American Journal of Primatology 69:877–889 (2007)

RESEARCH ARTICLE

# Treatment With CRH-1 Antagonist Antalarmin Reduces Behavioral and Endocrine Responses to Social Stressors in Marmosets (*Callithrix kuhlii*)

JEFFREY A. FRENCH[1–3*], JEFFREY E. FITE[1,2], HEATHER JENSEN[1], KATIE OPAROWSKI[1,2], MICHAEL R. RUKSTALIS[1,3], HOLLY FIX[1,3], BRENDA JONES[1,2], HEATHER MAXWELL[1,2], MOLLY PACER[1,2], MICHAEL L. POWER[4,5], AND JAY SCHULKIN[4,6]

[1]*Callitrichid Research Center, University of Nebraska at Omaha, Omaha, Nebraska*
[2]*Department of Psychology, University of Nebraska at Omaha, Omaha, Nebraska*
[3]*Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska*
[4]*Research Department, American College of Obstetrics and Gynecology, Washington, DC*
[5]*Department of Conservation Biology, Smithsonian National Zoological Park, Washington, DC*
[6]*Department of Physiology and Biophysics, Georgetown University, Washington, DC*

Corticotropin-releasing hormone (CRH) has multiple roles in coordinating the behavioral and endocrine responses to a host of environmental challenges, including social stressors. In the present study we evaluated the role of CRH in mediating responses to a moderate social stressor in Wied's black tufted-eared marmosets (*Callithrix kuhlii*). Male and female marmosets (n = 14) were administered antalarmin (a selective CRH-1 receptor antagonist; 50μg/kg, p.o.) or vehicle in a blind, counterbalanced, crossover design. One hr after treatment, marmosets were separated from long-term pairmates and then housed alone in a novel enclosure for 7 hr. Behavior was recorded during separation and upon reunion with the partner, and urine samples for cortisol assay collected before, during, and after the intervention. Separation from partners elevated urinary cortisol concentrations over baseline for both conditions, but antalarmin treatment reduced the magnitude of the elevation. Antalarmin also lowered rates of behavioral patterns associated with arousal (alarm and "e-e" vocalizations, object manipulate/chew), but had no effect on contact calls, locomotory activity or alertness. Although most patterns of social behavior upon reunion with the partner were not affected by antalarmin, antalarmin-treated marmosets displayed more sexual behavior (mounts and copulations) upon reunion. These data indicate that antagonism of the CRH-1 receptor acts to reduce the magnitude of both endocrine and

Contract grant sponsor: National Science Foundation (NSF); Contract grant number: IBN 00-91030; Contract grant sponsor: National Institutes of Health (NIH); Contract grant number: HD 42882.
Jeffrey E. Fite, Heather Jensen, Katie Oparowski, and Michael R. Rukstalis made similar contributions to the conduct and preparation of this study.

*Correspondence to: Jeffrey A. French, Psychology Department, University of Nebraska at Omaha, Omaha, NE 68182. E-mail: jfrench@mail.unomaha.edu

Received 22 May 2006; revised 21 August 2006; revision accepted 23 August 2006

DOI 10.1002/ajp.20385
Published online 30 March 2007 in Wiley InterScience (www.interscience.wiley.com).

behavioral responses to a moderate social stressor without causing any overall reduction in alertness or general activity. This supports the hypothesis that CRH, acting through its type 1 receptor, is involved in coordinating the responses to anxiety-producing events. These results further suggest that the marmoset is a useful model for exploration of the role of CRH in mediating the behavioral and neuroendocrine responses to psychosocial stressors, particularly in the context of heterosexual social relationships. Am. J. Primatol. 69:877–889, 2007. 

**Key words: social stress; HPA axis; primate; CRH; antalarmin**

## INTRODUCTION

Adaptive responses to adverse events are coordinated and integrated at multiple levels, including cognitive and affective processing in the central nervous system and the programming of peripheral endocrine responses to these events. Although many steroids and peptides play a role in coordinating these response systems, it is becoming increasingly apparent that corticotropin-releasing hormone (CRH) plays a key role in both the central and peripheral components of the response [Charmandari et al., 2005; Schulkin et al., 2005]. Neurons expressing CRH messenger RNA (mRNA) are widely distributed throughout the central nervous system in rodents, including the central nucleus of the amygdala and the bed nucleus of the stria terminalis, important nuclei for affective responding [e.g., Makino et al., 1994; Swanson & Simmons, 1989] as well as in the hypothalamus and pituitary, consistent with the proposed dual roles of CRH in stress reactivity. The distribution of CRH receptors in primate brain has also been explored, and the ubiquitous presence of CRH receptors throughout the midbrain, forebrain, and pituitary suggests a key role for this hormone in primates, as well [Kostich et al., 2004; Millan et al., 1987]. CRH-containing neurons in the paraventricular nucleus of the hypothalamus regulate activity in the hypothalamic-pituitary-adrenal (HPA) axis, and CRH-containing neurons in the amygdala in rodents are associated with fear-related behaviors [Schulkin et al., 2005].

