, self-grooming, body shake, and yawning. These behaviors, which are referred to as "displacement activities" in the ethological literature, seem to show a resemblance to clinical anxiety which is in some respects superior to that of conflict paradigms in rodents. First, displacement activities occur in response to common social situations not involving physical pain. Second, in nonhuman primates, these behaviors do not occur only as responses to acute frightening stimuli but also as responses to more subtle situations somehow perceived as threatening, but where no immediate threat is apparent to the animal. This finding is interesting in relation to the clinical notion that human anxiety often depends on the "meaning" attributed to a given stimulus or situation (13).

However, there is as yet no conclusive evidence that displacement activities are valid measures of anxiety in nonhuman primates. Pharmacological studies are an important component of the research strategy for validating displacement activities as a primate model of human anxiety. The present study aimed at assessing whether scratching by group-living monkeys is selectively diminished by lorazepam, a pharmacologic agent which is anxiolytic in humans. In addition to scratching, other social and nonsocial behaviors were recorded to exclude the possibility that changes in scratching were due to nonspecific or indirect effects of the drug.

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METHOD

Subjects and Housing

The subjects were 10 adult (4–9 years of age, 2.2–4.0 kg of weight) females belonging to a group of long-tailed macaques (*Macaca fascicularis*) housed in an indoor/outdoor cage at the C.R.E. Casaccia, ENEA. During data collection, the monkeys were confined in the outdoor part of the cage which consisted of two compartments, each measuring $8 \times 2 \times 2$ (H) m, connected by a door. Over the study period, the group numbered 37 monkeys, being composed of 3 adult males, 12 adult females (including the 10 subjects) and 22 immature individuals. Two adult females were excluded from the sample because of their reproductive condition (one female was in the later stages of pregnancy and the other was lactating her 30-day-old infant). Over the study period, none of the female subjects was a mother of an infant younger than 6 months of age. The monkeys were fed twice daily with commercial pellets and fresh fruit, with water freely available.

Drug Treatment and Behavioral Measures

Each animal was given two 120-min observation sessions. Thirty minutes before the session, the animal was injected IM with lorazepam (0.2 mg/kg) (test session) or with an equivalent volume of 0.9% saline (control session). One animal at a time was treated and sampled, and only one session a day was made. Consecutive sessions, both those involving the same subject and those involving different subjects, were divided by an interval of 2–3 days. Because of this short interval, the stage of the menstrual cycle was unlikely to cause changes in the female subjects' behavior over the period between the test session and the control session. To eliminate possible order effects, five subjects received lorazepam first and five subjects received saline first. Drug and vehicle treatments were administered in a counterbalanced order. Lorazepam was chosen because it is the only benzodiazepine that is predictably absorbed following intramuscular injection (6).

Observations were made between 12.30 and 14.30 hours. The rater was not blind to the treatment. In addition to scratching (a usually repeated movement of the hand or foot during which the fingertips are drawn across the individual's fur), a comprehensive list of social and nonsocial behaviors were recorded. However, in this paper, only the results concerning locomotion (walking, running, or climbing), passive grooming (the subject's fur is inspected and cleaned by another monkey through rhythmical patterns of fur manipulation) and passive aggression (the subject is threatened, chased, or physically attacked by another monkey) will be reported. The effect of drug treatment on these behaviors is relevant to an interpretation of the results concerning scratching because: 1) locomotion is a reliable behavioral measure of sedation [e.g., (14)]; 2) aggression and grooming have been shown to respectively increase (1) and decrease (12) scratching by the target animal, probably because of their opposite effects on the emotional state of the animal; in addition, both contact aggression (cuff, pinch, grab, pull, and bite) and grooming can alter the conditions of the target animal's pelage and/or skin, thus influencing the cutaneous stimuli that elicit scratching.

A combination of the "focal animal" and "complete record" sampling techniques (8) was used to record scratching, grooming, and aggression: the observer (G.S.) recorded the timing and, for grooming only, duration of every episode of these behaviors involving the focal subject. Locomotion was recorded using the instantaneous sampling technique: on the instant of each sample point (one sample point every minute, 120 sample points per session), the observer recorded whether or not the focal subject was moving.

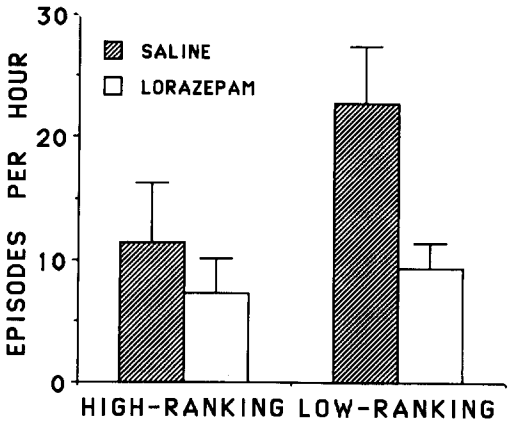

FIG. 1. Effect of acute administration of lorazepam (0.2 mg/kg IM) on the individual hourly rate (mean ± SEM) of scratching by high-ranking (N = 5) and low-ranking (N = 5), group-living macaque females.

