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Behavioral effects of buspirone in the marmoset employing a predator confrontation test of fear and anxiety

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Abstract

In order to further validate the recently developed marmoset (*Callithrix penicillata*) predator confrontation model of fear and anxiety, we investigated the behavioral effects of buspirone with this method. The apparatus consisted of three parallel arms connected at each end to a perpendicular arm, forming a figure-eight continuous maze. A taxidermized wild oncilla cat (*Felis tigrina*) was positioned facing a corner of the parallel arms, alternating between the left or right side of the maze among animals tested. All subjects were first submitted to seven 30-min maze habituation trials (HTs) in the absence of the predator, and then to five randomly assigned treatment trials (TTs) in the presence of the predator: three buspirone sessions (0.1, 0.5 and 1.0 mg/kg), saline and sham injection controls. Twenty minutes after treatment administration, the animal was released into the maze and had free access to the apparatus for 30 min. All trials were taped for later behavioral analysis. Buspirone significantly decreased the frequency of scent marking, while increasing the time spent in proximity to the 'predator' stimulus, indicating an anxiolytic effect. Neither locomotor activity, exposure to a novel environment, stimulus location and habituation, nor gender influenced the effects of the drug treatments. These results further validate this method and demonstrate the potential usefulness of this ethologically based paradigm to test anxiety and fear-induced avoidance in nonhuman primates and its susceptibility to anxiolytic pharmacological manipulations.

Keywords: Marmoset; Anxiety; Fear; Maze; Taxidermized predator; Confrontation; Serotonin; Buspirone

1. Introduction

Ethologically based models of anxiety attempt to approximate natural conditions under which such emotional states are elicited and thus hope to provide comparable results to human anxiety (Blanchard et al., 1998; File, 1980). In fact, various naturalistic models have been developed to test anxiety in rodents, including the social interaction tests, predator confrontations (odor, sound or presence), elevated plus- and T-maze, open field and conspecific confrontations (for reviews, see Blanchard et al., 1998; Griebel, 1995). In nonhuman primates, models like the human threat (Barnes et al., 1991; Carey et al., 1992; Costall et al., 1992; Jones et al., 1988; Newman and Farley, 1995; Walsh et al., 1995), social isolation (Newman and Farley, 1995; Smith and French, 1997; Smith et al., 1998), conspecific confrontation (Cilia and Piper, 1991; French and Inglett, 1991), and social interaction (Palit et al., 1998) have also been employed. Since nonhuman primates exhibit similar physiological and behavioral responses to anxiety-inducing situations as humans (Newman and Farley, 1995; Vellucci, 1990), they can provide important data of relevance to humans (Carey et al., 1992; Newman and Farley, 1995).

Recently, we have developed a new ethologically based method to study fear and anxiety in *Cerrado* marmosets (*Callithrix penicillata*) (Barros et al., 2000). The strategy employed was to expose these animals to a taxidermized predator (the wild oncilla cat *Felis tigrina*), known to elicit fear and anxiety responses in callitrichids (Barros et al., 2000; Emmons, 1987; Passamani, 1995). This predator confrontation model was shown to be sensitive to diazepam, indicating this method as a potentially useful experimental paradigm for studying anxiety and fear-induced avoidance

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in marmosets. Administration of diazepam significantly reduced scratching, while increasing the frequency of exploratory behaviors and the time spent near the location of the 'predator' (Barros et al., 2000).

One of the major drawbacks in many existing models of anxiety is that their validation is based essentially on their sensitivity to benzodiazepines (BZDs) (File, 1987; Griebel, 1995; Rodgers, 1997; Rodgers et al., 1997). Preclinical and clinical studies employing non-BZD drugs, like buspirone, have sometimes failed to demonstrate conclusive effects of these novel compounds using various methods (for review, see Griebel, 1995). Serotonin (5-hydroxytryptamine, 5-HT) has been repeatedly demonstrated as an essential component of the central network mediating fear and anxiety-induced behaviors in animals (e.g. Barrett and Vanover, 1993; Graeff et al., 1997), and in human pathological states of anxiety (Graeff et al., 1996). In fact, buspirone, a 5-HT_{1A} ligand, has become the most commonly employed alternative drug to classical BZDs in clinical treatments of anxiety (Lader, 1995).

