Journal of Neuroendocrinology
From Molecular to Translational Neurobiology

Journal of Neuroendocrinology **23**, 1139–1148

ORIGINAL ARTICLE

Inhibition of Maternal Behaviour by Central Infusion of Corticotrophin-Releasing Hormone in Marmoset Monkeys

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Journal of Neuroendocrinology

Stress can inhibit maternal behaviour and increase rates of child abuse in humans and other animals; however, the neuroendocrine mechanisms are not known. To determine whether corticotrophin-releasing hormone (CRH) plays a role in stress-induced disruption of maternal behaviour in primates, we characterised the effects of acute i.c.v. infusions of CRH on maternal and abusive behaviour in common marmoset monkeys (*Callithrix jacchus*). Nulliparous females were implanted with indwelling i.c.v. guide cannulae before conception. Between 18 and 58 days after the birth of her first infants, each female underwent a series of i.c.v. infusions of human CRH (0, 2, 8 and 25 μg) in 8 μl of artificial cerebrospinal fluid. In the 70 min after infusion, marmosets were tested with one of their infants, first in their home cage and, subsequently, in an unfamiliar cage in which the infant was confined in a transparent box on the cage floor. In the home cage, the highest dose of CRH significantly reduced the amount of time that mothers spent carrying their infants, as compared to vehicle alone, although it did not reliably affect aggression toward the infant or other behaviours. In the confined-infant test, the highest dose of CRH significantly reduced the amount of time that mothers spent on the cage floor, increased mothers' vocalisation rates, and tended to reduce their activity levels and time spent in proximity to their infant. Twenty-five micrograms of CRH also elicited significant elevations in plasma adrenocorticotrophic hormone and cortisol concentrations compared to vehicle. These results indicate that i.c.v.-administered CRH reduces maternal behaviour in marmoset mothers, in both familiar and unfamiliar environments, but does not increase infant abuse.

Key words: adrenocorticotrophic hormone, corticotrophin-releasing factor, cortisol, maternal care, stress.

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doi: 10.1111/j.1365-2826.2011.02153.x

Stress can inhibit maternal behaviour and increase the risk of offspring abuse in mammalian mothers. In women, for example, chronic stressors such as poverty and domestic violence lead to impaired maternal behaviour and increased abusive behaviour (1–3). Similarly, chronic psychosocial or environmental stressors, including crowding and lack of social support, elevate rates of infant abuse in nonhuman primates (4–6), and chronic stressors involving wet bedding and forced foraging (7) or limited nesting material (8) have been shown to decrease maternal behaviour and/or increase abusive behaviour in rats. Fewer studies have examined the effects of acute stress on mothering. In humans, however, the incidence of child abuse rises in families affected by natural disasters (9), whereas infant abuse may be triggered by acute social conflict or extragroup disturbances in macaque monkeys (5). Similarly, rat mothers exhibited reductions in maternal behaviour and/or increases in abusive behaviour immediately after acute restraint stress (10), during acute confinement in a novel chamber with limited bedding (11) or during acute exposure to predator odours (12).

The mechanisms by which chronic and acute stress disrupt maternal behaviour are not known. Most studies addressing this issue have focused on the glucocorticoid hormones (e.g. cortisol and corticosterone). Correlational studies in several nonhuman primate species have found that circulating or excreted cortisol concentrations are negatively associated with specific aspects of maternal behaviour (13). In the only experimental study in primates reported to date, chronic treatment with high doses of

glucocorticoids caused a modest reduction in rates of infant carrying in multiparous common marmoset (*Callithrix jacchus*) mothers (14). By contrast, among human mothers, circulating or salivary cortisol levels have been found to correlate positively with certain aspects of maternal behaviour or maternal attitudes (15–17). Finally, in rats, adrenalectomy decreased and corticosterone replacement increased maternal behaviour of postpartum females (18,19), whereas these effects were reversed in pup-sensitised virgin females (20). Thus, chronic changes in glucocorticoid levels apparently can either promote or inhibit maternal behaviour in mammals, possibly depending upon the mother's parity and species, as well as the time course of the hormonal change. The acute effects of glucocorticoids on maternal behaviour are not known.

Another likely candidate for mediating stress-induced inhibition of maternal behaviour is corticotrophin-releasing hormone (CRH). This neuropeptide regulates behavioural, hormonal, autonomic and immune responses to stress, both by acting: (i) on the anterior pituitary to stimulate the secretion of adrenocorticotrophic hormone (ACTH) and, subsequently, the adrenocortical secretion of glucocorticoids and (ii) in a number of hypothalamic and extrahypothalamic regions within the brain. In rodents and primates, i.c.v. CRH treatment has been shown to elicit many behavioural responses that are similar or identical to those exhibited in response to stressors and/or that are associated with anxiety (21,22). Conversely, a variety of specific CRH type-1 receptor antagonists have been found to elicit effects opposite to those of i.c.v. CRH treatment, and generally exert anxiolytic-like and antidepressant-like effects in rodents and primates (23,24).

Acute effects of i.c.v. CRH treatment on maternal behaviour have been investigated in sheep and rodents. Keverne and Kendrick (25) found that i.c.v. infusions of CRH tended to increase acceptance and decrease rejection of lambs by ovariectomised, oestrogen-treated ewes. By contrast, among rodents, CRH tends to inhibit components of maternal behaviour, although these effects may vary as a function of the female's reproductive state and previous experience with infants. Acute i.c.v. CRH treatment was found to inhibit pup-induced maternal behaviour in nulliparous, ovariectomised, oestrogen- and progesterone-treated rats that either did or did not have previous experience with pups (26). In rats lacking pup experience, moreover, CRH treatment significantly increased rates of infanticide (26). Acute i.c.v. CRH treatment has also been reported to reduce nursing behaviour in lactating rats (27) and to inhibit maternal aggression toward male intruders in lactating mice (28).

In the present study, we investigated the effects of acute i.c.v. CRH treatment on maternal behaviour in a nonhuman primate, the common marmoset. These small-bodied (approximately 350 g), New World monkeys live in small groups (approximately five to 16 individuals) in which the dominant female gives birth, usually to fraternal twins or triplets, at approximately 6-month intervals. Infants are weaned at about 8–10 weeks of age (29). All members of the social group, including the father and older siblings, contribute to infant care; however, mothers spend substantial amounts of time carrying their infants (approximately 30–40% of observation time during the first month postpartum and approximately 10–20% during the second month postpartum) (30,31). Such biparental and cooperative care of infants is unusual among primates and makes marmosets a particularly suitable model for human parental behaviour. Moreover, stress has been reported to increase rates of infant abuse and infanticide by marmoset parents and to markedly reduce infant survival rates (32), whereas chronic treatment with high doses of cortisol has been found to reduce mothers' rates of infant-carrying (14).

In the present study, we tested the hypothesis that acute, intracerebral elevations in CRH would inhibit maternal behaviour in marmoset mothers. Maternal behaviour in primates sometimes, but not always, shows an inverse relationship with abusive behaviour towards infants (33,34), and may be mediated by different neuroendocrine mechanisms than abusive behaviour (13); therefore, we also tested the hypothesis that acute CRH treatment would increase aggression by mothers towards their infants. Because previous studies have found that the behavioural effects of CRH treatment may differ with the degree of familiarity or stressfulness of the testing conditions (35–37), we characterised mothers' behaviour both in their familiar home cage and in an unfamiliar, presumably more anxiogenic test paradigm.