Receptor subtypes for CRH have been well characterized [Bale & Vale, 2004]. There are at least two receptors, CRH type 1 receptor (CRH-R1) with eight splice variants [Pisarchik & Slominski, 2001; Slominski et al., 2004] and three splice variants of the CRH type 2 receptor (CRH-R2$\alpha$, $\beta$, and $\gamma$) [Catalano et al., 2003]. The type 1 receptor has been associated with the anxiogenic effects of CRH, and the activation of the HPA axis. The synthesis, characterization, and use of the potent CRH type I receptor antagonist antalarmin [Webster et al., 1996] has provided substantial evidence supporting this association. In rats, antalarmin treatment chronically reduces levels of adrenocorticotropic hormone (ACTH) and corticosterone [Bornstein et al., 1998], reduces the ACTH response to forced swim stress and foot shock [Deak et al., 1999; Jutkiewicz et al., 2005], blocks the anxiogenic actions of CRH administration in the elevated plus maze [Zorrilla et al., 2002], blocks the expression of fear-related behavior potentiated by corticosterone treatment in the amygdala [Myers et al., 2005], and impairs the acquisition and maintenance of conditioned fear [Deak et al., 1999]. In mice, the administration of antalarmin immediately prior to an aggressive defeat in a social encounter reduces subsequent defeat-induced behavioral depression [Robinson et al., 2004]. Thus, antalarmin dampens activity in the HPA axis, and attenuates fear and anxiety states in rodent models, most likely mediated by nonhypothalamic CRH-responsive neural networks.

For human and nonhuman primates, social uncertainty is a potent activator of central and peripheral stress circuitry [Charmandari et al., 2005; Honess & Marin, 2006], and it is likely that CRH and the CRH-1 receptor play important roles in these response systems. CRH regulates HPA activity via its action on the CRH-1 receptor, since macaques pretreated with antalarmin show dose-dependent reductions in ACTH and cortisol levels following a standard CRH challenge [Broadbear et al., 2004]. Two recent studies have assessed the impact of antalarmin treatment on behavioral and endocrine responses to social stress in primates. In adult male rhesus macaques, acute administration of antalarmin reduced anxious behavior in the presence of unfamiliar male intruders, enhanced social exploration of the unfamiliar intruder, and also reduced both ACTH and cortisol responses to this social stressor. In addition, antalarmin treatment enhanced sexual motivation in males, as indexed by higher rates of masturbation [Habib et al., 2000]. Chronic treatment of rhesus macaques with antalarmin also affects behavioral, but not endocrine, reactivity to social stressors [Ayala et al., 2004]. During long-term (5-day) separation from groupmates, preadolescent male macaques treated with antalarmin exhibited higher levels of exploratory interaction with the environment than macaques treated with vehicle. However, antalarmin treatment did not alter the magnitude of either the short-term elevations in ACTH or the change in cortisol levels produced by separation from groupmates [Ayala et al., 2004]. The nature of the neuroendocrine and behavioral responses to presumed stressful events in primates appears to be modified by selective antagonism of the CRH-1 receptor system by antalarmin.

Social behavior and group structure in marmoset monkeys differ in important ways from that of macaques and other nonhuman primates commonly studied. Unlike most polygynous primates, in which significant and long-term social relationships are likely to be intrasexual [Smuts et al., 1987; Strier, 2003], the most prominent social relationship in marmosets is among socially monogamous breeding males and females. Social affiliation among heterosexual pairs has been well documented, and is characterized by selective association between partners, high levels of reciprocal grooming and food sharing, cooperative defense of territory, and aggressive exclusion of same-sex competitors [Anzenberger, 1985; Evans & Poole, 1984; French et al., 1995; Schaffner et al., 1995; Schaffner & French, 1997]. Perturbation of this relationship by separating long-term partners has been demonstrated to elicit significant behavioral and endocrine stress responses [Gerber et al., 2002; Norcross & Newman, 1999; Rukstalis & French, 2005; Shepherd & French, 1999; Smith et al., 1998], suggesting that heterosexual pairmate separation and social isolation is a useful model for assessing the role of CRH in mediating social stress reactivity.

While the overall distribution of CRH receptors in marmoset brain is not known, both the intermediate lobe and the anterior lobe of the marmoset pituitary contain receptors that bind $I^{125}$-labeled ovine CRH with high affinity, similar to cynomologous macaques and humans [Millan et al., 1987]. There is every expectation that CRH is involved in both HPA axis responses and behavioral responses to adverse, and therefore presumed stressful, stimuli in marmosets.

In the present study, we assessed the impact of acute modification of CRH activity with the CRH-1 receptor antagonist antalarmin on behavioral, social, and endocrine responses to separation from, and reunion with, significant social partners in Wied's black tufted-eared marmosets (*Callithrix kuhlii*). To the extent that CRH systems mediate these responses, we predicted that the

magnitude of responses associated with arousal and anxiety would be muted in animals receiving antalarmin, relative to vehicle-treated marmosets. Further, by monitoring behavioral indices of both arousal and general activity, we hoped to identify whether CRH antagonism with antalarmin produced selective effects on stress physiology and behavior, or had widespread, nonspecific effects on general arousal.

## METHODS

### Subjects

A total of 14 black tufted-ear marmosets (seven males and seven females) served as subjects for this experiment. Marmosets were maintained in unrelated male-female pairs; individual identities, ages, and the duration of pairing are provided in Table I. No offspring were maintained in the cages for any pair: males had been vasectomized or females ovariohysterectomized several years prior to this study. The marmosets had been paired an average of $4.3 \pm 1.0$ (mean $\pm$ standard error [SE], throughout) years, ranging from 1.5 to 9.7 yr. Even the shortest pairing duration was sufficient to produce stable pair relationships [Schaffner et al., 1995]. Animals were housed in large home enclosures ($3.0 \times 0.8 \times 2.0\,m^3$) with natural branches, nest boxes, foraging toys, and other enrichment devices. Multiple enclosures were maintained in colony rooms, and while marmoset pairs could hear and smell marmosets in neighboring enclosures, visual access to neighboring cages was minimized by suspending opaque barriers between cages. Further details on husbandry protocols can be found in Schaffner et al. [1995] and French et al. [1996].