Therefore, going further in the validation of the marmoset predator confrontation model as a new method to study anxiety and fear-induced avoidance, the aim of the present study was to test the effects of buspirone on the behavior of marmosets using this paradigm.

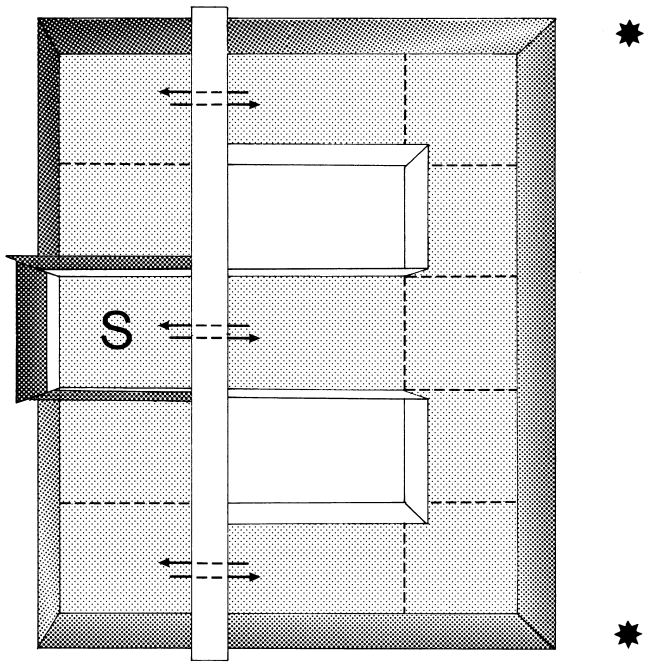

Fig. 1. Topview of the experimental apparatus (figure-eight continuous maze) employed in the marmoset predator confrontation model of fear/anxiety. (S) Start compartment; (✸) locations where the taxidermized wildcat could be positioned.

2. Materials and method

2.1. Subjects

Seven captive born and experimentally naive adult *Cerrado* marmosets (*Ca. penicillata*: four males and three females) were used as subjects. Animals weighed 300–400 g at the beginning of experiments and were housed in male/female pairs in cages ($2 \times 1.3 \times 2$ m). Maintenance and testing of subjects were done at the Primate Center, University of Brasília. Except during the 30-min experimental sessions, food and water were available ad libitum. The study was approved by the Animals Ethics Committee of the Institute of Biology, University of Brasília, Brazil.

2.2. Drugs

Buspirone (Bristol-Meyers) was dissolved in physiological saline and injected subcutaneously (sc) in 0.1, 0.5 and 1.0 mg/kg doses, in a volume of 1 ml/kg. Doses of buspirone are expressed as their base and saline was used as vehicle. All treatments were administered in each animal's home cage.

2.3. Apparatus

The experimental apparatus has been described in detail elsewhere (Barros et al., 2000). Briefly, it consists of a rectangular field (125×103 cm) divided into five arms by two holes and barriers, forming a figure-eight continuous maze (see Fig. 1). The apparatus, suspended 1 m from the floor, was divided into two parts (front and back chambers) by a concrete visual barrier (147 cm long, 8 cm wide, 218 cm high). The removable wire mesh start compartment, consisted of a rectangular arm (30 cm long, 25 cm wide, 35 cm high) with a central guillotine-type door. The front chamber, made of 4 mm transparent glass supported by a metal frame, had three parallel arms (40 cm long, 25 cm wide, 35 cm high), 25 cm apart, ending in a common perpendicular arm (125 cm long, 25 cm wide, 35 cm high). The two chambers were connected through holes in the visual barrier at each parallel arm.

Video cameras for monitoring and recording the experimental sessions were used, and a small taxidermized wild oncilla cat (*F. tigrina*) was placed outside the maze facing one corner of the parallel arms. The concrete barrier prevents view of the taxidermized cat as the subject enters the maze (see Barros et al., 2000), enabling a casual encounter through spontaneous exploration of the maze.