Materials and methods

Animals

We used five adult female common marmosets housed at the Wisconsin National Primate Research Center (WNPRC) at the University of Wisconsin (UW), Madison. At the outset of the experiment (implantation of i.c.v. cannulae), the animals were nulliparous, with a mean ± SEM age of 22.7 ± 2.0 months. Each female was pair-housed with an adult male indoors in an aluminium and wire mesh cage (61 × 91 × 183 cm) that permitted visual, auditory and olfactory contact between animals in different groups. The animals were fed Mazuri Hi-Fiber Callitrichid Diet (Mazuri, Richmond, IN, USA) supplemented with vitamin D and a small amount of fruit, at 12.30–13.30 h; however, food was typically available in the cages at all times. Water was available *ad lib.* Lights were on from 06.30 to 18.30 h, and room temperature and humidity were maintained at approximately 23 °C and 30–70%, respectively.

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, and were reviewed and approved by the UW-Madison Graduate School Animal Care and Use Committee. WNPRC is accredited by AAALAC as part of the UW-Madison Graduate School.

Design

Each female marmoset was implanted with an indwelling guide cannula in the third ventricle, as described below. Blood samples (0.15 ml; see below) were collected by femoral venipuncture twice/week from the time of cannula implantation until 14 days before the anticipated parturition date, and from 7 days after parturition until the end of data collection. These samples were assayed for plasma progesterone (see below) for monitoring of ovarian cycles and pregnancies. Ovulation was considered to have occurred on the day preceding a sustained rise in plasma progesterone concentrations to above 10 ng/ml (38). To ensure that the surgical procedures and anaesthesia did not affect pregnancy outcomes, marmosets were treated with cloprostenol sodium, a prostaglandin F2α analogue (0.75–1.0 μg, i.m., for up to two consecutive days, Estrumate; Schering-Plough, Pointe Claire, Quebec,

Canada), within 1 month after cannula-implantation surgery (approximately 15–30 days after the previous ovulation) to cause luteolysis and terminate the luteal phase or pregnancy (39). Each female's subsequent pregnancy was permitted to proceed to term.

Between 18 and 58 days after the birth of her first infants, each marmoset underwent a series of CRH infusions [0, 2, 8 and 25 μg of CRH in 8 μl of artificial cerebrospinal fluid (aCSF)], with each infusion followed by two behavioural tests and collection of two blood samples, as described below. The order of doses was approximately balanced across animals, and at least 3 days elapsed between successive tests on the same animal. Common marmosets lactate for approximately 65–90 days (29); therefore, we assume that all of the mothers were lactating throughout the period of testing. On completion of all testing, indwelling cannulae were surgically removed and the animals were returned to the WNPRC breeding colony.

Implantation of i.c.v. guide cannulae

We implanted marmosets with i.c.v. guide cannulae using methods modified from those described by Barnett *et al.* (40). Marmosets were anaesthetised with ketamine (15 mg/kg, i.m.) and placed in a stereotaxic apparatus. Anaesthesia was maintained with isoflurane (1–3% in oxygen; 0.6 l/min). Each animal was given dexamethasone (5 mg/kg, i.m.) and 5% dextrose (5 ml, s.c.) 14–18 h before surgery, and 5% dextrose (5 ml, s.c.), atropine (0.02–0.04 mg/kg, i.m.) and buprenorphine (0.01–0.03 mg/kg, i.m.) after anaesthesia induction. Fluid replacement was maintained throughout surgery by administration of 5% dextrose (5 ml/h, i.v.). Vital signs were monitored via pulse oximetry, and body temperature was maintained by a wrap-around body-heating apparatus thermostatically controlled at body temperature.

At the start of the procedure, 2% lidocaine was injected to the scalp ID. Presurgery X-rays of each marmoset's head were compared with X-ray ventriculograms from previous animals with comparable head size and shape, to enable accurate estimation of cannula length and coordinates for i.c.v. cannula placement. A guide cannula (22 gauge, 11–13 mm; Plastics One, Roanoke, VA, USA) was implanted into the third ventricle, and placement was verified by X-ray after infusion of radiopaque dye (20 μl infused over approximately 1 min, Omnipaque; Nycomed, Princeton, NJ, USA). After the position of the guide cannula was confirmed, the guide cannula was anchored in place using dental acrylic (Justi Products, Oxnard, CA, USA). The infusion cannula was then removed from the implanted guide cannula and replaced with a capped stylet. The acrylic was shaped to protect the indwelling cannula and provide easy access to the stylet for future infusions.

Acclimation of marmosets to i.c.v. infusion

The process of acclimating marmosets to i.c.v. infusion was undertaken 2–6 weeks after cannula implantation. Once weekly, between 07.00 and 12.00 h, each cannulated monkey was removed from its home cage and placed in a modified marmoset restraint apparatus (41). The monkey's head was transiently and gently restrained while the capped stylet was removed. After outflow of CSF from the guide cannula was observed, a sterile 28-gauge injection cannula, attached to a 25-μl Hamilton syringe, was inserted. A sterile solution of aCSF vehicle (injection volume: 8 μl) was manually infused slowly (over approximately 1 min) into the third ventricle. After infusion, the injection cannula was removed and a sterile capped stylet was re-inserted into the guide cannula. Aseptic technique was employed throughout the procedure.

CRH infusion

At 11.00 h, the marmoset was captured from her home cage and placed in a marmoset restraint tube (41) and the capped stylet was removed from the indwelling i.c.v. guide cannula. After outflow of CSF from the guide cannula was observed, a sterile infusion cannula, attached to a primed, 25-μl Hamilton syringe, was inserted into the guide cannula. Eight microliter of artificial CSF containing 0, 2, 8 or 25 μg ovine CRH (National Hormone and Peptide Program, Torrance, CA, USA) was infused into the third ventricle over 1 min, using aseptic technique. After infusion, the infusion cannula was left in place for 30 s to prevent backflow, before being replaced with a sterile stylet.

Behavioural tests

Immediately after the infusion procedures, the animal was placed in a nest-box from her home cage for 20 min, after which a blood sample (0.4 ml) was collected for subsequent analysis of ACTH and plasma cortisol concentrations. To prevent females from biting their infants, a polyethylene mesh 'hood' (diameter 8.0 cm; height 5.8–6.2 cm) (42) affixed to a plastic neck collar (inner diameter 3.2–3.6 cm; outer diameter 8.0 cm) was then placed over the marmoset's head. These hoods allowed the marmoset to see, hear and smell the infant but prevented biting (42). The female was then returned to her home cage, from which her cagemates [pairmate and infant(s)] had been removed. Opaque vinyl shower curtains had been hung in front of neighbouring cages, to prevent the female from interacting with other marmosets during testing.

After 10 min (approximately 35 min after i.c.v. infusion), one of the female's own infants was re-introduced into the home cage. The mother and infant were videotaped and their behaviour recorded on a laptop computer (see below) continuously for the next 15 min, after which time the infant was removed from the cage and the hood was removed from the mother.

Ten minutes after the end of the home-cage test, the mother and infant underwent a confined-infant test. This test was conducted in a 122 × 61 × 183 cm aluminium and wire mesh double cage in a room containing no other marmosets. A vertical, transparent Plexiglas partition divided the cage into two halves, connected only by a circular opening (15 cm diameter) at the bottom of the partition. The infant was confined in a closed, transparent Plexiglas box (15 × 15 × 18 cm) placed on the cage floor in the right half of the cage. At the outset of the test, the mother was released into the left half of the cage at a height of approximately 60–100 cm. The mother could see and hear the infant from any location in the double cage but had to descend to the cage floor and cross through the opening in the partition to approach the infant. Captive common marmosets and other callitrichids (marmosets and tamarins) typically spend relatively little time on the cage floor (43,44), and the amount of time spent on the floor has been suggested to decline under threatening conditions (43). The test continued for 10 min, during which time the mother's behaviour was recorded continuously on videotape and on a laptop computer. At the conclusion of the test, the mother was captured manually and a second blood sample (0.4 ml) was collected within 3 min.

