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Diazepam-induced decrease in anxiety-like behaviors of marmoset monkeys exposed to a novel open-field

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ABSTRACT

Unfamiliar environments can be a source of stress, fear and anxiety for marmoset monkeys. In spite of existing data, the influence of putative anxiolytics on the effects of novel environments has yet to be tested in primates. Therefore, the behavior of adult black tufted-ear marmosets to a single brief (15 min) exposure to a novel environment was analyzed in the presence and absence of diazepam (DZP). Marmosets were pre-treated with vehicle (n = 5) or diazepam (0.5 mg/kg, ip; n = 5) and submitted to a 15 min free exploration trial within a rectangular open-field arena. DZP-treated subjects, compared to vehicle controls, demonstrated significantly lower rates of (*phee*) contact calls and exploration, while a higher scan duration. Sojourn time in the arena's central zone was also significantly higher in the former group and sedation was not observed. Thus, pre-treatment with the benzodiazepine DZP decreased several anxiety-related behaviors induced by subjecting the marmosets to a new environment. The results also indicate that, as with rodent subjects, the open-field may provide a useful simple paradigm for assessing anxiety-like behaviors in this primate and, as such, constitutes a unique opportunity for direct comparative studies between rodents and marmoset monkeys in terms of anxiety and/or sedation.

1. Introduction

The forced confrontation with a novel environment, from which escape is prevented due to the presence of surrounding walls/barriers, has long been used to study emotionality in several animal species (Belzung, 1999; Prut and Belzung, 2003). For rodents, in particular, the anxiety-related behaviors resultant from these situations may be due to social isolation and/or agoraphobia, being reversed by standard anxiolytic treatments (i.e., benzodiazepines, 5-HT_{1A} receptor agonists; Prut and Belzung, 2003). However, the specific conditions under which the experiments are conducted (e.g., size/shape of the apparatus, presence of objects within the arena, lighting conditions, test duration), as well as the behavioral response observed and the effect of putative drug administration vary considerably depending on the animal model used. These typically range from rodents to several livestock species.

Yet unfamiliar environments can also be a potent source of stress, fear and anxiety for non-human primates. In marmoset monkeys, novel surroundings typically induce social (*phee*) contact calls, in addition to elevating cortisol and heart rate levels (Dettling, 2002; French et al., 2007; Gerber et al., 2002; Norcross and Newman, 1999; Rukstalis and French, 2005; Smith et al., 1998). Unfamiliar places may also require a more efficient and frequent surveillance of the environment (Barros et al., 2004a), being potentially seen as a situation that may increase the risk of predation. These small-bodied, arboreal and diurnal monkeys not only form stable extended family groups of 2–13 individuals (Stevenson and Rylands, 1988), with a complex parental care of offspring (Yamamoto, 1993), but they also suffer one of the highest rates of predation among primates (Cheney and Wrangham, 1987). Thus, isolation from group members and lack of established escape routes and/or protective shelters could be triggering the emotional response elicited during these situations (e.g., Barros et al., 2004a). Brief (15–30 min) repeated exposures of marmosets to an unknown environment rapidly lead to the habituation of the behavioral response (e.g., Barros et al., 2004b), thus minimizing costly anti-predation responses once a situation has been accurately established as being safe (Caine, 1998). This seems to be the case even among captive- and captive-born callitrichids (Barros et al., 2002; Buchanan-Smith, 1999; Caine, 1998; Koenig, 1998).

In spite of existing data related to the effects of environmental novelty in non-human primates, the influence of putative anxiolytics on such an experimental design has yet to be tested in monkeys. The open-field has long been employed with rats and mice for assessing exploratory- and anxiety-related behaviors. Therefore, the present study analyzed the behavioral response of individually tested adult black tufted-ear marmosets to a single brief (15 min) exposure to a novel open-field environment, in the presence and absence of the benzodiazepine diazepam.