2.4. Habituation to the maze

To avoid confounding effects of exposing the marmosets to a novel environment (maze) while measuring their response to a taxidermized predator, seven 30-min habituation trials (HTs) were given in the absence of the 'predator', with 48-h intervals between sessions. These trials are essential to reliably measure the marmoset's fear/anxiety

behaviors in response to the 'predator' stimulus, as these animals can predominantly engage in highly erratic locomotor patterns when first exposed to novel environments. Such behavior tends to decline to a baseline level by the seventh trial (Barros et al., 2000).

2.5. Experimental procedure

After HT, five treatment trials (TTs) were performed on each subject, including three doses of buspirone, saline and a sham injection trial. For HT, each marmoset was quickly captured in its own home cage, handled for 1 min, and then placed in a transportation cage (35 cm long, 20 cm wide, 23 cm high). For TT, after being captured, each animal was administered a treatment, and thereafter placed into the cage. After 20 min, for both HT and TT, the subject was released into the start compartment of the maze, thus commencing a 30 min trial. Barriers from this compartment were promptly removed upon the marmoset's exit. After the test session, the subject was returned to its home cage in the transportation cage.

The 'predator' was presented on either the left or right side of the maze among subjects. Sessions were observed through a closed-circuit television and taped for later analysis. Treatment and order of the subjects were pseudo-randomly assigned for each test day. Sessions were performed between 07:30 and 13:30 hours, with a 72-h interval between test days.

2.6. Behavioral and statistical analysis

The choice of the behaviors analyzed was based on information from the literature, pilot work testing various taxidermized predators as stimuli, and on a previous study of the effects of diazepam using this model (Barros et al., 2000). The figure-eight maze was divided into 13 sections. Locomotor activity (frequency and time spent in each section) was measured using the behavior analysis software CHROMOTRACK 4.02, and the frequency and duration of other behaviors were analyzed by the focal-all occurrences sampling method (Altman, 1974). The following behaviors were measured by an observer blind to the experimental treatment: (1) exploratory behavior: to smell and/or lick any part of the apparatus; (2) locomotor activity; (3) scent marking: to rub the anogenital region to any substratum; and (4) time spent in the vicinity of the 'predator'.

Statistical analysis was carried out using Friedman's test for repeated measures followed by Dunnett's or Tukey's test for pairwise comparisons. Level of significance was set at $P < .05$ and analysis are based on one-tailed levels of significance, except for the different time intervals on the locomotor activity and proximity to the 'predator'. Based on previous studies with buspirone (Costall et al., 1992), one-tailed probabilities were employed since an anxiolytic effect was expected after treatments.

3. Results

For each of the behavioral categories analyzed data were pooled into one group, as no significant differences in gender were observed. The results for scent marking and exploratory behaviors are presented as the averaged frequencies obtained over each 30-min treatment session. Furthermore, we analyzed the number of maze section crossings (locomotor activity) and the time spent in the section closest to the stimulus (proximity to 'predator'), right or left side, over the 30-min testing period for each habituation and treatment session. Analysis of the latter behaviors, divided into three 10-min time intervals, are also presented.

The administration of buspirone in doses of 0.5 and 1.0 mg/kg significantly decreased the frequency of scent marking as compared to saline control ($\chi^2 = 9.415$, $P < .05$; Fig. 2). A relative increase in the frequency of exploratory behaviors (to smell and/or lick the apparatus) was observed for the dose of 0.5 mg/kg, but failed to attain significance level ($\chi^2 = 1.586$, $P = .406$; Fig. 2).