### Antalarmin Treatment

The lipophilic nonapeptide corticotropin-releasing hormone type-1 receptor antagonist antalarmin was administered orally to marmosets at a dose of 50 mg/kg. Antalarmin for each marmoset was individually prepared by folding the appropriate dose of powdered antalarmin into a small volume (approximately 0.3 ml) of sugar-free marshmallow mix that was initially in a liquid state (to facilitate mixing) and hardened into a solid phase within 10 min. When marmosets were tested in the vehicle control condition, they received a similar volume of marshmallow only. Antalarmin-containing and vehicle treats were provided manually to each marmoset, and an observer ensured that the marmoset consumed the entire treat. One person (H.A.J.) prepared, coded, and administered the antalarmin and vehicle treats; all other human observers involved in the

**TABLE I. Demographic Characteristics of Marmoset Subjects***

| Pair | Male age | Female age | Length of pairing |
|---|---|---|---|
| Bud-Nel | 6.0 | 6.4 | 4.2 |
| Cal-Ith | 10.7 | 7.5 | 5.3 |
| Dej-Mol | 4.3 | 5.3 | 1.5 |
| Fli-Flo | 6.8 | 5.8 | 3.9 |
| Ken-Yaz | 12.6 | 10.9 | 9.7 |
| Nic-Ivy | 5.3 | 4.2 | 1.8 |
| Yen-Iza | 6.0 | 5.5 | 4.0 |
| Means | $7.4 \pm 1.2$ | $6.5 \pm 0.8$ | $4.3 \pm 1.0$ |

*All values represent years.

study were blind to treatment condition. Marmosets were provided antalarmin or vehicle at 0800 hr, immediately prior to morning feeding. Pharmacokinetic studies in rhesus macaques reveal that plasma concentrations of antalarmin are elevated within 60 min of oral treatment, and these concentrations reach peak levels 180 min following treatment and remain at these levels for at least 6 hr [Habib et al., 2000]. All marmosets were tested in both treatment conditions and half received antalarmin first and half received vehicle first. Only one member of each pair received vehicle or antalarmin for a given trial, so each heterosexual pair experienced four separation–isolation trials. Trials on each marmoset were separated by at least three weeks.

## Separation/Isolation Procedures and Behavioral Observations

After first-void urine collection and treatment with antalarmin or vehicle, marmosets were captured with a small net at 0900 hr, manually restrained, and removed from the net. They were placed in an epoxy-coated wire small housing cage (0.4 $m^3$) that was located in a room unfamiliar to the marmoset. Disposable plastic sheets were placed under each cage to facilitate urine collection. The small cages contained natural branches, a food bowl, and two water bottles. Although marmosets could hear loud vocalizations from their partners and other marmosets, animals that were separated had no visual contact with any conspecifics. Marmosets remained separated and isolated from their pairmates for 7 hr, and were returned to their home cages at 1600 hr.

Two sets of behavioral observations were designed to assess the impact of antalarmin on responses to partner separation and isolation. In the first, we determined the impact of antalarmin treatment on the behavioral responses of marmosets during the period of separation and isolation from their long-term pairmates. Marmosets exhibit a suite of behaviors during isolation from their social partner that includes increased arousal vocalizations ("e-e" and alarm chirps), increased behavioral agitation (pull/chew cage), elevated phee-calling (a putative contact vocalization), and higher rates of self-directed behavior such as scratching and self-grooming [Rukstalis et al., 2003; Shepherd & French, 1999]. These patterns were recorded, along with general measures of activity and arousal (locomotion, eating, active/alert, urination/defecation). Observers who were blind to the treatment condition recorded the occurrence or nonoccurrence of each of these patterns during 40 successive 15 sec intervals (one-zero scoring) at 0910 hr, 1300 hr, and 1550 hr.

A second set of observations quantified the impact of antalarmin treatment on the behavioral reactions of pairmates upon reunion from separation. Observers recorded all occurrences of the following patterns (definitions in Schaffner et al. [1995]): approach partner, leave partner, initiate and receive allogrooming, time in proximity, food sharing, and sexual behavior (mounts and copulations). Hinde indices [Hinde & Atkinson, 1970] were calculated to determine which partner (male or female) was responsible for the initiation and maintenance of close proximity. For each treatment cycle, pairs were observed for 20 min on the day prior to treatment/separation, the day of treatment/separation (immediately upon reunion at 1600 hr), and the day following treatment/separation. To control for potential circadian variation in sociosexual behavior, all observations were conducted at 1600 hr, which corresponded to the time of reunion on the treatment/separation day. For both sets of measures, training was instituted until interobserver reliability exceeded 0.85. A summary of the protocol and measurements are found in Figure 1.

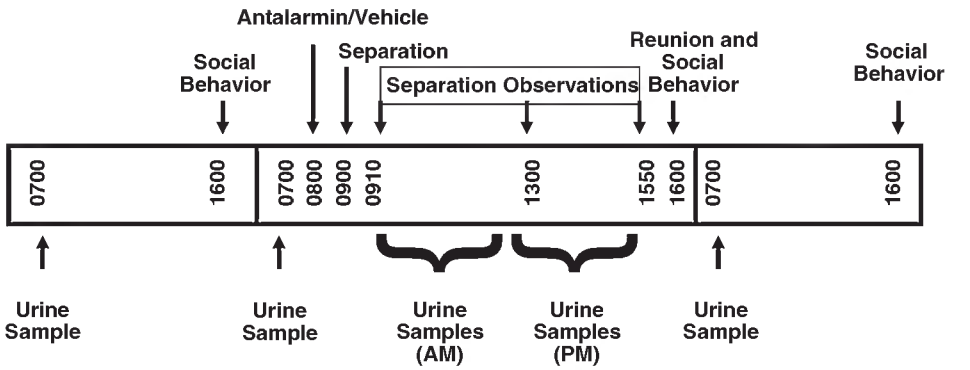


Fig. 1. Schematic of three-day experimental design. Each block represents one day, and the time of various manipulations are denoted by numbers within the blocks. All marmosets underwent two trial sequences, one after antalarmin treatment and one after treatment with vehicle. Order of treatment was counterbalanced among marmosets.

