

Short report

Rapid habituation of scan behavior in captive marmosets following brief predator encounters

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Abstract

Scan behavior in 10 captive predator-naive adult black tufted-ear marmosets (*Callithrix penicillata*) was investigated prior, during and following brief predator encounters (taxidermized oncilla cat — *Leopardus tigrinus*) versus neutral stimulus exposures (stuffed toy). For each stimulus, three 9 min home-cage trials were conducted ≥ 72 h apart. Each trial was divided into three consecutive 3 min intervals: pre-exposure baseline observation, stimulus exposure, and post-exposure observation period. Post-exposure scan duration increased during the first two predator confrontations, while scan frequency increased significantly only after the first. Scan behavior remained constant within the last predator encounter, as it also did within and between the three neutral stimulus exposures. Although marmosets scanned more often and significantly longer after encountering the predator than the neutral stimulus, this response rapidly habituated by the second trial. Therefore, black tufted-ear marmosets in a familiar environment rapidly habituate to brief repeated predator encounters, possibly minimizing anti-predation costs once the degree of a potential threat has been adequately assessed.

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1. Introduction

Callitrichids are small-bodied neotropical primates susceptible to a wide range of potential predators, including raptorial birds, snakes and felids (Emmons, 1987; Heymann, 1987, 1990). As direct observations of predation in the wild are rare, the influence of the risk predation upon callitrichids' behavioral ecology stems mainly from indirect evidences; i.e. from the diversity and complexity of their anti-predation strategies (Searcy and Caine, 2003). These range from careful selection of sleeping sites, retirement prior to sunset, huddled-group sleeping, and arising after dawn, to the formation of mixed-group associations, use of sentinels, emission of predator-specific vocalizations and high vigilance behavior (e.g. Caine, 1987; Ferrari and Lopes Ferrari, 1990; Hardie and Buchanan-Smith, 1997; Savage et al., 1996).

Events like unsuccessful attacks, various predator-related stimuli, and even sudden loud movements/noises alter ongo-

ing behaviors of these primates (Barros et al., 2002; Buchanan-Smith et al., 1993; Heymann, 1990; Searcy and Caine, 2003), many having long-lasting effects ranging from a few hours and days to several weeks (Caine, 1998; Hankerson and Caine, 2004; Heymann, 1990; Searcy and Caine, 2003). Accordingly, black tufted-ear marmosets demonstrated high constant scan rates even after repeated 30 min exposures to a novel environment (Barros et al., 2004a). However, scan behavior in this species habituated during the course of consecutive 30 min encounters with a taxidermized predator stimulus in a familiar surrounding (Barros et al., 2004b). Thus, following long-term exposures and when in familiar environments, marmosets may respond differently to the presence of direct predator cues, an aspect not very surprising. Important, however, are the reports that in the marmosets' natural milieu, confrontations with such stimuli are short lasting (e.g. 5–10 min; Heymann, 1987). Therefore, to determine the effects of a predator encounter under a more natural experimental design (familiar environment and fast confrontations) upon captive adult black tufted-ear marmoset (*Callithrix penicillata*) scan behavior, subjects were home-cage tested prior, during and following brief (3 min) repeated expo-

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tures to a taxidermized oncilla cat (*Leopardus tigrinus*) and the response pattern compared to that induced by a neutral object (small stuffed bear-like toy).

2. Material and methods

2.1. Subjects and maintenance

Ten experimentally naive adult black tufted-ear marmosets (*Callithrix penicillata*; four males, six females) were used as subjects. Marmosets were kept, and tests were conducted, at the Primate Centre of the University of Brasilia. Animals were housed in separate pairs, with or without juvenile offspring (which were not tested), in enclosures ($2 \times 1.3 \times 2$ m each) of a same colony room. This room forms an outdoor/semi-indoor housing system, so animals are exposed to natural light, temperature and humidity conditions (for further details see Barros et al., 2004a). The test procedures followed the regulations of the Animal Ethics Committee of the University of Brasilia, Brazil and the 'Principles of Laboratory Animal Care' (NIH publication No. 85-23, revised 1996).