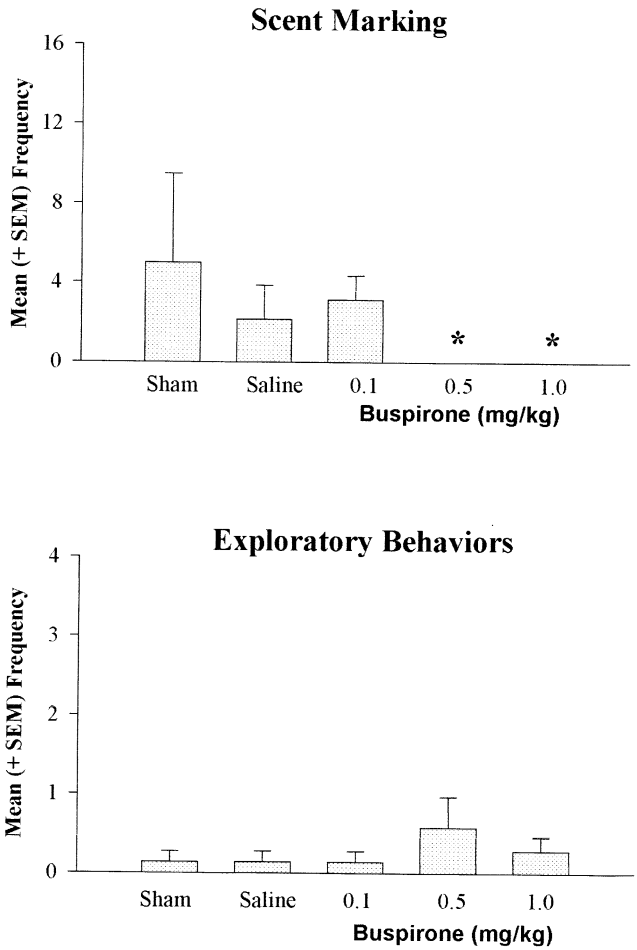

Fig. 2. Effects of treatments on the mean (+S.E.M.) frequency of scent marking (top) and exploratory behaviors (bottom) during 30-min sessions. (Friedman's test followed by Dunnett's one-tailed test. * $P < .05$ compared to saline control, $n = 7$).

Analysis of the time spent in the maze section closest to the taxidermized predator location indicated a significant increase at 0.1- and 0.5-mg/kg doses, compared to saline control ($\chi^2 = 10.185$, $P < .05$; Fig. 3a). Significant differences in this parameter were not observed during the HTs ($\chi^2 = 3.453$, $P > .1$; Fig. 3a), when the 'predator' stimulus was absent. In turn, analysis of the different time intervals (Fig. 3b) did not reveal significant differences between the three intervals of the HTs ($\chi^2 = 2.000$, $P = .486$), while indicating a tendency to increase the time spent in this section during the last 10 min of the buspirone sessions, although not significantly (control: $\chi^2 = 1.130$, $P = .620$; 0.1 mg/kg: $\chi^2 = 1.826$, $P = .486$; 0.5 mg/kg: $\chi^2 = 3.217$, $P = .237$; and 1.0 mg/kg: $\chi^2 = 0.778$, $P = .768$).

A significant decrease in locomotor activity was observed during the course of the HTs when compared to Trial 1 ($\chi^2 = 25.592$, $P < .05$; Fig. 4a). Furthermore, the number of maze section crossings tended to decrease after 10 min of exposure in each HT, except for HT1 (Fig. 4b). Buspirone treatment significantly decreased the level of locomotion