## Urine Sample Collection and Hormone Analysis

Urine samples were collected using noninvasive procedures developed in our laboratory [French et al., 1996]. Samples collected from marmosets in home cages (first-void) were collected when observers entered colony rooms at the time of light onset in the morning. Marmosets had been previously trained to urinate into a handheld aluminum pan in exchange for a mealworm or other treat. During separation from social partners, urine samples were collected by pipetting urine from the plastic sheets that had been placed underneath the small housing cages. For all samples, urine was pipetted into 1.5 ml microcentrifuge tubes, centrifuged at 2,000 *g* for 2 min to separate detritus, and then transferred to a clean tube and stored at –20°C until assay.

Urine samples were assayed for cortisol concentrations using an enzyme immunoassay previously validated for this species [Smith & French, 1997]. Briefly, microtiter plates were coated with anticortisol antibody (R4866, raised against a steroid bovine albumin [BSA] in rabbit, and provided by Dr. Bill Lasley and Coralie Munro from the University of California, Davis, Davis, CA). Samples were diluted 1:6,400 in distilled water, and assayed on plates along with standards (1,000–7.8 pg/well), and two concentrations of a quality control pool. Labeled cortisol (horseradish peroxidase) was added to wells during incubation. After separation of free from bound cortisol, substrate (hydrogen peroxide and 2,2'-azino-bis(3)-ethylbenzthiazoline-6-sulphonic acid) was added to each well, and absorbance (410 nm, reference 570 nm) was measured after approximately 1 hr incubation. A four-parameter sigmoid fit regression was used to calculate sample values. For additional details on the assay protocol and validation, see Smith and French [1997] and Smith et al. [1998]. Assay performance was reflected by intraassay coefficients of variation of 3.9% and 3.3% for a high concentration and low concentration pool. Interassay coefficients of variation for these pools were 8.7% and 13.3%, respectively. For each urine sample, variation in fluid intake and output was assessed by measuring urinary creatinine with a colorimetric assay using the Jaffé reaction (described in French et al. [1996]). Cortisol concentrations were adjusted by creatinine concentrations to produce values expressed as μg cortisol/mg creatinine. Samples that registered

creatinine values less than 0.1 mg/ml were eliminated from analysis because of the high likelihood of sample contamination by water.

## RESULTS

### Stress-Related Behavior During Separation/Isolation

Antalarmin treatment affected several measures of arousal while marmosets were separated and isolated from their long-term social partners (Table II). Vocal measures of arousal differed between treatments, in that rates of alarm chirping and emission of "e-e" vocalization during separation were significantly reduced when marmosets received antalarmin, relative to vehicle treatment. The occurrence of phee calling, in contrast, was not influenced by antalarmin treatment. The rate of "e-e" vocalizations in females was higher immediately after separation ($6.43 \pm 2.47$ intervals) than 3 or 6 hr into separation ($< 1.20$ intervals), while males had higher "e-e" rates 3 hr into separation ($6.57 \pm 2.04$ intervals) than early or late in the separation phase of the study ($< 2.8$ intervals; sex $\times$ time interaction $F(2,24) = 3.82$, $P = 0.036$).

Agitated and frantic pulling, biting, and scratching the separation cage also occurred significantly less frequently when the marmosets received antalarmin. Measures of general activity (locomotion), alertness (active-alert), and food consumption (eating) were not influenced by treatment condition. No sex differences in any measure, or interactions between sex and treatment condition, were noted for any of the activity measures during separation. Rates of self-directed behavior during separation were higher 3 and 6 hr after separation (1200 hr: $1.64 \pm 0.44$ intervals; 1500 hr: $1.11 \pm 0.51$ intervals) when compared to self-directed behavior immediately following separation at 0900 hr ($0.25 \pm 0.18$ intervals; $F(2,24) = 6.26$, $P = 0.006$).

### Sociosexual Pair Behavior Upon Reunion

Separation from social partners altered patterns of maintaining close social proximity to partners in marmosets. Figure 2 presents the Hinde indices for reunions on the day of treatment, and comparable measures on undisturbed marmoset pairs the day prior to and the day following separation. Under undisturbed conditions, males play a predominant role in maintaining proximity between pairmates, but separation produces a significant shift such that females

**TABLE II. Behavioral Responses of Marmosets During Separation From Partner***

| Behavioral measure | Antalarmin (50 mg/kg) | Vehicle | *P* |
|---|---|---|---|
| Alarm chirp | $1.14 \pm 0.79$ | $4.26 \pm 1.70$ | 0.03 |
| "e-e" vocalization | $1.09 \pm 0.55$ | $5.64 \pm 1.64$ | 0.008 |
| Phee calling | $1.33 \pm 0.76$ | $1.14 \pm 0.76$ | n.s. |
| Pull-chew cage | $0.26 \pm 0.12$ | $2.24 \pm 0.81$ | 0.029 |
| Self-directed behavior | $0.88 \pm 0.43$ | $1.12 \pm 0.37$ | n.s. |
| Eating | $0.79 \pm 0.28$ | $0.86 \pm 0.28$ | n.s. |
| Locomotion | $6.64 \pm 1.88$ | $7.78 \pm 2.16$ | n.s. |
| Urinate-defecate | $0.95 \pm 0.19$ | $1.09 \pm 0.32$ | n.s. |
| Active-alert | $38.12 \pm 0.58$ | $36.26 \pm 1.31$ | n.s. |

