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# Multiple presentations reduce the behavioral impact of protected predator exposure in rats☆

Raymond F. Genovese*, Christina C. Johnson, Christine A. Tobin, Sangeeta Gauchan

*Behavioral Biology Branch, Center for Military Psychiatry and Neuroscience, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500, USA*

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ABSTRACT

Exposure of rats to a predator species, such as a cat, or stimuli associated with a predator species has been used to model the effects of traumatic stress. We further investigated this procedure to determine if the behavioral effects from such exposure could be increased by multiple exposures. In rats ($n = 8$ for each treatment group), we evaluated single (1×) and multiple (1×/day for 3 consecutive days [3×] and 2×/day for 3 consecutive days [6×]) exposures using cats and soiled cat litter. All exposures were 15 min in duration and the rats were directly exposed to the cats but in a protected fashion that did not allow the predator to physically injure the rat. Sham exposures were conducted using similar conditions without the presence of the predator or litter. The effects of the exposures were evaluated using an elevated plus maze (EPM). Sessions on the EPM were conducted before the exposures and at various times after the exposure. Difference scores (post–pre) were calculated for dependent measures from the EPM, and statistical analyses compared the slopes and intercept values derived from regression functions from these scores over the post-exposure sessions. During the first 30 days after exposure, a significant reduction in activity on the EPM was observed for the 1× treatment and a smaller reduction was observed for the 3× treatment, but no reduction was observed for the 6× and sham control treatments. Thus, increasing the number of exposures did not increase the magnitude of the effect but, instead, resulted in a decrease. These results show that adaptation to the effects of the predator exposure occurred with repeated sessions.



## 1. Introduction

Predator exposure procedures are valued for the development of animal models of traumatic stress. Because the exposure is ethologically relevant, there is an assumption that the procedure represents the critical feature of a traumatic event. That is, exposure to the predator can be reasonably expected to be perceived as a life-threatening event. In a typical procedure, rats are exposed a single time to cats as the predator. Such procedures have been shown to produce a variety of effects, including deficits in behavioral performance (Adamec and Shallow, 1993; Sandi et al., 2005; Woodson et al., 2003), neuroendocrine changes such as increases in corticosterone (Adamec et al., 2006; Blanchard et al., 1998; Figueiredo et al., 2003) and other cytochemical changes (Adamec et al., 2006, 2005; Blundell and Adamec, 2007, 2006; Figueiredo et al., 2003). Furthermore, such changes have frequently been demonstrated to be persistent, even after a brief predator exposure session (e.g., Adamec and Shallow, 1993).

While a number of reports of exposure of rats directly to cats exist in the literature as either unprotected (e.g., Adamec et al., 2006; Adamec and Shallow, 1993) or protected exposures (e.g., Sandi et al., 2005; Woodson et al., 2003), many other studies have used indirect methods of exposure. For example, exposure to the urine of cats (in the form of soiled litter) has also been an effective stressor in adult (Cohen et al., 2006, 2004) and juvenile (Cohen et al., 2007; Tsoory et al., 2007) rats. Exposure to cat fur/dander has also been used as an effective stressor (Munoz-Abellan et al., 2010; Wright et al., 2008). Additionally, component fox odor (Endres et al., 2005; Thomas et al., 2006) and ferret odor (Campeau et al., 2008; Masini et al., 2005) have been effectively used as stressors

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* Corresponding author. Tel.: +1 301 319 9721; fax: +1 301 319 9905.
*E-mail address:* Raymond.F.Genovese.Civ@mail.mil (R.F. Genovese).

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in rats. While it is likely that substantial differences in impact exist between these procedures (e.g., see Blanchard et al., 2003), it is clear that exposure to predators and stimuli associated with predators are stressful events in rats.

We further investigated the predator exposure procedure in rats using cats as the predator species. Specifically, we were interested in determining if repeated predator exposures would enhance the behavioral effects of exposure in rats. With regard to the utility of an animal model, the severity of the behavioral impact of a traumatic stressor is an essential consideration since clinically; the impact of traumatic stress can be both life altering and long-lasting as is the case with Post-Traumatic Stress Disorder (PTSD) (American Psychiatric Association, 2000). Repeated exposure to psychological trauma is also integral to some situations (in both a civilian and military context) that can lead to PTSD and related stress disorders. Therefore, the ability to enhance the behavioral impact of exposures as well as to evaluate the effects of repeated exposures can increase the utility of the model. To maximize the salience of the exposure, we conducted the procedure using physical proximity of the predators with rats being exposed in the normal living environment of cats. Additionally, we specifically added olfactory cues associated with the cats (used litter) to the exposure apparatus. To prevent acute physical injury which could potentially confound the interpretation of the results, however, the exposures were conducted in a protected fashion that did not allow significant physical contact between the predators and the rats.

