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# Repeated cocaine administration in marmoset monkeys induces hypervigilance-related behaviors, but no changes in locomotion and cortisol levels

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ABSTRACT

Although cocaine induces several behavioral and hormonal effects, little is known about non-contingent repeated administrations in non-human primates. Therefore, we analyzed behavioral (locomotion, vigilance) and hormonal (cortisol) responses of adult black tufted-ear marmosets during repeated administrations and withdrawal trials. The subjects were divided into two groups (saline or cocaine 5 mg/kg, ip) and submitted to nine treatment trials and four withdrawal trials in the absence of any treatment in an open-field arena. Blood samples were obtained on five different time points of the procedure to evaluate the effects of repeated cocaine treatment on basal cortisol levels. Cocaine repeatedly administered to drug-naïve marmosets induced a slow-onset hypervigilance effect (i.e., scan — long-lasting sweeping movements of the head directed at the environment; and glance — single rapid movement of the head directed at the environment), with no concomitant change in locomotion. Treatment cessation during withdrawal immediately reversed the cocaine-induced hypervigilance effect. Cortisol levels remained constant throughout the procedure. Therefore, marmosets seem to have a similar behavioral — but not hormonal — response as humans and other nonhuman primates repeatedly injected with cocaine, but differ from rats in their absence of hyperlocomotor activity. The development of hypervigilance with repeated application may constitute a unique measure to assess cocaine-induced changes in behavior in the marmoset and other nonhuman primates.



## 1. Introduction

Psychostimulants, such as cocaine, induce several behavioral and hormonal effects in rodents, nonhuman primates (NHP) and humans. They typically enhance locomotion in rodents, whereas increases in stereotyped psychotomimetic or hallucinatory-like behaviors have been reported in NHP and humans (Bradberry, 2007; Castner and Williams, 2007; Leyton, 2007). In NHP "checking of the environment" has been reported, although stereotyped grooming, oral movements (e.g., oralbuccal dyskinesias, vacuous chewing) and manipulation of objects, as well as the tracking and/or grasping of non-existent stimuli may also occur (e.g., Farfel et al., 1992; Ridley et al., 1982). Direct dopaminergic agonists repeatedly injected in monkeys result in similar behavioral patterns (Kleven and Woolverton, 1990). Several of these behavioral changes are observed regardless of whether the drug is applied by contingent (self-administered) or noncontingent (experimenter-administered) means (Steketee and Kalivas, 2011).

Acute cocaine administration also activates the hypothalamic–pituitary–adrenal (HPA) axis, leading to an increased release of corticosterone in rodents (Mello and Mendelson, 2002) and cortisol in NHP (Sarnyai et al., 1996) and humans (Baumann et al., 1995). Whether the release is more pronounced after contingent than noncontingent administration remains a controversial issue (e.g., Palamarchouk et al., 2009). Nonetheless, activation of the HPA axis has been associated to the neural mechanisms of cocaine reinforcement and relapse (Marinelli and Piazza, 2002). The hormonal effects of repeated cocaine exposures and withdrawal in NHP have not been addressed.

Due to considerable differences between rodents and primates, including the morphological organization, connectivity and prenatal development of the mesocortical DA system (Berger et al., 1991; Haber and McFarland, 1999; Joel and Weiner, 2000), the dynamics of dopamine release and uptake in the striatum (Cragg et al., 2000), the genetic homology of the dopamine transporter (Miller et al., 2001), the density and distribution of receptors within addiction-related neural circuits (e.g., Camps et al., 1990) and brain metabolic responses to psychostimulants (e.g., Lyons et al., 1996), NHP may help bridge

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the gap between pre-clinical and clinical studies on drug addiction, particularly the small neotropical marmoset monkey (Maior et al., 2011). However, little is still known about the behavioral and hormonal effects of repeated cocaine administrations in this NHP.