*Rates reflect the mean $\pm$ SEM of the number of 15 sec intervals (out of 40) during which behavior was expressed. n.s., not significant.

play a substantially greater role in maintaining proximity ($F(2,24) = 6.90$, $P = 0.004$). However, treatment with antalarmin did not alter the magnitude or direction of this effect. Antalarmin likewise had no impact on the other measures of pair social behavior, such as allogrooming, time in proximity, number of male approaches, or number of female approaches. However, sexual behavior upon reunion tended to be affected by both separation and by antalarmin treatment. Rates of sexual behavior (mounts+copulations) tended to be higher in the observation immediately following reunion relative to the days prior to and following separation (Day effect; $F(2,12) = 3.05$, $P = 0.08$). Antalarmin treatment tended to potentiate this effect (Day × Treatment interaction, $F(2,12) = 3.59$, $P = 0.06$). Post-hoc contrasts revealed that rates of sexual behavior did not differ

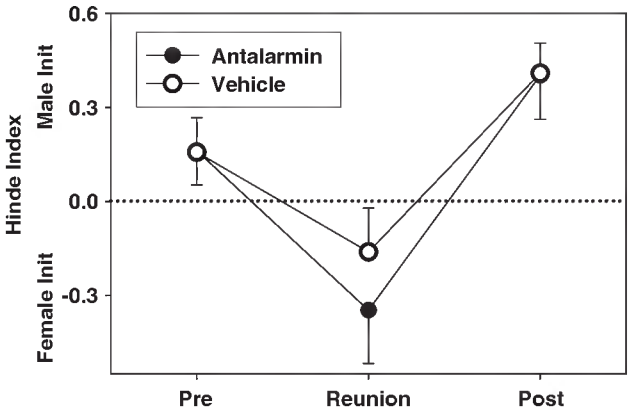


Fig. 2. Hinde indices for marmoset pairs. Positive values indicate male initiative in initiating and maintaining contact between pairmates, while negative values indicate female initiative. Observations were conducted at 1600 hr on the day prior to treatment/separation from partner (Pre), immediately upon reunion with the partner on the day of treatment/separation (Reunion), and on the day after treatment/separation (Post). Vertical lines indicate ±1 SEM.

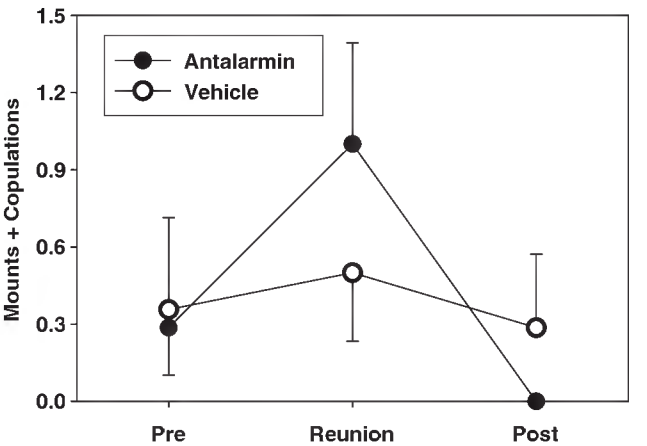


Fig. 3. Rates of sexual behavior between male-female partners (sum of mounts +copulations) in marmosets receiving antalarmin or vehicle treatment. See Fig. 2 for description of observations.

by treatment on the day prior to or the day after separation ($t(6)$'s $<0.25$, not significant [n.s.]), but antalarmin-treated marmosets exhibited two-fold higher rates of sexual behavior upon reunion than did vehicle-treated marmosets ($t(6) = 2.65$; $P = 0.038$; Fig. 3). Interestingly, this effect did not differ with regard to whether the male or female partner received antalarmin (Day × Treatment × Sex of Treated Marmoset $F(2,12) = 0.74$, n.s.).

### Cortisol Responses to Separation/Isolation

As in previous studies on marmosets, social separation and exposure to novel housing produced elevations in cortisol concentrations. In both treatment conditions, both absolute cortisol concentrations ($F(3,39) = 14.21$, $P<0.001$) and percent change in cortisol from baseline ($F(3,39) = 12.94$, $P<0.001$) were higher in both morning and afternoon samples following social separation than in samples collected prior to, or the morning after, separation. Since baseline concentrations in vehicle- and antalarmin-treated marmosets differed significantly ($t(13) = 2.42$, $P<0.03$), we assessed antalarmin effects by analyzing the impact of drug treatment on percent change from baseline. When cortisol responses to isolation were corrected for contemporary baseline concentrations, significant differences in cortisol response emerged as a consequence of treatment condition ($F(3,39) = 3.76$, $P = 0.018$; Fig. 4). Marmosets treated with antalarmin exhibited significantly dampened urinary cortisol responses to separation (approximately 200% over baseline), relative to the marked elevations in cortisol during separation when they received vehicle treatment (approximately 400% over baseline). Antalarmin treatment did not alter return to baseline the morning following separation. Cortisol concentrations in marmosets on the day after separation approached preseparation baseline concentrations, and did not differ between treatment conditions.