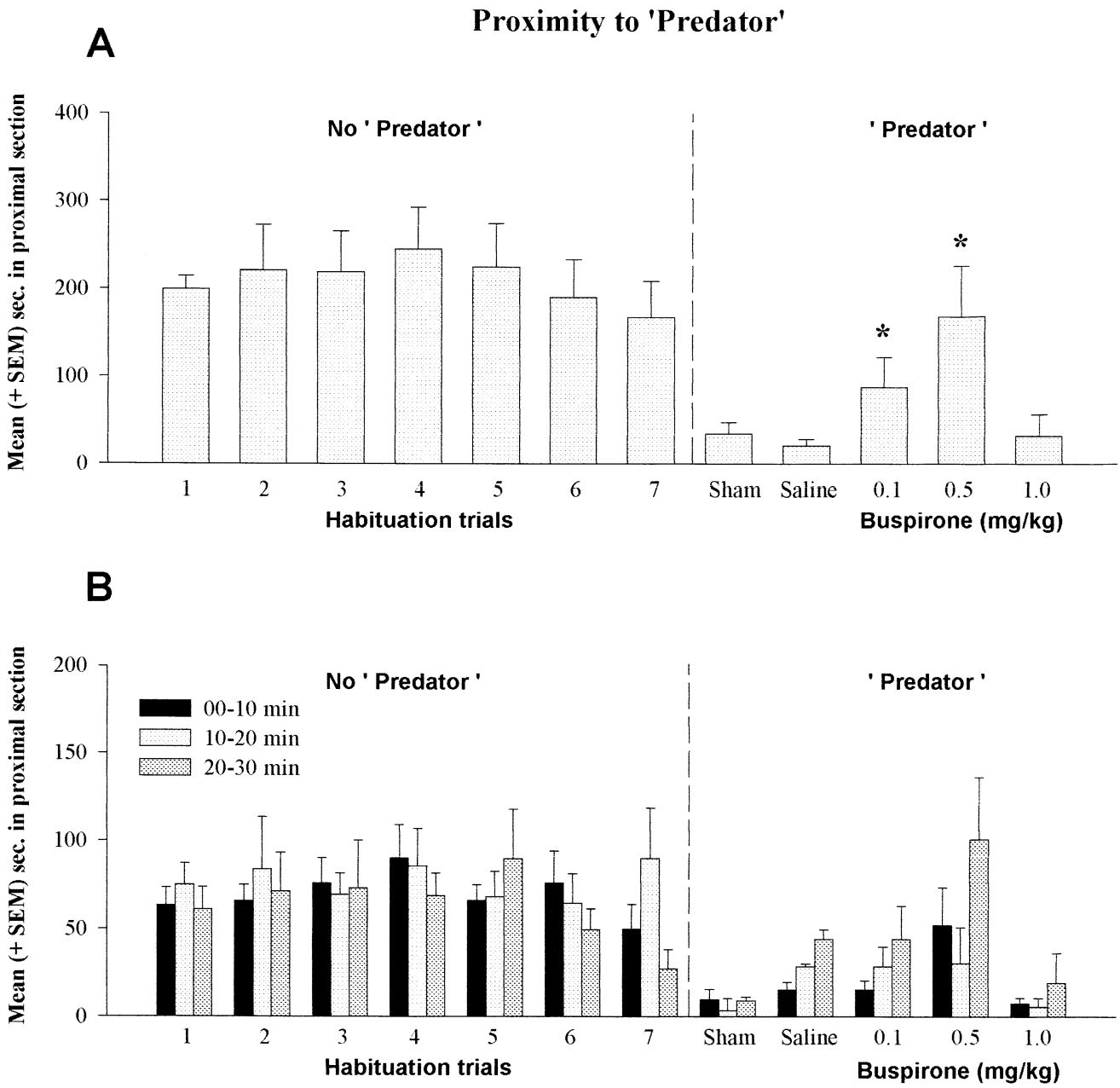

Fig. 3. Effect of habituation trials (left) and control and buspirone treatment sessions (right) on the mean (+S.E.M.) seconds spent in the maze section closest to the 'predator' stimulus during 30 min (A) or three 10-min time intervals (B). (Friedman's test followed by Dunnett's one-tailed or Tukey's two-tailed test. * $P < .05$ compared to saline control, $n = 7$).