## 2. Materials and methods

### 2.1. Subjects

This study was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 and 2011 edition. All procedures were reviewed and approved by the Institute's Animal Care and Use Committee, and performed in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Adult male Sprague–Dawley rats (Charles River, Wilmington, MA) were used and were ~8 weeks of age at the start of the study. Rats were individually housed and maintained in a temperature and humidity controlled vivarium under a 12-h L:12-h D cycle (lights on at 06:00 h) and water was always available in the home cages. Rats were fed rodent chow (PMI Nutrition International, St. Louis, MO) and were mildly food restricted such that body weights were no more than 15% below the expected *ad libitum* feeding weights (as provided by the breeder) toward maintaining an equivalent level activity for all rats over the course of the study. The mean and SEM of the rat's weights at the beginning and end of the evaluation period were 238.2 ± 3.0 and 297.1 ± 1.3 g, respectively.

### 2.2. Predator exposure

Rats were randomly assigned to treatment groups consisting of 1 (1×), 3 (one per day for three consecutive days, 3×) or 6 (two per day for three consecutive days, 6×) predator exposures, or a control treatment that received 6 (two per day for three consecutive days) sham exposures (CON). Four cats (three ovariectomized females and one orchidectomized male) ~10 years old were used as predators. Cats were pair housed (female/female and female/male) in kennels; each measuring 1.19 m (W) × 3.05 m (L) and 2.12 m (H) and the housing remained unchanged for the duration of the study. Each exposure took place using one pair of cats and the choice of which kennel (i.e., which pair) was balanced to maximize equal numbers of exposures to both pairs of cats for each treatment condition.

For the predator exposure, rats were placed in isolator cages and transported to the room housing the cat kennels. From the isolator cages, individual rats were placed in a small animal cage (21.4 cm × 14 cm × 15.2 cm with ~1 cm spacing between cage bars) in which fresh soiled cat litter (Paperchip Soft, Shepherd Specialty Papers, Watertown, TN) was sifted to remove clumps of feces and placed in a quantity sufficient to cover the floor of the cage. The cage did not represent restraint as it was large enough to allow the rat to easily turn around completely and to rise up, but otherwise restricted substantial locomotion. The cage prevented the cats from physically attacking the rat, but otherwise freely allowed the transmission of visual, olfactory and auditory stimuli. To begin the exposure, the cage was placed near the center of the kennel and food treats were placed around the perimeter of the cage to facilitate the presence of the cats in the vicinity of the rat. The duration of each exposure was 15 min, after which the rat was returned to the transport cage and then moved to the home cage. Sham exposures were conducted by placing the rat in an identical apparatus (used only for the control treatment) to that used for predator exposures, but without litter. Control rats were placed in a separate room from the normal housing room which never contained a predator. The duration of the sham exposures was also 15 min. Exposures generally took place at the same time each day. For single exposure treatments this was ~10:00 h and for twice daily exposure treatments it was ~10:00 and ~14:00 h. On exposure days, cats were fed normally, but always shortly after the exposure sessions were completed.

### 2.3. Elevated plus maze

Sessions on the elevated plus maze (EPM) were conducted using a commercial maze (model EPM1000, Kinder Scientific, Poway, CA). The maze has four arms, each measuring 55.9 cm × 11.4 cm. Two of the arms ("closed arms"), which face each other, have opaque side walls measuring 45.7 cm. The remaining two arms ("open arms"), which also face each other, do not have side walls. The maze is elevated to a height of 80 cm. The floor of each arm and the intersection area (10.2 cm × 10.2 cm) contains photo-emitter/detector pairs that are monitored by software to measure movement (as basic activity counts) and position within the maze. Illumination in the room containing the maze was measured at 432 lx at the maze intersection.

Rats were placed at the intersection point at the beginning of the session and allowed to explore the maze undisturbed for 15 min, after which, they were returned to their home cage. A pre-exposure baseline session on the maze was conducted three days before predator or sham exposures began. Subsequently, multiple sessions on the maze were conducted beginning 24 h after the last predator or sham exposure. Post-exposure test sessions were conducted with all rats at the following time points (in hours) relative to the (last) predator or sham exposure: 24, 132, 192, 300, 360, 480, 528, 660, 696. All sessions on the maze were 15 min in duration.

### 2.4. Data analysis

Dependent measures collected from the maze sessions included the total number of activity counts per session and activity counts in each of the three maze areas (closed arms, open arms and intersection). Time (s) spent in each of the three maze areas was also calculated. Data from maze sessions following predator or sham exposure were calculated for each rat as a difference score from the baseline session conducted before the exposures. One way ANOVA was used to assess differences in baseline performance between