Behavioural data were recorded on a laptop computer by trained observers using the JWATCHER event-recorder programme (45). Animals had been habituated to the observers at least 2 weeks before testing. Behaviours scored during home-cage tests and confined-infant tests are described in Table 1. A number of additional behaviours were included in our original ethogram but were observed too infrequently to permit statistical analysis for home-cage tests [groom infant, nurse infant, lick infant, huddle with infant, reject infant, attack infant, attempt to bite infant, cuff infant, refuse to carry infant, withdraw from infant, infant on female's hood, vocal threat, facial submit, autogroom, scratch self, scentmark, genital present, ear-tufts flick, lipsmack, long call and infant squawk; for descriptions, see (14,48)] or for confined-infant tests (vocal threat, ear-tufts flick, scentmark, scratch self and autogroom).

Table 1. Behaviours Scored in Home-Cage (HC) and Confined-Infant (CI) Tests.

Behavior	Measure	Definition	Test(s)
Carry infant	Duration	Infant has all four limbs on mother's body	HC
Solicit infant	Frequency	Position body directly above or against infant and/or attempt to pull infant onto body; may or may not result in infant climbing onto female's body	HC
Inspect infant	Frequency	Push face against or toward infant or container in which infant is confined, and/or use hands to investigate infant; excludes grooming	HC
Approach infant	Frequency	Move to within 10 cm of infant	HC
Proximity to infant	Duration	Any part of female's body, excluding tail, is within 10 cm of infant (HC) or of container in which infant is confined (CI)	HC, CI
Attempt to retrieve infant	Frequency	Pull on or otherwise manipulate infant container in an apparent attempt to reach the infant	CI
Bristle strut	Duration	Arching posture and/or strut locomotion and/or general piloerection	HC, CI
Manipulate hood	Frequency	Scratch, grab, pull, bite at, or otherwise manipulate hood covering head	HC
Long call (phee)	Frequency	Long, high-pitched, whistle-like contact call; most commonly performed during separation from a familiar groupmate(s) (46)	HC, CI
Chirp	Frequency	Any tsee, tsik, twitter, or chirp vocalisation; associated with high arousal; may be used as alarm/mobbing calls (46,47)	HC, CI
Ngä by infant	Frequency	Infantile squeal; associated with distress or used as a contact call (46)	CI
On cage floor	Duration	Female has all four limbs on floor of cage	CI
In left-hand cage	Duration	Female is in left half of double cage (opposite side from confined infant)	CI
In right-hand cage	Duration	Female is in right half of double cage (side containing confined infant)	CI
Locomotion	1-min scan	Female is engaged in locomotion or other whole-body movement at 1-min scan	HC, CI

Blood collection

Marmosets were restrained in a marmoset restraint device (41) while blood was collected from the femoral vein into a heparinised syringe and immediately placed on ice. These blood-sampling procedures do not elevate plasma cortisol concentrations in marmosets in our colony that have previously undergone frequent blood collection (38,49). Blood samples to be assayed for ACTH and cortisol were processed as described previously (50): blood was centrifuged at 887 ***g*** for 15 min at 4 °C, and the plasma fraction was removed. The portion for ACTH assay was centrifuged again at 4075 ***g*** for 10 min at 4 °C, and subsequently stored at −80 °C. Plasma to be assayed for cortisol was separated after the first centrifugation and stored at −20 °C. Samples to be assayed only for progesterone were centrifuged at 268 ***g***. for 10 min, and the plasma fraction was removed and stored at −20 °C.

Hormone assays

Blood samples were assayed in duplicate for plasma cortisol using an antibody-coated-tube radioimmunoassay (RIA) kit (GammaCoat; DiaSorin Corp., Stillwater, MN, USA) that had been fully validated for use with marmoset plasma, as described previously (38). The assay sensitivity at 90% binding was 0.1 ng/tube (1.0 μg/dl), and intra- and inter-assay coefficients of variation (CVs) were 5.77% and 7.91%, respectively.

Plasma ACTH concentrations were measured by an RIA that had been fully validated for marmoset plasma (50). Assay sensitivity at 90% binding was 0.5 pg/tube (6.7 pg/ml), and intra- and inter-assay CVs were 3.07% and 7.18%, respectively.

Plasma progesterone concentrations were measured in duplicate aliquots using a heterologous enzymeimmunoassay that was fully validated for marmoset plasma (38). The assay sensitivity at 90% binding was 3.6 pg/tube (2.7 ng/ml), and intra- and inter-assay CVs were 4.7% and 13.7%, respectively.

Statistical analysis

One of the five female marmosets implanted with an indwelling cannula had offspring delivered by Caesarean section as a result of difficulties with parturition; however, all five females reared healthy twins (n = 3) or triplets (n = 2), as is typical for this species.

Behavioural data were analysed nonparametrically. ACTH and cortisol concentrations were log-transformed to increase normality and homogeneity of variance, and were analysed by paired t-tests and ANOVAS. Nontransformed values are presented for ease of interpretation. Analyses were performed using SYSTAT, version 12 (Systat Software, Chicago, IL, USA), and $P < 0.05$ (two-tailed) was considered statistically significant.

Results

Behaviour in the home-cage test

Initial analyses indicated that only the 25-μg dose of CRH reliably altered behaviour and circulating hormone concentrations compared to vehicle. Therefore, all subsequent analyses compared only these two conditions.

The results of the home-cage test are presented in Table 2 and Fig. 1. When tested in her home cage with one of her infants, each female marmoset spent less time carrying the infant after i.c.v. infusion of 25 μg of CRH than after infusion of artificial CSF vehicle alone (Wilcoxon test, $z = -2.023$, $P = 0.043$; Fig. 1A). Neither the time at which mothers first retrieved their infants nor the total number of carrying bouts differed reliably between the CRH and vehicle conditions; however, one of the five females (not the female whose infants were delivered by Caesarean section) never carried

Table 2. Behaviours (Median, Range) of Female Marmosets Tested in their Home Cage with One of their Infants After i.c.v. Infusion of 8 μl of Artificial Cerebrospinal Fluid (aCSF) Vehicle Alone or 8 μl of aCSF Containing 25 μg of Corticotrophin-Releasing Hormone (CRH).

Behaviour	Vehicle	25 μg of CRH	P (Wilcoxon)
Carry infant – total duration[a]	0.10 (0.01–0.99)	0.02 (0.00–0.86)	**0.043**
Carry infant – mean duration per bout[b]	46.34 (6.42–892.54)	18.49 (0.00–386.41)	**0.043**
Proximity to infant[c]	0.57 (0.07–1.00)	0.07 (0.00–0.86)	0.066
Latency to retrieve infant[b]	7.46 (3.03–9.06)	5.20 (1.66–900.00)	0.500
Approach infant[d]	17 (1–31)	2 (1–7)	0.225
Solicit infant[d]	5.0 (0–6)	1.0 (0–2)	0.103
Locomotion[c]	0.07 (0.00–0.43)	0.20 (0.00–0.36)	0.498
Bristle strut[a]	0.50 (0.00–0.99)	0.96 (0.72–0.99)	0.138
Vocalisation (chirp + long call)[d]	0 (0–146)	4 (0–105)	1.000

[a]Proportion of time. [b]Number of seconds. [c]Proportion of instantaneous scans. [d]Total number of occurrences. Significant results ($P < 0.05$) are indicated in bold.

her infant after treatment with 25 μg of CRH, and the remaining four females all had longer mean durations of carrying bouts in the vehicle condition than in the CRH condition (Wilcoxon test, $z = -2.023$, $P = 0.043$). Consequently, mothers tended to be in proximity to their infants on more 1-min scans after infusion of vehicle than after infusion of 25 μg of CRH; however, this difference did not quite reach statistical significance (Wilcoxon test, $z = -1.841$, $P = 0.066$; Fig. 1B).