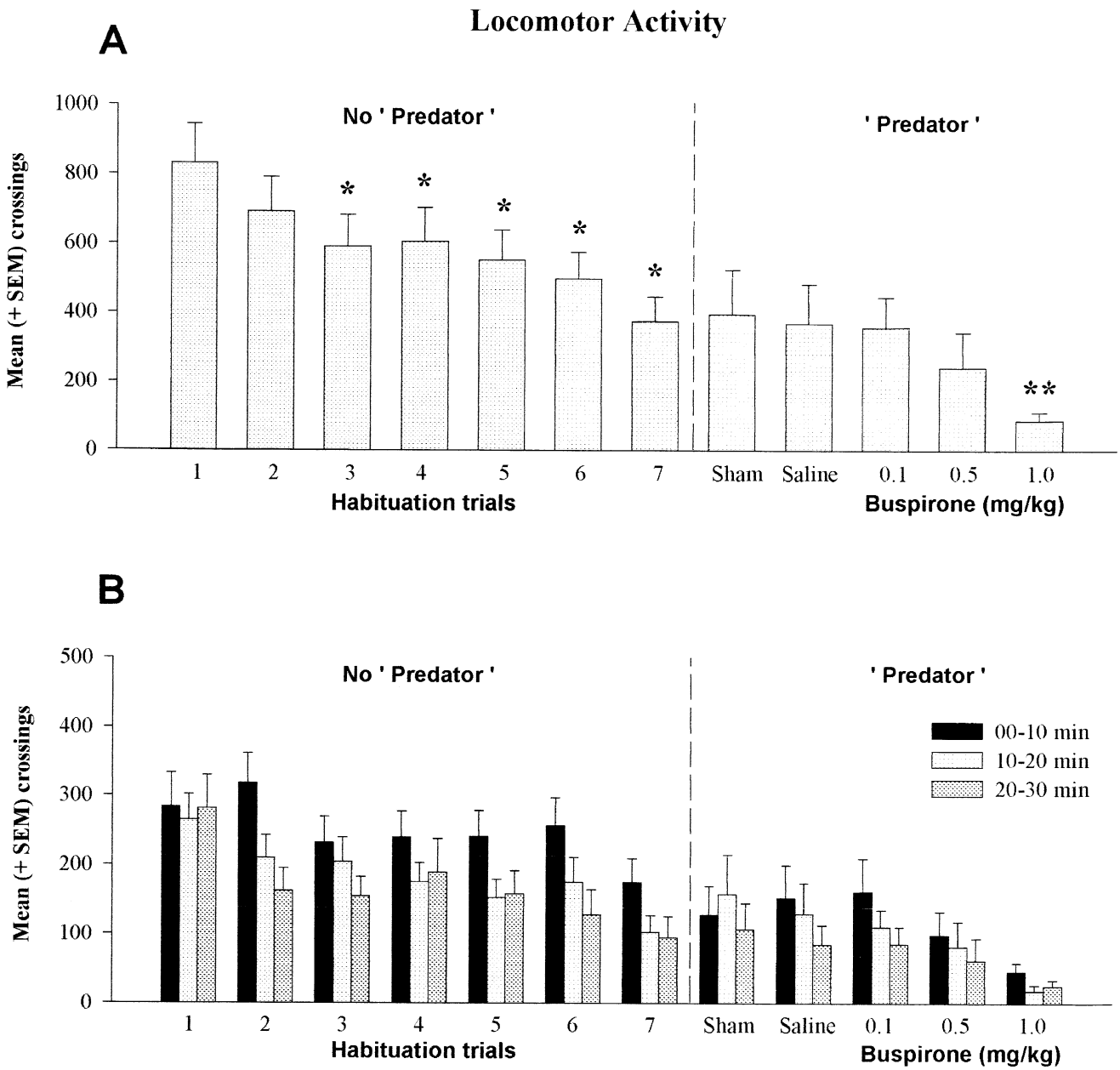

Fig. 4. Mean (+S.E.M.) locomotor activity as defined by the number of the 13 maze sections that were crossed over 30-min periods (A) or during three 10-min time intervals (B). (Left) The seven habituation trials; (right) control and buspirone treatment sessions. (Friedman's test followed by Dunnett's one-tailed or Tukey's two-tailed test. $*P<.05$ compared to habituation trial 1, $**P<.05$ compared to saline control, $n=7$).

only at the 1.0-mg/kg dose, compared to saline control ($\chi^2=9.663$, $P<.05$; Fig. 4a), and no significant decrease during the time course of each trial was observed (control: $\chi^2=5.407$, $P=.085$; 0.1 mg/kg: $\chi^2=4.667$, $P=.112$; 0.5 mg/kg: $\chi^2=3.714$, $P=.192$; and 1.0 mg/kg: $\chi^2=5.556$, $P=.085$; Fig. 4b).