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2. Methods

2.1. Subjects and housing conditions

Ten, experimentally-naïve, adult black tufted-ear marmosets (*Callithrix penicillata*) were used, weighing 290–390 g at the beginning of the study. The marmosets were housed in pairs at the Primate Center of the University of Brasilia in cages (2×1.3×2 m each) of the same colony room. This room, which consisted of two parallel rows of 12 cages, separated by a common wire-mesh enclosed central corridor, formed an outdoor/semi-indoor housing system; thus, marmosets were exposed to natural light, temperature and humidity conditions. Each home-cage consisted of two parallel concrete walls (separating adjacent cages), a wire-mesh front, back and top, a suspended wooden nest-box, several wooden perches at different heights, a food tray (where food bowl was placed), a PVC feeding tube (for dry food pellets) hung from the wire mesh top, and a layer of saw dust on the floor. Additionally, a solid roof 50–150 cm above the wire-mesh top covered two thirds of all cages. Food was provided twice a day, at 07:00 h and 13:00 h, consisting of a mixture of fresh fruits and vegetables, with mealworms, boiled eggs, various nuts and/or cooked chicken breast given three times a week. Water and dry food pellets were available ad libitum. Housing conditions complied with the regulations of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA). All procedures employed were approved by the Animal Ethics Committee of the University of Brasilia, complied with the 'Brazilian Principles of Laboratory Animal Use' (COBEA) and followed the NIH guidelines for care and use of laboratory animals.

2.2. Drugs

Diazepam (DZP; 0 and 0.5 mg/kg; Compaz, Brazil) was dissolved in a solution of phosphate buffered saline with 1% Tween 80 (Sigma-Aldrich, USA) and injected intraperitoneally (ip) 30 min prior to the behavioral testing in a volume of 1.0 mL/kg. The dose used was based on similar behavioral studies with marmosets (Cagni et al., 2009; Carey et al., 1992).

2.3. Apparatus

Testing was conducted in a rectangular open-field (OF) arena (130×75×40 cm) suspended 1.2 m from the floor. Three of its walls were made of aluminum, whereas the fourth was of 4 mm transparent glass. This glass wall made up one of the two widest sides of the rectangular arena (i.e., 130 cm). The top consisted of the same glass material and the bottom was made of 2.5 cm^2 wire-mesh. The apparatus had a guillotine-type door located on the wall opposite the one made of glass, which served as its entry/exit point. Except for the glass wall and top, the OF arena was painted white to enhance the automated video-tracking of the marmosets.

The apparatus was set-up in a test-room located in a building 50 m away from the callitrichid colony facility. The marmosets were transported to and from the test-room in a stainless steel transportation-cage (35×20×23 cm) containing a guillotine-type door. This cage did not allow marmosets to see their surrounding while being transported. Two 100 W light bulbs, fixed on opposite walls of the test-room, acted as a light source. The OF arena was monitored via a closed-circuit system using two digital cameras (Fire-i, Unibrain, USA), one mounted approximately 1 m directly above the apparatus (top-view) and one located 1.5 m away from its glass wall (side-view). Both cameras were connected to a PC laptop located outside the test-room, from where all trials were observed and recorded. The marmosets were tracked automatically (via top-view camera) using the AnyMaze software (Stoelting Co., USA). An observer, using the side-view camera, manually scored specific behaviors that could not be automatically distinguished by the software. Also, for the behavioral analyses (see Section 2.4), the same software divided the OF arena into 15 square sections of equal dimensions (26×25 cm).

2.4. Behavioral procedure and analyses

Marmosets were randomly assigned to one of two treatment groups: vehicle (n=5) or DZP (n=5). All subjects were submitted to a single 15 min trial of free exploration of the entire OF arena. This trial consisted in capturing the subject in its home-cage, administering the pre-established treatment (i.e., DZP or vehicle) and then releasing it back into its original home-cage. Following a 30 min interval, the subject was captured once again, placed in the transport-cage, taken to the test-room and released into the apparatus. At the end of the 15 min trial, the subject was promptly returned to its home-cage. The order in which subjects were tested was randomly assigned on each day and sessions were held between 13:30 and 17:00 h.

The AnyMaze software (Stoelting Co., USA) automatically tracked the marmosets' sojourn time and number of entries in each of the 15 sections of the OF arena, as well as the distance and average speed traveled within the apparatus. In addition, an experienced observer (with a 95% intra-rater reliability) scored, with the same program, the following behaviors by pressing keys on the keyboard upon occurrence of the appropriate response: (1) Long call vocalization, the frequency of long *phee* contact calls; (2) Exploratory activity, the frequency of leg-stands (to raise the body into a bipedal position) and the smelling/licking of any part of the apparatus; (3) Glance, the frequency of a single rapid upward or downward movement of the head directed at the environment, while the animal remained stationary; and (4) Scan, the frequency and duration of long-lasting (>5 s) sweeping upward or downward movements of the head directed at the environment, while the animal remained stationary. Also, the OF apparatus was divided into two zones, peripheral and central, comprising 80% and 20% of its total area, respectively. Sojourn and number of entries in the peripheral zone were determined as the total time spent and frequency in the 12 outer sections of the OF arena, respectively, relative to the total number of sections in this zone. The frequency and time spent in the three inner sections of the apparatus, also relative to the number of sections in this zone, was calculated for the central zone.