Mothers never performed aggressive behaviours (attack, attempt bite, cuff, ear-tufts flick, vocal threat) toward their infants after infusion of either 25 μg of CRH or vehicle. Moreover, mothers never withdrew from or refused to carry their infants in either condition, and only two mothers rejected their infants (i.e. attempted to force the infant off of the mother's body), one after CRH infusion and one after vehicle infusion.

Each of the five mothers performed one or more 'abnormal' behaviours in the home cage, including frothing at the mouth (n = 3), head-shaking (n = 3), crouching (n = 3) and lying down (n = 4), after infusion of 25 μg of CRH, whereas these behaviours were never seen (except for a single head-shake) after vehicle infusion. No other behaviours differed significantly between the 25 μg of CRH and vehicle conditions (Fig. 1C, D and Table 2).

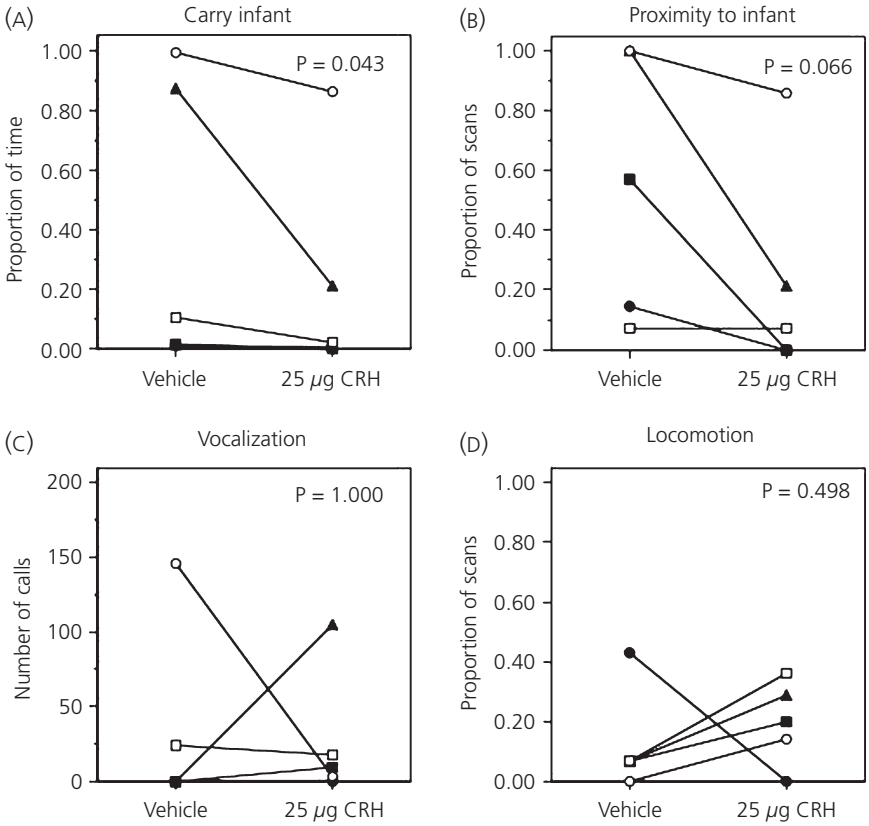

Fig. 1. Individual behavioural scores of five marmoset mothers during a 15-min test in their home cage with one of their infants, after i.c.v. infusion of 8 μl of artificial cerebrospinal fluid (aCSF) vehicle alone or 8 μl of aCSF containing 25 μg of corticotrophin-releasing hormone (CRH). (A) Proportion of time spent carrying the infant, (B) proportion of 1-min instantaneous scans in which the mother was in proximity to or in contact with the infant, (C) number of vocalisations (chirps + long calls) emitted by the mother, (D) proportion of 1-min instantaneous scans in which the mother was engaged in locomotion or other whole-body movement. Each line represents one mother.

Behaviour in the confined-infant test

Results of the confined-infant test are presented in Table 3 and Fig. 2. In this test, marmoset mothers had to descend to the floor of a double cage and cross through an opening in a transparent partition to approach their infant, which was confined in a transparent box on the floor of the right-hand cage. After i.c.v. infusion of vehicle alone, all five mothers descended to the floor of the divided cage, and three of the females crossed into the right-hand cage, where they spent most of the remaining test period. By contrast, after i.c.v. infusion of 25 μg of CRH, only one of the five mothers descended to the cage floor, and none crossed into the right-hand cage or approached the confined infant. Consequently, mothers spent significantly less time on the cage floor after CRH treatment than after vehicle treatment (Wilcoxon test, z = 2.023, P = 0.043; Fig. 2A). Furthermore, mothers showed nonsignificant tendencies to spend more time in the left-hand cage and less time in proximity to the confined infant (Fig. 2B and Table 3). Marmoset mothers performed significantly more vocalisations (chirps + long calls) after treatment with CRH than after treatment with vehicle (Wilcoxon test, z = 2.023, P = 0.043; Fig. 2C) and showed a strong tendency to engage in less locomotion after CRH treatment (Fig. 2D); however, this trend was not quite significant. Again, most of the females exhibited 'abnormal' behaviours, including frothing at the mouth (n = 2), head-shaking (n = 3) crouching (n = 1) and lying down (n = 1), after infusion of 25 μg of CRH, although none of these behaviours were observed after vehicle infusion.

Plasma ACTH

Plasma ACTH concentrations were determined in four of the five female marmosets both 20 min after infusion of vehicle or CRH (before the home-cage and confined-infant tests) and approximately 70 min after infusion (after both behavioural tests); plasma volumes from the fifth animal were insufficient for the ACTH assay. ANOVA on log-transformed ACTH concentrations indicated that ACTH levels were significantly influenced by both treatment ($F_{1,3}$ = 11.546, P = 0.043) and time since CRH or vehicle infusion ($F_{1,3}$ = 17.155, P = 0.026) but not by a treatment × time interaction ($F_{1,3}$ = 0.541, P = 0.515). ACTH levels were higher after infusion of 25 μg of CRH than after infusion of vehicle, and were higher 70 min after infusion than 20 min after infusion (Fig. 3).

Table 3. Behaviours (median, range) of Female Marmosets in the Confined-infant Test After i.c.v. Infusion of 8 μl of Artificial Cerebrospinal Fluid (aCSF) Vehicle Alone or 8 μl of aCSF Containing 25 μg of Corticotrophin-Releasing Hormone (CRH).

Behaviour	Vehicle	25 μg of CRH	P (Wilcoxon)
On cage floor[a]	0.25 (0.08–0.65)	0.00 (0.00–0.14)	**0.043**
In left-hand cage[a]	0.29 (0.04–1.00)	1.00 (1.00–1.00)	0.104
Proximity to confined infant[a]	0.17 (0.00–0.85)	0.00 (0.00–0.00)	0.109[b]
Bristle strut[a]	0.41 (0.16–0.71)	0.79 (0.06–1.00)	0.138
Locomotion[c]	0.13 (0.00–0.56)	0.00 (0.00–0.11)	0.063
Vocalisation (chirp + long call)[d]	12 (0–69)	84 (14–823)	**0.043**
Infant ngä[d]	13 (1–128)	4 (0–20)	0.138

[a]Proportion of time. [b]Behaviour was performed by only three marmosets in the vehicle condition and none in the CRH condition. [c]Proportion of instantaneous scans. [d]Total number of occurrences. Significant results (P < 0.05) are indicated in bold.