4. Discussion

The present study indicates that the new test of fear and anxiety, the marmoset predator confrontation in the figure-eight maze, is sensitive to serotonergic pharmacological manipulations, inducing significant dose-dependent changes in the behavioral repertoire of the animals tested.

Scent marking, a common behavior in marmosets, disappeared after the administration of buspirone (0.5 and 1.0 mg/kg). In the first validating study of this model, scent marking was also reduced by diazepam treatments, although not statistically significant (Barros et al., 2000). This anxiety-related behavior in marmosets has been shown to increase under various stressful conditions (Epple et al., 1993; Smith et al., 1998). Furthermore, scent marking in marmosets has been shown to be

sensitive not only to BZDs, but also to serotonergic drug manipulations (Barnes et al., 1991; Cilia and Piper, 1991; Costall et al., 1992).

Buspirone at 0.5 mg/kg also induced an increase in exploratory behaviors (to lick and/or smell the apparatus), although not significantly (possibly due to the small sample size). This behavioral pattern is considered an indicator of anxiety levels in rhesus monkeys (Suomi et al., 1981), and has also been demonstrated to increase in marmosets after the administration of diazepam employing our paradigm (Barros et al., 2000). A decrease in exploration under stressful situations has been an indicator of anxiety in many rodents models, in which anxiogenic and anxiolytic compounds have effectively altered the frequency of this behavior (e.g. Bättig, 1969).

The lack of a baseline frequency of scratching observed in our study (data not shown), contrasts with the dose-dependent effect initially obtained for this behavior when first validating the method with diazepam (Barros et al., 2000). The present experiment was conducted during a different period of the year than the previous study, corresponding precisely with the rainy and dry seasons, respectively. These very distinct and opposite seasons are typical for the *Cerrado* region in which *C. penicillata* naturally occur, and are known to significantly influence a wide range of behavioral parameters in callitrichids (Ferrari and Diego, 1992). As the animals used in this study are maintained in indoor–outdoor housing facilities, they are also susceptible to such climatic variations, possibly influencing the baseline levels of scratching observed for this behavior. Previous studies where significant effects on scratching were demonstrated, have in general been conducted under controlled environmental conditions (Cilia and Piper, 1991; Diezinger and Anderson, 1986; Schino et al., 1991; Troisi et al., 1991) where temperature and humidity were not considered influencing factors. Further research, considering such variables, would be necessary for a more reliable evaluation of the effect of climatic conditions on the behavioral parameters observed in this model.

A significant increase in the time spent in the vicinity of the 'predator' after 0.1 and 0.5 mg/kg administration of buspirone indicates that this drug had an effective anxiolytic action on the subjects tested. The same effect was observed in our first study employing diazepam (Barros et al., 2000). The observed tendency to increase the time spent near the stimulus during the last 10 min of each session may relate to the pharmacokinetics of buspirone. Alternatively, possible effects of habituation may have induced the enhanced time in proximity to the 'predator' within each treatment session. However, other reports have also revealed anxiolytic effects for subcutaneously administrated buspirone in marmosets after 47 min (Barnes et al., 1991; Costall et al., 1992), corresponding to the 40–50-min post-administration time interval found in this study, suggesting an anxiolytic rather than a habituation effect.

Place preference (right or left side of the maze) does not confound the results obtained for proximity to the taxidermized predator, as the location of the stimulus was alternated between these two sides among subjects. Therefore, proximity may be a measure of anxiety in this model, in which an increase in the time spent close to the 'predator' indicates an anxiolytic effect.

The behavioral changes observed after the administration of buspirone are also not due to effects of the drug on locomotor activities. This behavior was only significantly altered at the highest dose (1.0 mg/kg), which had a sedative effect. Such an effect has also been observed for the same dose of this drug in other marmosets studies (Barnes et al., 1991; Costall et al., 1992). Changes in the behavioral repertoire are then primarily due to anxiolytic effects of drug administrations, and the results obtained for all behaviors analyzed indicate 0.5 mg/kg as the most effective dose in this new method.