Data Analysis

Social status has previously been shown to be an important determinant of drug effects in monkeys, with dominant and subordinate animals exhibiting quantitatively different responses to the same drug, and in some cases even opposite effects [e.g., (3,14)]. Therefore, on the basis of the outcome of dyadic (i.e., involving two subjects) agonistic interactions, the subjects were ranked in a linear dominance hierarchy and divided into two dominance groups (high-ranking, N = 5, and low-ranking, N = 5). The rank of each subject in the female dominance hierarchy was as follows: high-ranking group, females Nos. 2, 3, 4, 5, and 6; low-ranking group, females Nos. 7, 8, 10, 11, and 12. Behavioral data were subjected to 2 (saline vs. lorazepam) × 2 (high- vs. low-ranking) analyses of variance (ANOVAs) with repeated measures on the drug treatment factor.

RESULTS

Acute administration of lorazepam reduced the frequency of scratching by the subjects, with low-ranking females showing a more marked response to the drug, as indicated by a two-way ANOVA that found a significant effect for treatment, $F(1,19) = 18.23$, $p = 0.003$, and a significant interaction effect (treatment × rank), $F(1,19) = 5.15$, $p = 0.05$ (Fig. 1). We made a pairwise comparison between the rates of scratching by low- and high-ranking females after saline administration to test the hypothesis that low-ranking animals are generally more anxious. The difference between the groups reached trend levels of significance [one-tailed, $t(8) = 1.67$, $p = 0.06$].

Statistical analysis indicated that lorazepam did not exert any significant effect on other behaviors that could influence the rate of scratching by the subjects. No significant changes were observed in the measure of locomotor activity [treatment effect: $F(1,19) = 0.39$, ns; interaction effect: $F(1,19) = 0.91$, ns]. This indicates that sedation was not responsible for the observed changes in scratching behavior. In addition, because lorazepam did not influence the amounts of either grooming [treatment effect: $F(1,19) = 1.00$, ns; interaction effect: $F(1,19) = 0.25$, ns] or aggression [treatment effect: $F(1,19) = 0.63$, ns; interaction effect: $F(1,19) = 0.94$, ns] addressed to the female subjects, the decrease in scratching cannot be ascribed to changes in the subjects' emotional and/or physical conditions caused by the effects of the drug on these social behaviors.

DISCUSSION

Lorazepam treatment selectively diminished scratching behavior by group-living female monkeys. The finding that the drug effect was status-dependent is consistent with the hypothesis that the decrease in scratching was due to the anxiolytic properties of lorazepam: especially low-ranking animals, who are expected to experience anxiety-provoking situations more frequently than high-ranking animals, showed a marked reduction in scratching. The effect of lorazepam on scratching was not due to sedation or mediated by the influence of the drug on other behaviors that can alter the emotional state of the animal or the cutaneous stimuli eliciting scratching.

In rodents, physiological and pharmacological data confirm the ethological finding that displacement activities including scratching are related to increased arousal or emotionality [e.g., (4,5)]. In contrast, comparable data for nonhuman primates are scarce and, moreover, they derive from studies involving chair-restrained subjects. Redmond and Huang (10) found that, in the stump-tailed monkey, electrical and pharmacological activation of the locus coeruleus, the major brain noradrenergic nucleus, elicited scratching and other behaviors similar to those observed in monkeys exposed to natural threats in the wild. Ninan et al. (9) showed that administration of β-CCE, an anxiogenic substance, to rhesus monkeys produced a behavioral syndrome reminiscent of fear or anxiety which also included scratching. To our knowledge, the present study is the first pharmacological study involving group-living animals to demonstrate that scratching may be a behavioral manifestation of anxiety in monkeys.

Taken together, the behavioral, physiological, and pharmacological data support the validity of scratching as a measure of anxiety. However, among nonhuman primates, scratching is not only a displacement activity but also a body care pattern. This should be taken into account in studies using scratching as a measure of anxiety. Individuals who exhibit different proportions of the two forms of scratching are expected to show a differential response to antianxiety drugs, as was probably the case for the high- and low-ranking females of this study. Especially animals performing high levels of displacement scratching are optimal subjects for pharmacological studies. In addition, it is important to control for those factors that can influence the causation of normal scratching (e.g., excluding from the sample animals with cutaneous lesions).

Provided that these methodological aspects are taken into account, studies aimed at investigating anxiety in nonhuman primates could include scratching among the behavioral measures considering that it offers peculiar advantages such as spontaneous occurrence in social settings, elicitation by mild stressors, and easy recording.

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