Plasma cortisol

Plasma cortisol concentrations were available for all five female marmosets before behavioural tests and from only four of the five females after behavioural tests under each treatment condition (vehicle, 25 μg of CRH). Therefore, we used paired t-tests to analyse log-transformed cortisol values. Cortisol levels did not change reliably between the two blood samples after infusion of either vehicle (t_3 = −1.486, P = 0.234) or 25 μg of CRH (t_3 = −1.869, P = 0.158) (Fig. 4). Cortisol concentrations 20 min after i.c.v. infusion (before behavioural tests) were significantly higher after treatment with 25 μg of CRH than with vehicle (t_4 = −3.122, P = 0.035). A similar trend was seen 70 min after infusion (after both behavioural tests) but did not reach statistical significance (t_2 = −3.165, P = 0.087).

Discussion

The results of the present study provide the first direct evidence that CRH can inhibit maternal behaviour in primates. When tested in their home cage, marmoset mothers showed a marked reduction in the amount of time spent carrying their infant after i.c.v. infusion of 25 μg of CRH compared to infusion of vehicle alone, reflecting a reduction in the average duration of carrying bouts. This appeared to be a relatively specific effect; CRH did not elicit significant changes in locomotion or in behaviours likely to be associated with anxiety, such as vocalisation, scratching or bristle strutting. Moreover, CRH did not increase abusive behaviour by mothers: marmosets were never observed to perform aggression towards their infants after either vehicle or CRH infusion.

We also found evidence that CRH reduced females' infant-directed behaviour in the confined-infant test, in which mothers were housed in a novel cage in an unfamiliar room, and could approach their confined (but visible and audible) infant only by descending to the cage floor and crossing through a hole in a partition. After CRH treatment, mothers spent significantly less time on the cage floor and, consequently, were less likely to spend time in proximity to their infant than after vehicle treatment; however, this latter trend did not reach statistical significance. CRH treatment also caused a significant increase in vocalisation rates and a near-significant decrease in rates of locomotion, suggesting that CRH elicited anxiety and behavioural inhibition in marmosets in this unfamiliar, presumably anxiogenic test paradigm. It is unclear whether the reduction in mothers' infant-directed behaviours resulted solely from this increase in anxiety or behavioural inhibition, or whether CRH also directly inhibited maternal motivation in this test.

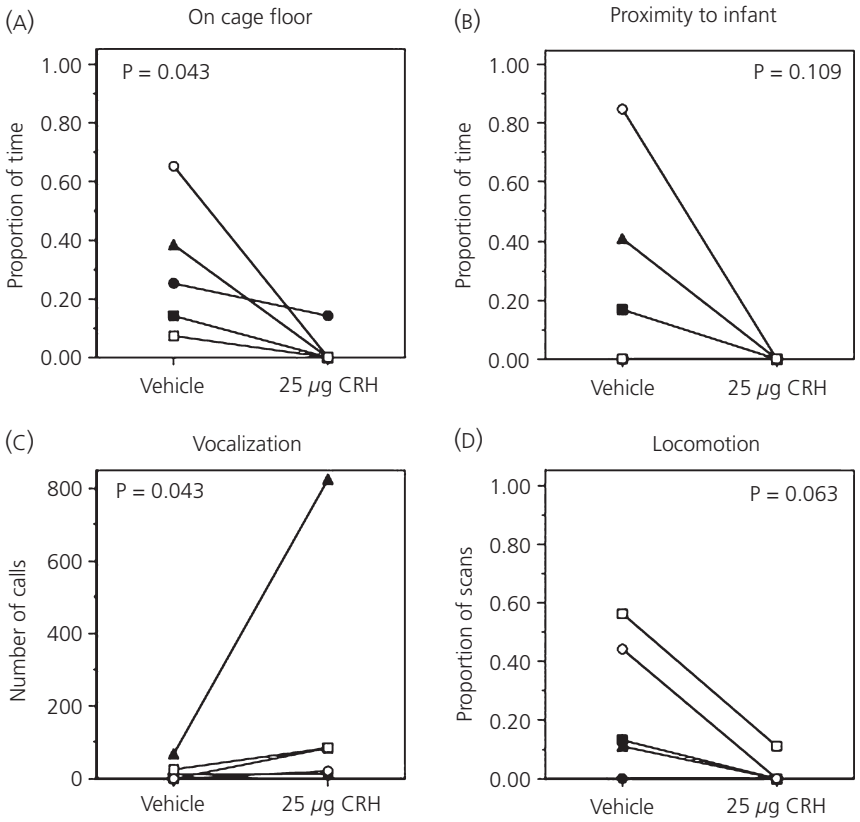

Fig. 2. Individual behavioural scores of five marmoset mothers during a 10-min confined-infant test, after i.c.v. infusion of 8 μl artificial cerebrospinal fluid (aCSF) vehicle alone or 8 μl of aCSF containing 25 μg of corticotrophin-releasing hormone (CRH). (A) Proportion of time spent on the floor of the cage, (B) proportion of time spent in proximity to the container in which the infant was confined, (C) number of vocalisations (chirps + long calls) emitted by the mother, (D) proportion of 1-min instantaneous scans in which the mother was engaged in locomotion or other whole-body movement. Each line represents one mother.

Our results are consistent with previous findings suggesting that the behavioural effects of CRH may differ between familiar and unfamiliar, or more and less anxiogenic, test environments (35). For example, Strome *et al.* (37) found that i.c.v. CRH infusion tended to increase anxiety-like behaviours and to decrease externally oriented behaviours in rhesus monkeys, both when the animals were housed with their familiar social group and when they were housed alone; however, CRH increased depressive-like behaviours only in the social-housing condition. In addition, Kalin *et al.* (36) found that i.c.v. CRH treatment tended to increase behavioural arousal in rhesus monkeys restrained in chairs but to increase depressive-like behaviour in the same animals when they were tested in their home cage. As stated by Broadbear (22), 'As a general rule, administration of CRH increases the level of arousal in unstressed animals, and enhances the stress-related behaviour under conditions of pre-existing or co-administered stress' (p. 2320). Thus, our findings in marmosets suggest that CRH can inhibit maternal behaviour both under nonstressful conditions, possibly through direct actions on the neural circuitry underlying maternal behaviour, and under stressful conditions, perhaps by activating competing emotional/behavioural states such as anxiety or behavioural inhibition. Importantly, however, the effects of CRH on marmoset mothers' behaviour in the home-cage test and the confined-infant test in the present study cannot be compared directly, for several reasons. First, mothers were able to interact physically with their infants in the home-cage test but not in the confined-infant test. Second, it is possible that the order of testing or time since i.c.v. infusion differentially influenced the results of the two tests.

In the present study, as in previous studies (21), CRH infusion into the cerebral ventricles elevated circulating concentrations of ACTH and cortisol. Consequently, we were unable to determine whether CRH altered maternal behaviour via direct actions within the brain, or indirectly via actions of cortisol or other hormones of the hypothalamic-pituitary-adrenal (HPA) axis. Circumstantial evidence suggests that central effects of CRH are more likely to account for the observed behavioural changes. First, behavioural differences were observed within 35–50 min after infusion of vehicle and CRH, whereas classic, genomically-mediated effects of cortisol and other steroid hormones typically require one or more hours to develop (51,52). It remains possible, however, that cortisol may have affected the marmosets' behaviour through more rapidly acting, nongenomic mechanisms, presumably mediated by membrane receptors (51,52). Second, numerous studies have shown that manipulations of the CRH systems of the brain have pronounced effects on behavioural responses to stress that are independent of changes in circulating ACTH or glucocorticoid concentrations (35).