## DISCUSSION

CRH is a critical mediator of central and peripheral responses to distressful and threatening stimuli, and there is growing evidence that CRH plays an

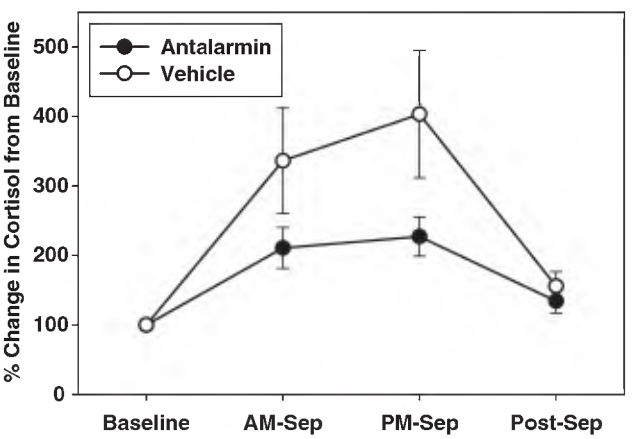


Fig. 4. Percent elevation from baseline (0700 hr) in urinary cortisol concentrations in samples collected from 0900 to 1200 hr (AM-Sep), 1201–1600 hr (PM-Sep), and first void the morning after separation (0700 hr; Post-Sep).

important role in mediating socially-induced stress in nonhuman primates. The present experiment confirms and extends this supposition in a monogamous, pair-bonded primate. Upon separation and isolation from long-term pairmates, marmosets display a set of behaviors that reflect arousal and agitation, and also exhibit significantly elevated urinary cortisol concentrations. Treatment of marmosets with the CRH-1 receptor antagonist antalarmin reduced the intensity of these responses. Vocalizations associated with stress and arousal, and agitated pulling and biting at the separation cage were emitted at lower rates in antalarmin-treated marmosets than in marmosets receiving vehicle. Further, while marmosets in both treatment conditions exhibited elevated urinary cortisol, the magnitude of the HPA response to partner separation was lower in the antalarmin treatment, relative to vehicle. Finally, rates of sexual behavior upon reunion of partners tended to be higher than on days when marmosets had not been separated, and antalarmin treatment tended to potentiate this effect.

Marmosets typically show elevated sexual behavior following a temporary separation from their heterosexual pairmates [Shepherd & French, 1999]. In the present study, blockage of CRH-1 receptor activity in marmosets via antalarmin potentiated this effect, with pairs in which one member of the partner received antalarmin showing higher levels of sexual behavior upon reunion relative to vehicle-treated marmosets. In rhesus macaques, sexual behavior is typically suppressed in animals exposed to psychosocial stressor, but macaques pretreated with antalarmin exhibit high levels of sexual activity [Habib et al., 2000]. In rodent models, CRH is considered inhibitory for male sexual behavior [Dornan & Malsbury, 1989]. Intracerebroventricular (ICV) infusion of CRH inhibits estrus in female hamsters, and astressin (a CRH-1 and CRH-2 receptor antagonist) is effective in inducing sexual receptivity in nonresponding females [Jones et al., 2002]. However, in the musk shrew, ICV treatment with CRH facilitates female sexual responsiveness [Schiml & Rissman, 2000]. The enhancement of sexual activity in both marmosets and macaques by CRH-1 receptor antagonism points to the potential importance of CRH circuitry in the regulation of primate sexual behavior. Possible hypotheses include that suppression of CRH neural circuits may lead to a relative enhancement of other neural networks that are involved in sexual behavior, perhaps networks associated with oxytocin. Of course sexual behavior may have an element of anxiety associated with it, which might be inhibitory. Suppressing the CRH-mediated fear and anxiety circuitry merely may serve to release this inhibition.

The data presented here, along with other studies, provide strong evidence that function in the HPA axis in primates can be modified with antalarmin. Under the conditions in this experiment, acute treatment of marmosets with antalarmin influenced both the cascade of endocrine events in the HPA axis that culminates in the release of cortisol and the behavioral responses to a psychosocial stressor, in that behavioral indices of stress and agitation were reduced, as was the magnitude of the cortisol response. Acute pretreatment of macaques with antalarmin blocks the CRH-induced elevation in circulating ACTH and cortisol [Broadbear et al., 2004]. Acute treatment of male macaques with antalarmin likewise impacted both behavioral and endocrine endpoints during exposure to an intense social stressor—exposure to an unfamiliar conspecific. Macaques that received antalarmin immediately prior to exposure to the stranger showed lower rates of fear and anxiety, enhanced levels of exploration, and dampened ACTH and cortisol responses to the stressor, relative to animals receiving vehicle [Habib et al., 2000]. In contrast, however, chronic treatment of macaques with antalarmin for 14 days prior to exposing them

to a different social stressor (separation from established cagemates) blocked behavioral, but not HPA, responses to the stressor [Ayala et al., 2004]. Because of differences in stressors and species among these studies, it is impossible to assess whether mode of treatment (acute vs. chronic) is responsible for the differences in the efficacy of CRH-1 receptor antagonism on behavioral and endocrine responses to stressors, but this is a critical issue for future examination.

While few studies on rodents or nonhuman primates report nonspecific effects of antalarmin on alertness or general activity, a recent report suggests that nonspecific effects may be a concern, at least in primates. Broadbear et al. [2004] treated macaques with varying doses of antalarmin and anecdotally noted behavioral responses immediately following treatment. Animals receiving the highest doses (10 mg/kg, intravenously [i.v.]) appeared highly sedated within 30 min of injection and were unresponsive to environmental stimuli for a period of approximately 60 to 90 min. The sedative-like effect of antalarmin waned with repeated injection of antalarmin. Neither of the other studies on macaques reported any sedative or tranquilizing effects of antalarmin administered orally at a dose of 20 mg/kg. We specifically included quantitative measures in the present study to assess potential nonspecific effects of antalarmin in marmosets. Relative to behavioral profiles under vehicle control treatment, marmosets that received antalarmin were equally active (measured by locomotion and eating), were equally alert (assessed by visual scanning or active engagement in behavior), and engaged in equal rates of contact calling (phee calls). The lack of effect of antalarmin on the rate of phee calls is interesting, as it implies that the rate of phee calling is not driven by anxiety, and may simply reflect the individual's assessment of being out of sight from their pairmate. Thus, it would appear that the reduction in fear/anxiety behavior we noted was attributable to the specific effects of antalarmin, presumably on midbrain and forebrain circuitry that coordinates adaptive behavioral responses to significant psychosocial stressors.