2.5. Statistical analysis

The time spent and number of entries in the peripheral and central zones of the OF arena were analyzed using a mixed design two-way analysis of variance (ANOVA), with treatment group (DZP and vehicle) as the independent factor, and zone (peripheral and central) as the repeated measure variable. Post-hoc comparisons were performed using the *t*-test (for the group factor) and paired *t*-test (for the zone factor). The remaining parameters were compared using the *t*-test. Significance level for all tests was set at $p \leq 0.05$.

3. Results

DZP-treated marmosets (0.5 mg/kg), compared to vehicle controls, demonstrated significantly lower rates of social contact vocalizations ($t_8 = 2.63$, $p<0.05$; Fig. 1) and exploration within the test apparatus ($t_8 = 2.46$, $p<0.05$; Fig. 1). On the other hand, the former group spent significantly more time scanning the environment than the vehicle-treated animals ($t_8 = -3.27$, $p<0.05$; Fig. 1).

Sojourn time within the OF arena (Fig. 2) differed significantly between groups ($F_{1,8} = 4.64$, $p<0.05$) and zones ($F_{1,8} = 25.02$, $p<0.001$), with a significant group vs. zone interaction ($F_{1,16} = 9.30$,

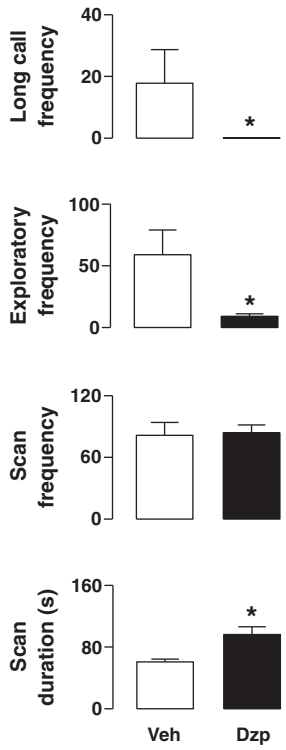

Fig. 1. Mean (+SEM) long call, exploration and scan frequency, as well as scan duration (in seconds), observed in the vehicle- (Veh) and diazepam-treated marmosets (Dzp; 0.5 mg/kg) during a single exposure to an open-field arena. n = 5/group; *p<0.05 vs. vehicle.

p<0.01). Accordingly, DZP-treated animals and those injected with vehicle spent equivalent amounts of time in the peripheral zone of the apparatus, while in the central part of the OF arena the former demonstrated a significantly higher occupancy than the latter group (p<0.05). Also, marmosets injected with vehicle spent significantly less time in the central part of the apparatus than in its peripheral area, whereas DZP-treated subjects divided their time equally between both zones. In terms of frequency, both groups demonstrated a significantly higher number of entries into the central zone of the OF arena than in its outer region ($F_{1,8} = 98.48$, p<0.001), with no significant group effect ($F_{1,8} = 2.89$, p = 0.09) or group vs. zone interaction ($F_{1,16} = 2.57$, p = 0.11). For the remaining behavioral parameters

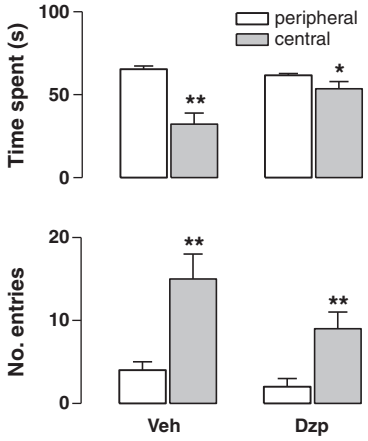

Fig. 2. Mean (+SEM) time spent (in seconds) and number of entries in the peripheral and central zones of the apparatus, relative to the number of sections in each zone, observed in the vehicle- (Veh) and diazepam-treated marmosets (Dzp; 0.5 mg/kg) during a single exposure to an open-field arena. n = 5/group; *p<0.05 vs. vehicle, **p<0.05 vs. its respective peripheral zone.

scored, significant between-group differences were not observed (distance traveled: $t_8 = 1.91$, p = 0.09; number of sections crossed: $t_8 = 0.92$, p = 0.39; travel speed: $t_8 = 1.88$, p = 0.10; glance frequency: $t_8 = 1.35$, p = 0.21; scan frequency: $t_8 = -0.16$, p = 0.87; Table 1 and Fig. 1).