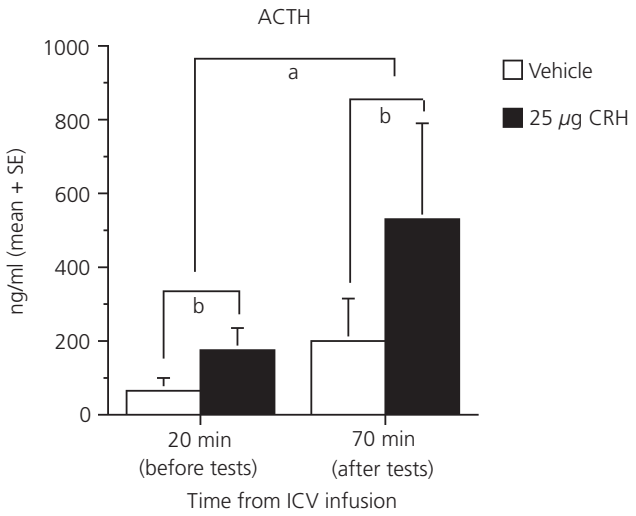

Fig. 3. Plasma adrenocorticotrophic hormone (ACTH) concentrations in four female marmosets approximately 20 min (before behavioural tests) and 70 min (after behavioural tests) after i.c.v. infusion of 8 μl of artificial cerebrospinal fluid (aCSF) vehicle alone or 8 μl of aCSF containing 25 μg of corticotrophin-releasing hormone (CRH). a, First (20 min post-infusion) versus second (70 min post-infusion) ACTH value: P = 0.026. b, Vehicle versus 25 μg of CRH: P = 0.043.

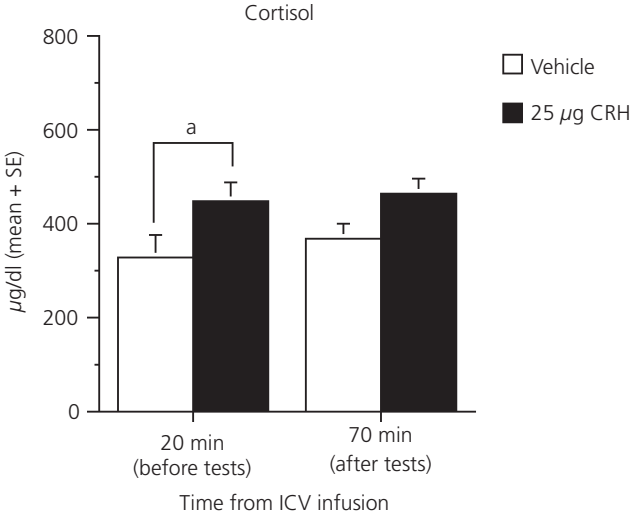

Fig. 4. Plasma cortisol concentrations in four female marmosets approximately 20 min (before behavioural tests) and 70 min (after behavioural tests) after i.c.v. infusion of 8 μl of artificial cerebrospinal fluid (aCSF) vehicle alone or 8 μl of aCSF containing 25 μg of corticotrophin-releasing hormone (CRH). a, 20 min post-infusion, vehicle versus 25 μg of CRH: P = 0.035.

Finally, studies using conditional mutant mice, in which either CRH type-1 receptor expression was blocked or CRH expression was increased selectively in specific, extrahypothalamic brain regions, have provided strong evidence that CRH can modulate anxiety-related behaviour independently of HPA activation (53,54).

In addition to elevating plasma ACTH and cortisol concentrations and reducing infant-directed behaviours, CRH treatment in the present study stimulated the display of several 'abnormal behaviours' in female marmosets, including frothing at the mouth, head-shaking, crouching and lying down. Similar effects of CRH have been reported in other species. In rats, CRH infusion into the nucleus accumbens shell or the lateral ventricles stimulated unfocused oral movements (e.g. nondirected chewing or licking) as well as tremors of the jaw or forepaws (55,56), and CRH has been implicated in mediating such behaviours as salivation and chewing in response to opiate withdrawal (57). In rhesus macaques, i.c.v. CRH treatment increased the frequency of several body postures, including huddling, self-clasping and slouching against the wall, which were considered to be depressive-like or anxiety-like (37). The significance of the abnormal behaviours that we observed in marmosets is not clear. Head-shaking might be related to the wet-dog shake, which has been described as an anxiety-related behaviour in marmosets (58). The remaining behaviours, however, have not been associated with stress, anxiety-like or depressive-like states, or other affective conditions in marmosets. Notably, the CRH dose that reliably elicited behavioural, ACTH and cortisol changes in female marmosets was higher, when corrected for body mass, than those typically used in previous studies of rodents and monkeys (21,22).

To our knowledge, only four previous studies have examined the effects of exogenous CRH treatment on maternal behaviour. Keverne and Kendrick (25) investigated the impact of acute i.c.v. CRH treatment on maternal behaviour in nulliparous and multiparous ewes that had been ovariectomised and treated with oestrogen, both with and without vaginocervical stimulation. Although the data were difficult to interpret, CRH tended to increase acceptance and decrease rejection of lambs in both multiparous and nulliparous ewes. By contrast to these findings in sheep, studies of rodents have consistently found that CRH inhibits components of maternal behaviour. Pedersen *et al.* (26) reported that acute i.c.v. CRH treatment significantly delayed the onset of pup-induced maternal behaviour and increased rates of infanticide in nulliparous, ovariectomised, oestrogen- and progesterone-treated female rats, and delayed the re-emergence of maternal behaviour in these females after their initial experience with pups. Notably, in both rats and sheep, the specific effects of CRH depended, to some extent, on the females' parity or previous experience with infants (25,26).

In another study of rats, Almeida *et al.* (27) found that acute i.c.v. CRH infusion tended to inhibit maternal behaviour, while increasing arousal and general behavioural activation, in lactating dams. Finally, Gammie *et al.* (28) found that acute i.c.v. CRH treatment significantly inhibited maternal aggression toward a male intruder in lactating mice but did not appear to alter other components of maternal behaviour. Importantly, these other components were not analysed quantitatively; therefore, subtle effects of CRH on the dams' behaviour toward pups might have been missed. Nonetheless, in conjunction with the results obtained in the present study, these previous findings in rodents suggest that acute elevations of i.c.v. CRH levels may cause mild or moderate impairments in the *maintenance* of maternal behaviour, but do not increase infant abuse, in lactating females. By contrast, the *onset* of maternal behaviour in nonlactating females, especially those lacking previous experience with infants, might be more sensitive to disruption

by CRH, possibly through potentiation of neophobic responses to novel stimuli from infants (59).

In addition to these experimental studies, a substantial body of indirect evidence implicates a possible role for CRH, particularly chronic elevations of CRH, in inhibiting maternal behaviour. Several conditions characterised by CRH hypersecretion, including numerous neuropsychiatric disorders, stress and anxiety, are associated with impairments in maternal behaviour (1–3,60). Further indirect evidence for an inhibitory effect of CRH on maternal behaviour comes from studies of early childhood trauma. In humans, nonhuman primates and rodents, traumatic events early in life, such as maternal deprivation or abuse, lead to chronic dysregulation of the CRH systems and HPA axis in adulthood (22,61,62). Importantly, individuals who experience trauma early in life frequently exhibit impaired parental behaviour as adults, which may be associated with depressive symptoms or anxiety (61–64). In rhesus monkeys, abusive mothers that were themselves abused as infants had higher CSF CRH concentrations than non-abusive mothers that were not abused as infants; moreover, CSF CRH levels were positively correlated with the females' overall frequencies of aggressive behaviour (65).