Therefore, in the present study we analyzed locomotor and vigilance-related behaviors of adult black tufted-ear marmosets during repeated systemic cocaine administrations and withdrawal trials. The levels of circulating cortisol were also measured during five different time points over the course of the experimental trials to evaluate the effects of repeated cocaine treatment on basal cortisol levels.

## 2. Methods

### 2.1. Subjects and housing conditions

We used 10, experimentally-naïve, adult black tufted-ear marmosets (*Callithrix penicillata*), weighing 290–410 g at the beginning of the study. They were housed in pairs at the Primate Center of the University of Brasilia in cages (2×1.3×2 m each) of a 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, the 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 meal-worms, 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

Cocaine hydrochloride (0 and 5 mg/kg; Sigma-Aldrich, USA) was dissolved in phosphate buffered saline and injected intraperitoneally, in a volume of 1.0 mL/kg, 5 min prior to the behavioral testing.

### 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., 130×40 cm). The top consisted glass and the bottom was made of 2.5 $cm^2$ wire-mesh. The apparatus had a guillotine-type door located on the wall directly opposite the one made of glass, which served as its entry/exit point. With the exception of 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 covered aluminum transportation-cage (35×20×23 cm) containing a guillotine-type door. 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 1.5 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 below), the same software divided the OF arena into 15 square sections of equal dimensions (26×25×40 cm).

### 2.4. Behavioral procedure and analyses

The marmosets were randomly assigned to one of two treatment groups: saline ($n=5$) or cocaine ($n=5$). All subjects were initially submitted to nine 15-min test trials and then to four 15-min withdrawal trials. All trials were held at 48 h intervals. Each trial consisted in capturing the subject in its home-cage and administering either cocaine or saline. The marmoset was then placed into the transportation-cage, taken to the test-room and released into the OF arena after a 5 min interval. At the end of the 15 min trial, the subject was promptly returned to its home-cage. As no treatment was administered prior to the four withdrawal trials, the marmosets were taken directly to the OF arena in the transportation-cage. The order in which subjects were tested was randomly assigned on each day and the sessions were held between 13:30 and 17:00 h.

The AnyMaze software (Stoelting Co., USA) automatically tracked the marmosets' entry into each of the virtual 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 vigilance-related behaviors by pressing keys on the keyboard upon occurrence of the appropriate response: (1) *Leg stand*, the frequency of raising the body into a bipedal position; (2) *Glance*, the frequency of a single rapid upward or downward movement of the head directed at the environment, while the animal remained stationary; and (3) *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. Vigilance behaviors were based on marmoset ethograms (Stevenson and Poole, 1976; Stevenson and Rylands, 1988) and previous reports in this species (Barros et al., 2004a, 2004b, 2008).

### 2.5. Blood sampling and cortisol assay

Five blood samples were obtained from each marmoset as follows: the first sample was taken one week prior to the test-trials (baseline), the second sample after test-trial 1, the third sample after test-trial 4, the fourth sample after the last withdrawal-trial and the fifth sample was taken 12 weeks later. All sampling were done between 09:00 and 11:00 h. Thus, except for the initial baseline sample, blood was taken the morning after the behavioral trials as to evaluate the effects of repeated cocaine treatment on basal cortisol levels.

For each blood sampling, the marmoset was captured in its home-cage and conveyed to an adjacent procedure room where it was anesthetized with isoflurane via inhalation. A 1.0 mL blood sample was then collected via femoral venipuncture and immediately placed into a tube containing a gel barrier and clot-promoting additives for total serum analyses. Following its recovery (<5 min), the subject was released back into its home-cage, monitored for 15 min and given vitamin supplements. The sampling procedure (i.e., time elapsed between entering the home-cage to capture the animal and the end of the venipuncture) was determined, as this may possibly influence the hormone content observed. On average, the latency was 4.02±0.59 min (mean±SEM), an interval previously reported not to exert a significant influence on cortisol assays in marmosets (Saltzman et al., 1994). All procedures were conducted in the presence of the Primate Center's veterinarian.