4. Discussion

In the present study, adult marmoset monkeys were individually exposed to a novel OF arena during a single 15 min interval. Those who had been previously administered the putative anxiolytic DZP (0.5 mg/kg) demonstrated significantly less anxiety-related behaviors than the ones treated with vehicle. Accordingly, DZP-treated marmosets, compared to the vehicle group, demonstrated significantly lower rates of social (*phee*) contact calls and exploratory behaviors within the apparatus, while a higher scan duration. In the latter group, the rates observed for these behaviors were similar to previous reports in treatment-free subjects of the same species, using a similar protocol and apparatus (Barros et al., 2004b, 2007). In fact, when exposed to such conditions, both group- and singly-housed marmosets of several ages typically demonstrate high frequencies of social contact calls (Barros et al., 2004b; Epple, 1968; Goedeking and Newman, 1987; Jones et al., 1993; Norcross and Newman, 1993, 1999; Norcross et al., 1999; Smith et al., 1998). Novel environments are also reported to induce exploration (Barros et al., 2002, 2003, 2004b) and inhibit vigilance-related behaviors in this simian (Barros et al., 2004a), which in turn are reversed as the degree of familiarity increases due to repeated exposures. Benzodiazepine treatment, on the other hand, reversed distress vocalization in different primate species (reviewed by Miczek et al., 1995) and increased vigilance in marmosets submitted to a predatory stress (Barros et al., 2007).

DZP treatment also led to a significantly longer sojourn time in the central zone of the novel OF apparatus, compared to marmosets that received vehicle. The latter group spent significantly more time in the outer than inner sections of the rectangular arena. Changes in zone occupancy – due to DZP administration – were not accompanied by modifications in entry frequency, compared to the profile of the vehicle-treated subjects. Accordingly, both groups entered the central zone of the OF arena more frequently than the peripheral area, possibly as an alternative short route between opposite sides of the apparatus. A similar profile is typically observed in rodents tested under comparable conditions, with rats/mice having an innate fear of open spaces. Thus, an increase in central occupancy is viewed as an indicator of anxiolysis in this type of setup (for review see Prut and Belzung, 2003). Although not necessarily true for all primates, unfamiliar open spaces may pose a considerable threat to the small arboreal neotropical marmoset, considering their high rate of predation (Cheney and Wrangham, 1987) — particularly by raptorial birds (e.g., Heymann, 1990). Thus, fear of unknown open spaces may also be a critical innate response in callitrichid monkeys.

Therefore, results from the present study indicate that pre-treatment with the benzodiazepine DZP decreased several anxiety-related behaviors induced by subjecting marmoset monkeys to a new environment. Importantly, this anxiolytic-like profile occurred in the absence of any

Table 1
Behavioral response to a single exposure to a novel open-field arena in vehicle- and diazepam-treated marmoset monkeys.

Behavior	Treatment group[a]	
	Vehicle	Diazepam
Distance traveled (in meters)	75 ± 16	54 ± 14
Number of sections crossed	316 ± 62	240 ± 56
Travel speed (in m/s)	0.11 ± 0.04	0.05 ± 0.02
Glance frequency	20 ± 5	15 ± 1

[a] mean ± SEM.

confounding sedative effect, common for benzodiazepines (e.g., Rudolph and Möhler, 2006), with the distance traveled, number of sections crossed and travel speed being similar in all subjects. In rodents, DZP typically induces a similar anxiolytic-like response, with a higher sojourn time/entry in the central zone being the main (and oftentimes the sole) reported response (reviewed in Prut and Belzung, 2003). Accordingly, the present results also indicate that – as with rodent subjects – the OF arena may provide a useful simple paradigm for assessing anxiety-like behaviors in this primate species and, as such, constitutes a unique opportunity for direct comparative studies between rodents and marmoset monkeys in terms of anxiety and/or sedation.

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