In summary, correlational findings from humans and nonhuman primates, as well as direct experimental evidence from rodents, suggest that both acute and chronic hyperactivity of the CRH systems may mediate stress- or anxiety-induced deficits in maternal behaviour. The present findings provide the first direct evidence for such an effect in primates, and indicate that acute intracerebral CRH elevations can impair maternal behaviour under both baseline and anxiogenic conditions. Additional studies will be needed to identify the mechanisms and sites of these acute effects and to determine whether chronic elevations of CRH maintain disruption of maternal behaviour in primates.

Acknowledgements

This research was supported by NIH grant MH075973 and was conducted at a facility, the Wisconsin National Primate Research Center at the University of Wisconsin, Madison (WNPRC), that was constructed with support from NIH Research Facilities Improvement Program grants RR15459-01 and RR020141-01. We thank F. H. Wegner, D. J. Wittwer and D. E. Green in the WNPRC Assay Services laboratories for assistance with hormone assays, and the WNRPC Veterinary Services and Animal Care staff for assistance with animals. We further thank three anonymous reviewers for helpful comments on an earlier draft of the manuscript. Preparation of the manuscript was facilitated by the staff and resources of the Lawrence Jacobsen Library at WNPRC.

Received 19 November 2010,
revised 11 April 2011,
accepted 3 May 2011

References

1 Tolan P, Gorman-Smith D, Henry D. Family violence. *Annu Rev Psychol* 2006; **57**: 557–583.
2 Cohen LR, Hien DA, Batchelder S. The impact of cumulative maternal trauma and diagnosis on parenting behavior. *Child Maltreat* 2008; **13**: 27–38.
3 Taylor CA, Guterman NB, Lee SJ, Rathouz PJ. Intimate partner violence, maternal stress, nativity, and risk for maternal maltreatment of young children. *Am J Public Health* 2009; **99**: 175–183.
4 Reite M, Caine NG. *Child Abuse: The Nonhuman Primate Data*. New York, NY: Alan R. Liss, 1983.
5 Maestripieri D, Carroll KA. Behavioral and environmental correlates of infant abuse in group-living pigtail macaques. *Inf Behav Devel* 1998; **21**: 603–612.
6 Maestripieri D, Carroll KA. Child abuse and neglect: usefulness of the animal data. *Psychol Bull* 1998; **123**: 211–223.
7 Léonhardt M, Matthews SG, Meaney MJ, Walker C-D. Psychological stressors as a model of maternal adversity: diurnal modulation of corticosterone responses and changes in maternal behavior. *Horm Behav* 2007; **51**: 77–88.
8 Ivy AS, Brunson KL, Sandman C, Baram TZ. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. *Neuroscience* 2008; **154**: 1132–1142.
9 Keenan HT, Marshall SW, Nocera MA, Runyan DK. Increased incidence of inflicted traumatic brain injury in children after a natural disaster. *Am J Prev Med* 2004; **26**: 189–193.
10 Yamada K, Santo-Yamada Y, Wada K. Restraint stress impaired maternal behavior in female mice lacking the neuromedin B receptor (NMB-R) gene. *Neurosci Lett* 2002; **330**: 163–166.
11 Roth TL, Sullivan RM. Memory of early maltreatment: neonatal behavioral and neural correlates of maternal maltreatment within the context of classical conditioning. *Biol Psychiatry* 2005; **57**: 823–831.
12 Sukikara MH, Mota-Ortiz SR, Baldo MV, Felicio LF, Canteras NS. The periaqueductal gray and its potential role in maternal behavior inhibition in response to predatory threats. *Behav Brain Res* 2010; **209**: 226–233.
13 Saltzman W, Maestripieri D. The neuroendocrinology of primate maternal behavior. *Prog Neuropsychopharmacol Biol Psychiatry*, in press.
14 Saltzman W, Abbott DH. Effects of elevated circulating cortisol concentrations on maternal behavior in common marmoset monkeys (*Callithrix jacchus*). *Psychoneuroendocrinology* 2009; **34**: 1222–1234.
15 Fleming AS, Steiner M, Anderson V. Hormonal and attitudinal correlates of maternal behaviour during the early postpartum period in first-time mothers. *J Reprod Infant Psychol* 1987; **5**: 193–205.
16 Fleming AS, Steiner M, Corter C. Cortisol, hedonics, and maternal responsiveness in human mothers. *Horm Behav* 1997; **32**: 85–98.
17 Krpan KM, Coombs R, Zinga D, Steiner M, Fleming AS. Experiential and hormonal correlates of maternal behavior in teen and adult mothers. *Horm Behav* 2005; **47**: 112–122.
18 Rees SL, Panesar S, Steiner M, Fleming AS. The effects of adrenalectomy and corticosterone replacement on maternal behavior in the postpartum rat. *Horm Behav* 2004; **46**: 411–419.
19 Graham MD, Rees SL, Steiner M, Fleming AS. The effects of adrenalectomy and corticosterone replacement on maternal memory in postpartum rats. *Horm Behav* 2006; **49**: 353–361.
20 Rees SL, Panesar S, Steiner M, Fleming AS. The effects of adrenalectomy and corticosterone replacement on induction of maternal behavior in the virgin female rat. *Horm Behav* 2006; **49**: 337–345.
21 Dunn AJ, Berridge CW. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Res Rev* 1990; **15**: 71–100.
22 Broadbear JH. Corticotropin-releasing hormone in nonhuman primates. *Front Biosci* 2006; **11**: 2303–2335.
23 Holmes A, Heilig M, Rupniak NMJ, Steckler T, Griebel G. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends Pharmacol Sci* 2003; **24**: 580–588.
24 Rotzinger S, Lovejoy DA, Tan LA. Behavioral effects of neuropeptides in rodent models of depression and anxiety. *Peptides* 2010; **31**: 736–756.

25 Keverne EB, Kendrick KM. Morphine and corticotrophin-releasing factor potentiate maternal acceptance in multiparous ewes after vaginocervical stimulation. *Brain Res* 1991; **540**: 55–62.

26 Pedersen CA, Caldwell JD, McGuire M, Evans DL. Corticotropin-releasing hormone inhibits maternal behavior and induces pup-killing. *Life Sci* 1991; **48**: 1537–1546.

27 Almeida OFX, Yassouridis A, Forgas-Moya I. Reduced availability of milk after central injections of corticotropin-releasing hormone in lactating rats. *Neuroendocrinology* 1994; **59**: 72–77.

28 Gammie SC, Negron A, Newman SM, Rhodes JS. Corticotropin-releasing factor inhibits maternal aggression in mice. *Behav Neurosci* 2004; **118**: 805–814.

29 Tardif SD, Smucny DA, Abbott DH, Mansfield K, Schultz-Darken N, Yamamoto ME. Reproduction in captive common marmosets (*Callithrix jacchus*). *Comp Med* 2003; **53**: 364–368.

30 Tardif SD, Carson RL, Gangaware BL. Comparison of infant care in family groups of the common marmoset (*Callithrix jacchus*) and the cotton-top tamarin (*Saguinus oedipus*). *Am J Primatol* 1986; **11**: 103–110.