2.2. Procedure

A taxidermized wild oncilla cat (*Leopardus tigrinus*) and a small stuffed bear-like toy (neutral stimulus) were used as stimuli. Both were positioned on a fixed platform 50 cm above ground level and 50 cm away from the home-cage's front wire mesh. To isolate individuals from a same home-cage and prevent stimuli from being viewed by other members of the colony room, an isolation curtain was placed around the home-cage of the subjects' being tested during each trial. All members of a same pair were exposed and evaluated simultaneously. Two observers, one for each focal animal, stood 1 m behind the enclosure's back wire mesh scoring behaviors. For each pair and stimulus, three 9 min trials were held 72 and 96 h (average = 78 h) between 'predator' encounters. Each trial was divided into three consecutive 3 min intervals: (a) a pre-exposure baseline observation, (b) a stimulus exposure, and (c) post-exposure observation period. Following the baseline interval, the stimulus covered with a cloth was placed on the fixed platform, and once the cloth was removed, the exposure interval began. At the end of this interval, the stimulus was covered and removed from the platform, thereupon beginning the post-exposure interval. The sequence of stimuli presentations (predator versus toy) and pair order were randomly established. Each pair was tested only once a day between 13:00 and 15:00 p.m. Marmosets were initially habituated to the presence of the observers and the isolation curtain.

Scan frequency and duration were scored by the observers, with a 95% inter-observer reliability. Based on previous reports by Caine (1984) and Koenig (1998), visual scanning was defined as a ≥ 5 s continuous sweeping or other visible movement of the head directed at the environment, while the subject remained stationary. Scans made during foraging or directed at conspecifics and observers were not recorded.

Data are presented as the absolute mean \pm S.E.M. As the data were found to be normally distributed and with equal variance, parametric tests were employed for statistical analysis. Thus, scan frequency and duration were analyzed separately for possible differences within and between trials by means of one-way repeated measures analysis of variance (RM ANOVA). Further post hoc comparisons were performed using Tukey's multiple all-pairwise comparisons. A $p < 0.05$ was used for statistical significance.

3. Results

Data from all ten subjects were pooled into one group as the small number of male subjects tested ($n = 4$) precluded analysis of possible sex differences. Furthermore, for the neutral stimulus (stuffed bear-like toy) data were also pooled together as no significant differences were observed between the three trials (data not shown).

A post-exposure increase in scan duration was observed solely for the first two predator stimulus confrontations (P_1 and P_2 ; Fig. 1A), compared to their respective pre-exposure and exposure intervals, almost attaining significant values (P_1 : $F_{9,2} = 3.127$, $p = 0.068$; P_2 : $F_{9,2} = 2.892$, $p = 0.081$). Power analysis of these comparisons revealed that they were below the desired value of 0.800 ($P_1 = 0.369$; $P_2 = 0.332$), possibly due to small sample size. Within the last predator encounter, on the other hand, scan duration remained constant (P_3 : $F_{9,2} = 0.687$, $p = 0.516$), similar to the result observed when the neutral stimulus was encountered (N : $F_{9,2} = 0.117$, $p = 0.891$). Furthermore, marmosets scanned the environment longer only after the first two encounters with the predator, compared to the neutral stimulus, almost attaining significance levels ($F_{9,3} = 2.317$,

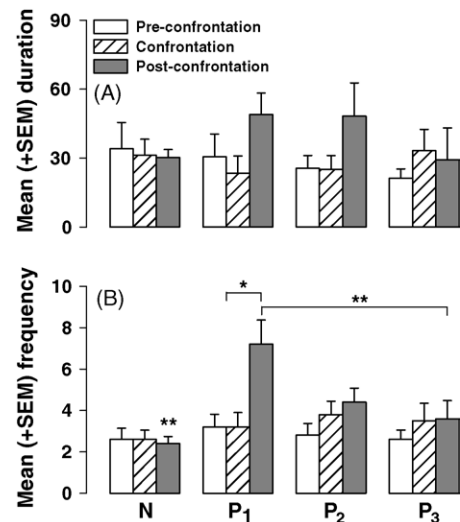


Fig. 1. Mean (+S.E.M.) total scan duration in seconds (A) and scan frequency (B) during each of the three 9 min predator (P_1 – P_3) and the neutral (N) stimulus exposure trials, divided into three consecutive 3 min intervals (pre-confrontation, confrontation and post-confrontation). For the neutral stimulus condition, the three trials conducted were pooled together as no significant differences among the three trials were observed (see text): * $p < 0.05$ vs. P_1 pre-exposure and exposure intervals; ** $p < 0.05$ vs. P_1 post-exposure interval.

$p=0.098$). For the remaining intervals, scan duration rates were similar between the two stimuli tested (pre-exposure: $F_{9,2}=0.490$, $p=0.692$; exposure: $F_{9,3}=0.552$, $p=0.651$).