In summary, our results confirm the observation that peripherally-administered antalarmin impacts the coordination of responses to stressors. The marmoset is a highly relevant model for studying stress reactivity and anxiety in the context of a strong heterosexual attachment system, and our data are the first to demonstrate that CRH plays a role in orchestrating both the behavioral and endocrine responses to separation from a long-term pairmate. Glucocorticoids are important mediators of pair-bond formation and maintenance in other monogamous mammals (e.g., prairie voles; DeVries et al. [1995, 2002]). In Wied's black tufted-ear marmosets, cortisol levels are high during the first weeks of pair formation, and tend to decrease as the social relationship becomes established [Schaffner & French, 2004]. The results in this work showing that the CRH-1 receptor antagonist antalarmin can alter the behavioral and endocrine consequences of a temporary separation from the pairmate adds further support an important role for CRH in mediating significant aspects of social relationships.

## ACKNOWLEDGMENTS

The protocol was reviewed and approved by the University of Nebraska at Omaha Institutional Animal Care and Use Committee. The work was supported in part by grants from the National Science Foundation (NSF) (IBN 00-91030) and National Institutes of Health (NIH) (HD 42882) awarded to J.A.F. The antalarmin used in this study was synthesized by Dr. Kenner C. Rice of the

National Institutes of Health. We acknowledge the comments of two anonymous reviewers whose critiques improved this article.

## REFERENCES

Anzenberger G. 1985. How stranger encounters of common marmosets (*Callithrix jacchus*) are influenced by family members: the quality of behavior. Folia Primatol 45: 204–224.

Ayala AR, Pushkas J, Higley JD, Ronsaville D, Gold PW, Chrousos GP, Pacak K, Calis KA, Gerald M, Lindell S, Rice KC, Cizza G. 2004. Behavioral, adrenal, and sympathetic responses to long-term administration of an oral corticotropin-releasing hormone receptor antagonist in a primate stress paradigm. J Clin Endocrinol Metab 89: 5729–5737.

Bale TL, Vale WW. 2004. CRF and CRF receptors: role in stress responsivity and other behaviors. Ann Rev Pharmacol Toxicol 44:525–557.

Bornstein SR, Webster EL, Torpy DJ, Richman SJ, Mitsiades N, Igel M, Lewis DB, Rice KC, Joost HG, Tsokos M, Chrousos GP. 1998. Chronic effects of a nonpeptide corticotropin-releasing hormone Type I receptor antagonist on pituitary-adrenal function, body weight, and metabolic regulation. Endocrinol 139:1546–1555.

Broadbear JH, Winger G, Rivier JE, Rice KC, Woods JH. 2004. Corticotropin-releasing hormone antagonists, astressin B and antalarmin: differing profiles of activity in rhesus monkeys. Neuropsychopharmacology 29:1112–1121.

Catalano RD, Kyriakou T, Chen J, Easton A, Hillhouse EW. 2003. Regulation of corticotrophin-releasing hormone type 2 receptors by multiple promoters and alternative splicing: identification of multiple splice variants. Mol Endocrinol 17:395–410.

Charmandari E, Tsigos C, Chrousos G. 2005. Endocrinology of the stress response. Ann Rev Physiol 67:259–284.

Deak T, Nguyen KT, Ehrlich AL, Watkins LR, Spencer RL, Maier SF, Licinio J, Wong M-L, Chrousos GP, Webster E, Gold PW. 1999. The impact of the nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. Endocrinol 140:79–86.

DeVries AC, DeVries MB, Taymans S, Carter CS. 1995. Modulation of pair bonding in female prairie voles (*Microtus ochrogaster*) by corticosterone. Proc Natl Acad Sci USA 92:7744–7748.

DeVries AC, Guptaa T, Cardillo S, Cho M, Carter CS. 2002. Corticotropin-releasing factor induces social preferences in male prairie voles. Psychoneuroendocrinology 27: 705–714.

Dornan WA, Malsbury CW. 1989. Neuropeptides and male sexual behavior. Neurosci Biobehav Rev 13:1–15.

Evans S, Poole TB. 1984. Long-term changes and maintenance of the pair-bond in common marmosets *Callithrix jacchus jacchus*. Folia Primat 42:33–41.

French JA, Schaffner CM, Shepherd RE, Miller ME. 1995. Familiarity with intruders modulates agonism toward outgroup conspecifics in Wied's black tufted-ear marmoset (*Callithrix kuhli*). Ethology 99: 24–38.

French JA, Brewer KJ, Schaffner CM, Schalley J, Hightower-Merritt DL, Smith TE, Bell SM. 1996. Urinary steroid and gonadotropin excretion across the reproductive cycle in female Wied's black tufted-ear marmosets (*Callithrix kuhli*). Am J Primatol 40:231–245.

Gerber P, Schnell CR, Anzenberger G. 2002. Behavioral and cardiophysiological responses of common marmosets (*Callithrix jacchus*) to social and environmental changes. Primates 43:201–216.