31 Ximenes MFFM, Sousa MBC. Family composition and the characteristics of parental care during the nursing phase of captive common marmosets (*Callithrix jacchus*). *Primates* 1996; **37**: 175–183.

32 Johnson EO, Kamilaris TC, Carter S, Gold PW, Chrousos GP. 'Environmental stress' and reproductive success in the common marmoset (*Callithrix jacchus jacchus*). *Am J Primatol* 1991; **25**: 191–201.

33 Maestripieri D. Parenting styles of abusive mothers in group-living rhesus macaques. *Anim Behav* 1998; **55**: 1–11.

34 Maestripieri D, Megna NL. Hormones and behavior in rhesus macaque abusive and nonabusive mothers. 2. Mother-infant interactions. *Physiol Behav* 2000; **71**: 43–49.

35 Heinrichs SC, Menzaghi F, Pich EM, Britton KT, Koob GF. The role of CRF in behavioural aspects of stress. *Ann NY Acad Sci* 1995; **771**: 92–104.

36 Kalin NH, Shelton SE, Kraemer GW, McKinney WT. Corticotropin-releasing factor administered intraventricularly to rhesus monkeys. *Peptides* 1983; **4**: 217–220.

37 Strome EM, Whelor GHT, Higley JD, Loriaux DL, Suomi SJ, Doudet DJ. Intracerebroventricular corticotropin-releasing factor increases limbic glucose metaoblism and has social context-dependent behavioral effects in nonhuman primates. *Proc Natl Acad Sci USA* 2002; **99**: 15749–15754.

38 Saltzman W, Schultz-Darken NJ, Scheffler G, Wegner FH, Abbott DH. Social and reproductive influences on plasma cortisol in female marmoset monkeys. *Physiol Behav* 1994; **56**: 801–810.

39 Summers PM, Wennink CJ, Hodges JK. Cloprostenol-induced luteolysis in the marmoset monkey (*Callithrix jacchus*). *J Reprod Fertil* 1985; **73**: 133–138.

40 Barnett DK, Bunnell TM, Millar RP, Abbott DH. Gonadotropin-releasing hormone II stimulates female sexual behavior in marmoset monkeys. *Endocrinology* 2006; **147**: 615–623.

41 Hearn JP. Restraining device for small monkeys. *Lab Anim* 1977; **11**: 261–262.

42 Saltzman W, Abbott DH. Diminished maternal responsiveness during pregnancy in multiparous female common marmosets. *Horm Behav* 2005; **47**: 151–163.

43 McKenzie SM, Chamove AS, Feistner ATC. Floor-coverings and hanging screens alter arboreal monkey behavior. *Zoo Biol* 1986; **5**: 339–348.

44 Buchanan-Smith HM, Shand C, Morris K. Cage use and feeding height preferences of captive common marmosets (*Callithrix j. jacchus*) in two-tier cages. *J Appl Anim Welf Sci* 2002; **5**: 139–149.

45 Blumstein DT, Daniel JC. *Quanitifying Behavior the JWatcher Way*. Sunderland, MA: Sinauer, 2007.

46 Epple G. Comparative studies on vocalization in marmoset monkeys (Hapalidae). *Folia Primatol* 1968; **8**: 1–40.

47 Cross N, Rogers LJ. Mobbing vocalizations as a coping response in the common marmoset. *Horm Behav* 2006; **49**: 237–245.

48 Saltzman W, Severin JM, Schultz-Darken NJ, Abbott DH. Behavioral and social correlates of escape from suppression of ovulation in female common marmosets housed with the natal family. *Am J Primatol* 1997; **41**: 1–21.

49 Saltzman W, Schultz-Darken NJ, Wegner FH, Wittwer DJ, Abott DH. Suppression of cortisol levels in subordinate female marmosets: reproductive and social contributions. *Horm Behav* 1998; **33**: 58–74.

50 Saltzman W, Prudom SL, Schultz-Darken NJ, Wittwer DJ, Abbott DH. Social suppression of cortisol in female marmoset monkeys: role of circulating ACTH levels and glucocorticoid negative feedback. *Psychoneuroendocrinology* 2004; **29**: 141–161.

51 Schumacher M. Rapid membrane effects of steroid hormones: an emerging concept in neuroendocrinology. *Trends Neurosci* 1990; **13**: 359–362.

52 Rose JD. Corticosteroid actions from neuronal membrane to behavior: neurophysiological mechanisms underlying rapid behavioral effects of corticosterone. *Biochem Cell Biol* 2000; **78**: 307–315.

53 Müller MB, Zimmermann S, Sillaber I, Hagemeyer TP, Deussing JM, Timpl P, Kormann MSD, Droste SK, Kühn R, Reul JMHM, Holsboer F, Wurst W. Limbic corticotropin-releasing hormone receptor 1 mediates anxiety-related behavior and hormonal adaptation to stress. *Nat Neurosci* 2003; **6**: 1100–1107.

54 Lu A, Steiner MA, Whittle N, Vogl AM, Walser SM, Ableitner M, Refojo D, Ekker M, Rubenstein JL, Stalla GK, Singewald N, Holsboer F, Wotjak CT, Wurst W, Deussing JM. Conditional mouse mutants highlight mechanisms of corticotropin-releasing hormone effects on stress-coping behavior. *Mol Psychiatry* 2008; **13**: 1028–1042.

55 Holahan MR, Kalin NH, Kelley AE. Microinfusion of corticotropin-releasing factor into the nucleus accumbens shell results in increased behavioral arousal and oral motor activity. *Psychopharmacology* 1997; **130**: 189–196.

56 Jones DNC, Kortekaas R, Slade PD, Middlemiss DN, Hagan JJ. The behavioural effects of corticotropin-releasing factor-related peptides in rats. *Psychopharmacology* 1998; **138**: 189–196.

57 Iredale PA, Alvaro JD, Lee Y, Terwilliger R, Chen YL, Duman RS. Role of corticotropin-releasing facotr receptor-1 in opiate withdrawal. *J Neurochem* 2000; **74**: 199–208.

58 Cilia J, Piper DC. Marmoset conspecific confrontation: an ethologically-based model of anxiety. *Pharmacol Biochem Behav* 1997; **58**: 85–91.

59 Pryce CR. A comparative systems model of the regulation of maternal motivation in mammals. *Anim Behav* 1992; **43**: 417–441.

60 Oyserman D, Mowbray CT, Meares PA, Firminger KB. Parenting among mothers with a serious mental illness. *Am J Orthopsychiatry* 2000; **70**: 296–315.

61 Champagne F, Meaney MJ. Like mother, like daughter: evidence for non-genomic transmission of parental behavior and stress responsivity. *Prog Brain Res* 2001; **133**: 287–302.

62 Claes SJ. Corticotropin-releasing hormone (CRH) in psychiatry: from stress to psychopathology. *Ann Med* 2004; **36**: 50–61.

63 Fleming AS, Kraemer GW, Gonzalez A, Lovic V, Rees S, Melo A. Mothering begets mothering: the transmission of behavior and its neurobiology across generations. *Pharmacol Biochem Behav* 2002; **73**: 61–75.

64 Suomi SJ, Ripp C. A history of motherless mother monkey mothering at the University of Wisconsin Primate Laboratory. In: Reite M, Caine NG, eds. *Child Abuse: The Nonhuman Primate Data*. New York, NY: Alan R. Liss, 1983: 49–78.

65 Maestripieri D, Lindell SG, Ayala A, Gold PW, Higley JD. Neurobiological characteristics of rhesus macaque abusive mothers and their relation to social and maternal behavior. *Neurosci Biobehav Rev* 2005; **29**: 51–57.