Possible confounding effects of exposing these animals to a novel environment were minimized by prior exposure to the apparatus, in the absence of the 'predator' (HTs). Novel environments can be a potent source of stress among marmosets, where increased locomotor activity is a predominant feature of their behavioral repertoire (Smith et al., 1998). This parameter decreased not only between HTs, but also within each trial, reaching stable baseline levels after the seventh trial and 10 min after initial exposure, respectively. Habituation to the maze environment was also observed in the first validating study of this method (Barros et al., 2000), which may in fact be employed as a useful experimental method for investigating different aspects of habituation learning to a novel environment. In addition, male and female marmosets did not differ significantly on any of the behavioral categories observed, consistent with previous reports employing the same method (Barros et al., 2000), and other experimental models (Barnes et al., 1991; Carey et al., 1992; Cilia and Piper, 1991; Costall et al., 1992; Jones et al., 1988; Smith et al., 1998).

The value of studying serotonin's influence in animal models is greatly supported by the fact that the 5-HT system retains various primitive aspects across species, suggesting similar physiological and behavioral roles among vertebrates (Jacobs and Fornal, 1999; Jacobs and Azmitia, 1992), particularly mammals. However, various studies carried out in rodents, pigeons and nonhuman primates employing different, and even the same paradigms, have often led to conflicting and paradoxical results, suggesting that the role of 5-HT in anxiety is more complex than that initially envisioned (for reviews, see Blanchard et al., 1998; Graeff et al., 1997; Griebel, 1995). Discrepancies in data concerning the effects of 5-HT on anxiety may actually be due to the fact that different models, and sometimes the same model, may be measuring different types of anxiety (Barrett and Vanover, 1993; File, 1995; Handley and McBlane, 1993; Handley et al., 1993; Rodgers, 1997) and therefore are not readily comparable.

Models based on ethologically elicited anxiety (e.g. conspecific confrontations and antipredator responses)

allow differentiation between the various types of anxiety, eliciting relevant defensive behaviors, each differentially sensitive to specific drug manipulations (Blanchard et al., 1998). When trying to evaluate anxiety such an array of responses is more informative than a single parameter, since, more often than not, different aspects of the same pathological state may respond differently to distinct pharmacological manipulations (Blanchard et al., 1993). Furthermore, mammals are highly dependent on behavioral adaptations for self-protection (Blanchard et al., 1993). Defense behaviors and their neural substrates are highly conserved among mammals (Davis, 1992; LeDoux, 1995), susceptible to selective pressures (Blanchard et al., 1990; Nesse, 1999), and are thought by some authors to be the 'primitive' basis of anxiety disorders (Darwin, 1872; Deakin and Graeff, 1991; Nesse, 1999). In regard to callitrichids, these animals are susceptible to a broad range of potential predators due to their small size, and predation has therefore had an important influence in the evolution of their defensive responses, among other aspects (Caine, 1990; Ferrari and Lopez Ferrari, 1990). Thus, such features make defense behaviors a prime target for the development of new animal models of anxiety and for investigative studies about its etiology and possible future treatments (Rodgers, 1997).

At the same time, antipredator models tend to give more consistent results when compared to conspecific confrontations (Blanchard et al., 1998). The use of stimuli that one would normally encounter in the environment of the studied species, such as taxidermized predators, approximate normal situations in which such defensive behaviors are elicited, and therefore can provide more valid data (Blanchard et al., 1998; File, 1980). The predator confrontation model used here can be regarded as a useful method for studying anxiety in marmosets. It provides a substantial behavioral repertoire, which has been associated with fear/anxiety situations in captive, as well as wild marmosets (Epple, 1975; Stevenson and Poole, 1976; Stevenson and Rylands, 1988), and has now been shown to be sensitive to pharmacological manipulations of the serotonergic and BZD systems.

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