Habib KE, Weld KP, Rice KC, Pushkas J, Champoux M, Listwak S, Webster EL, Atkinson AJ, Schulkin J, Contoreggi C, Chrousos GP, McCann SM, Suomi SJ, Higley JD, Gold PW. 2000. Oral administration of a corticotropin releasing hormone receptor antagonist significantly attenuates behavioral neuroendocrine, and autonomic responses to stress in primates. Proc Natl Acad Sci USA 97: 6079–6084.

Hinde RA, Atkinson S. 1970. Assessing the roles of social partners in maintaining mutual proximity, as exemplified by mother-infant relations in rhesus monkeys. Anim Behav 18:169–176.

Honess PE, Marin CM. 2006. Behavioural and physiological aspects of stress and aggression in nonhuman primates. Neurosci Biobehav Rev 30:390–412.

Jones JE, Pick RR, Davenport MD, Keene AC, Corp ES, Wade GN. 2002. Disinhibition of female sexual behavior by a CRH receptor antagonist in Syrian hamsters. Am J Physiol Regul Integr Comp Physiol 283:591–597.

Jutkiewicz EM, Wood SK, Woods JH, Houshyar H, Hsin L-W, Rice KC. 2005. The effects of CRF antagonists, antalarmin, CP154,526, LWH234, and R121919, in the forced swim

test and on swim-induced increases in adrenocorticotropin in rats. Psychopharmacology 180:215–223.

Kostich WA, Brzanna R, Lu NZ, Largent BL. 2004. Immunohistochemical visualization of corticotropin-releasing factor type 1 (CRF1) receptors in monkey brain. J Comp Neurol 478:111–125.

Makino S, Gold PW, Schulkin J. 1994. Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. Brain Res 640:105–112.

Millan MA, Samra AB, Wynn PC, Katt KJ, Aguilera G. 1987. Receptors and actions of corticotropin-releasing hormone in the primate pituitary gland. J Clin Endocrinol Metab 64:1036–1041.

Myers DA, Gibson M, Schulkin J, Greenwood van-Meerveld B. 2005. Corticosterone implants to the amygdale and type 1 CRH receptor regulation: effects on behavior and colonic sensitivity. Behav Brain Res 161:39–44.

Norcross JL, Newman JD. 1999. Effects of separation and novelty on distress vocalizations and cortisol in the common marmoset (*Callithrix jacchus*). Am J Primatol 47: 209–222.

Pisarchik A, Slominski AT. 2001. Alternative splicing of CRH-R1 receptors in human and mouse skin: identification of new variants and their differential expression. FASEB J 15:2754–2756.

Robinson CL, Meyerhoff JL, Saviolakis GA, Chen WK, Rice KC, Lumley LA. 2004. A CRH1 antagonist into the amygdale of mice prevents defeat-induced defensive behavior. Ann NY Acad Sci 1032:324–327.

Rukstalis MR, Fite JE, French JA. 2003. Social change affects vocal structure in a callitrichid primate (*Callithrix kuhlii*). Ethology 109:1–14.

Rukstalis MR, French JA. 2005. Vocal buffering of the stress response: exposure to conspecific vocalizations moderates urinary cortisol excretion in isolated marmosets. Horm Behav 47:1–7.

Schaffner CM, Shepherd RE, Santos CV, French JA. 1995. Development of heterosexual social relationships in Wied's black tufted-ear marmoset (*Callithrix kuhli*). Am J Primatol 36:185–200.

Schaffner CM, French JA. 1997. Group size and aggression: 'recruitment incentives' in a cooperatively breeding primate. Anim Behav 54:171–180.

Schaffner CM, French JA. 2004. Behavioral and endocrine responses in male marmosets to the establishment of multimale breeding groups: evidence for non-monopolizing facultative polyandry. Int J Primatol 25: 703–732.

Schiml PA, Rissman EF. 2000. Effects of gonadotropin-releasing hormones, corticotropin-releasing hormone, and vasopressin on female sexual behavior. Horm Behav 37: 212–220.

Schulkin J, Morgan MA, Rosen JB. 2005. A neuroendocrine mechanism for sustaining fear. Trends Neurosci 28:629–635.

Shepherd RE, French JA. 1999. Comparative analysis of sociality in lion tamarins (*Leontopithecus rosalia*) and marmosets (*Callithrix kuhlii*): responses to separation from long-term pairmates. J Comp Psychol 113:24–32.

Slominski A, Pisarchik A, Tobin DJ, Mazurkiewicz JE, Wortsman J. 2004. Differential expression of a cutaneous corticotrophin-releasing hormone system. Endocrinology 145:941–950.

Smith TE, French JA. 1997. Social and reproduction conditions modulate urinary cortisol excretion in black tufted-ear marmosets (*Callithrix kuhli*). Am J Primatol 42: 253–267.

Smith TE, McGreer-Whitworth B, French JA. 1998. Close proximity of the heterosexual partner reduces the physio logical and behavioral consequences of novel-cage housing in black tufted-ear marmosets (*Callithrix kuhli*). Horm Behav 34:211–222.

Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TE, editors. 1987. Primate societies. Chicago: University of Chicago Press. 564p.

Strier KB. 2003. Primate behavioral ecology. 2nd ed. Boston: Allyn and Bacon Press. 422p.

Swanson LW, Simmons DM. 1989. Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: a hybridization histochemical study in the rat. J Comp Neurol 285:413–435.

Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. 1996. In vivo and in vitro characterization of antalarmin, a nonpeptide corticotrophin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. Endocrinology 137:5747–5750.

Zorrilla EP, Valdez GR, Nozulak J, Koob GF, Markou A. 2002. Effects of antalarmin, a CRF type 1 receptor antagonist, on anxiety-like behavior and motor activation in the rat. Brain Res 952:188–199.