In addition, the first encounter with the predator stimulus significantly influenced the marmosets' scan frequency (P_1 : $F_{9,2}=6.761$, $p=0.006$ Fig. 1B). Subsequent analysis revealed a post-exposure increase in this parameter, compared to its pre-exposure and exposure intervals. Subsequent encounters with this stimulus and all exposures to the neutral object did not alter scan frequency (P_2 : $F_{9,2}=1.973$, $p=0.168$; P_3 : $F_{9,2}=0.719$, $p=0.501$; N : $F_{9,2}=0.159$, $p=0.854$). In addition, marmosets also scanned the environment significantly more often following the first than the last predator encounter, as well as more frequently than after the neutral stimulus ($F_{9,3}=6.580$, $p=0.002$). For the remaining intervals, scan frequency rates were similar between the two stimuli tested (pre-exposure: $F_{9,3}=0.302$, $p=0.824$; exposure: $F_{9,3}=0.660$, $p=0.584$).

4. Discussion

The marmosets' tested in the present experiment increased their scanning rate immediately after an encounter with a taxidermized predator stimulus (wild oncilla cat). Such a response was not observed when subjects were exposed to the neutral stuffed toy stimulus, indicating a specificity of this response to the type of stimulus presented and not a mere response to novelty. In fact, learning to correctly differentiate between potentially threatening and non-threatening stimuli has been observed in this (Barros et al., 2002) and other marmoset species (Caine, 1998; Koenig, 1998). Furthermore, the scan increase observed only after encounters, but not during encounters, may be due to increases in other behavioral patterns during the actual confrontations, and thus does not necessarily imply that marmosets were not overall vigilant under this condition. Behaviors such as locomotion, vocalizations and mobbing-related responses are commonly seen in this species when confronted with different 'predator' stimuli (Barros et al., 2002). A fast approach-withdrawal pattern was indeed observed during confrontations (albeit not quantified, as the focus was only on the scan behavior), consistent with a mobbing response described for feral marmoset due to the presence of a wild cat (Passamani, 1995). In addition, locomotion has been found to negatively correlate with levels of scanning in previous studies (Barros et al., 2004a). Furthermore, Treves (2000) pointed out that scan behavior defined solely on the basis of specific scan duration criteria may under-represent the variability of this complex behavior in primates. In marmosets, more specifically, one point of variability may be between the frequency/duration of the location scanned (aerial versus terrestrial), as suggested by Barros et al. (2004a).

Interestingly, however, was the rapid habituation (<3 trials) of the marmosets' scan response following repeated confrontations with the taxidermized predator stimulus. As the preceding encounter did not result in an actual attack by the 'predator', and the fact that oncilla cats are known to prey upon species <100 g (Sunquist and Sunquist, 2002), the observed decrease in scanning after the second/third encounter may represent a rapid assessment of a decrease in predation risk and/or of the predator

type. As anti-predation responses can be costly and disruptive, fast and accurate assessments of potential threats may minimize such factors, increasing energy allocation towards other essential nondefensive activities, such as foraging and reproduction (Kavaliers and Choleris, 2001). In fact, callitrichids seem to rapidly resume various interrupted activities once a potential threat has been adequately evaluated (Caine, 1998). However, the possibility that such rapid habituation may actually reflect the short duration of the exposures (i.e. 1 min) and/or the small interval between trials (i.e. 78 h on average) should also be carefully considered. As previous studies with black tufted-ear marmoset employing a longer confrontation design (30 min) also reported a habituation effect (Barros et al., 2004b), such factor may not be as relevant to captive marmosets. Albeit speculative, such aspects of exposure/inter-exposure duration, as well as specific predator type, warrant further studies and should be carefully considered when investigating the determinants of vigilance behavior in primates and possibly other animals.

On the other hand, long-term effects of predator encounters, specifically upon vigilance behavior, have also been shown (Caine, 1998; Barros et al., 2004a; Hankerson and Caine, 2004). These seemingly disparate findings are thought to relate to significant differences between testing conditions, such as degree of familiarity with the surroundings (home-cage versus novel environment) and, again, the interval between consecutive confrontations (hours–days). However, one should be cautious when interpreting these differences in light only of a familiar versus novel environment, considering that wild animals may respond differently to such aspects than captive subjects. For feral animals, specific circumstances of the predator encounter (e.g. visibility, whether the predator was seen moving off or not) may have a more substantial impact upon scanning behavior. Furthermore, specific components of the marmosets' scan behavior may be related to the general predator type (e.g. snake, cat or raptorial bird), which differed significantly within the previous studies with *Callithrix* (Barros et al., 2004a; Caine, 1998; Hankerson and Caine, 2004).

Thus, in the present study, a rapid habituation effect was observed upon brief encounters with a cat stimulus when in a familiar environment. Such conditions of habitat familiarity and rapid encounters may be more directly related to the marmosets' natural environment than other experimental designs (e.g. long confrontations). Future research employing repeated direct or cued confrontations with a predator stimulus should consider a possible rapid habituation effect as an influencing factor upon the animals' short- and long-term behavioral response. Further comparative studies on exposure duration, interval between consecutive confrontations and the specific nature of the predator stimulus may significantly contribute to the understanding of the determinants of vigilance behavior in different animal species.

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References

- Barros, M., Boere, V., Mello Jr., E.L., Tomaz, C., 2002. Reactions to potential predators in captive-born marmosets (*Callithrix penicillata*). *Int. J. Primatol.* 23, 443–454.
- Barros, M., Alencar, C., Tomaz, C., 2004a. Differences in aerial and terrestrial visual scanning in captive black tufted-ear marmosets (*Callithrix penicillata*) exposed to a novel environment. *Folia Primatol.* 75, 85–92.
- Barros, M., de Souza Silva, M.A., Huston, J.P., Tomaz, C., 2004b. Multi-behavioral analysis of fear and anxiety before, during and after experimentally induced predatory stress in *Callithrix penicillata*. *Pharmacol. Biochem. Behav.* 78, 357–367.
- Buchanan-Smith, H.M., Anderson, D.A., Ryan, C.W., 1993. Responses of cotton-top tamarins (*Saguinus oedipus*) to faecal scents of predators and non-predators. *Anim. Welfare* 2, 17–32.
- Caine, N.G., 1984. Visual scanning by tamarins: a description of the behavior and tests of two derived hypothesis. *Folia Primatol.* 43, 59–67.
- Caine, N.G., 1987. Vigilance, vocalization, and cryptic behavior at retirement in captive groups of red-bellied tamarins (*Saguinus labiatus*). *Am. J. Primatol.* 12, 241–250.
- Caine, N.G., 1998. Cutting costs in response to predatory threat by Geoffroy's marmosets (*Callithrix geoffroyi*). *Am. J. Primatol.* 46, 187–196.
- Emmons, L.H., 1987. Comparative feeding ecology of felids in a neotropical rainforest. *Behav. Ecol. Sociobiol.* 20, 271–283.
- Ferrari, S.F., Lopes Ferrari, M.A., 1990. Predator avoidance behavior in the buffy-headed marmoset, *Callithrix flaviceps*. *Primates* 31, 323–338.
- Hankerson, A., Caine, N., 2004. Pre-retirement predator encounters alter the morning behavior of captive marmosets (*Callithrix geoffroyi*). *Am. J. Primatol.* 63, 75–85.
- Hardie, S.M., Buchanan-Smith, H.M., 1997. Vigilance in single and mixed-species groups of tamarins (*Saguinus labiatus* and *S. fuscicollis*). *Int. J. Primatol.* 18, 217–234.
- Heymann, E.W., 1987. A field observation of predation on a moustached tamarin (*Saguinus mystax*) by an anaconda. *Int. J. Primatol.* 8, 193–195.
- Heymann, E.W., 1990. Reactions of wild tamarins, *Saguinus mystax* and *Saguinus fuscicollis*, to avian predators. *Int. J. Primatol.* 11, 327–337.
- Kavaliers, M., Choleris, E., 2001. Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neurosci. Biobehav. Rev.* 25, 577–586.
- Koenig, A., 1998. Visual scanning by common marmosets (*Callithrix jacchus*): functional aspects and the special role of adult males. *Primates* 39, 85–90.
- Passamani, M., 1995. Field observation of a group of Geoffroy's marmosets mobbing a margay cat. *Folia Primatol.* 64, 163–166.
- Savage, A., Snowdon, C.T., Giraldo, L.H., Soto, L.H., 1996. Parental care patterns and vigilance in wild cotton-top tamarins (*Saguinus oedipus*). In: Norconk, M.A., Rosenberger, A.L., Garber, P.A. (Eds.), *Adaptive Radiations of Neotropical Primates*. Plenum Press, New York, pp. 187–199.
- Searcy, Y.M., Caine, N.G., 2003. Hawk calls elicit alarm and defensive reactions in captive Geoffroy's marmosets (*Callithrix geoffroyi*). *Folia Primatol.* 74, 115–125.
- Sunquist, M., Sunquist, F., 2002. *Wild Cats of the World*. University of Chicago Press, New York.
- Treves, A., 2000. Theory and method in studies of vigilance and aggregation. *Anim. Behav.* 60, 711–722.