Specific Neuroendocrine Mechanisms Not Involving Generalized Stress Mediate Social Regulation of Female Reproduction in Cooperatively Breeding Marmoset Monkeys[a]

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Competition within a social group can have dramatic consequences for an individual's fertility and fecundity. Social competition is certainly one of the major environmental selection pressures determining individual reproductive success.[1] Recently, increasing numbers of studies employing genetic criteria have supported the principle that reproductive benefits accrue to socially dominant individuals.[2,3] At the proximate level, dominant females have traditionally been thought to gain reproductive advantages over subordinate females as a result of (1) harassment-induced stress inhibiting ovulation in subordinate females (e.g., cynomolgus monkeys, *Macaca fascicularis*),[4] (2) harassment-related pregnancy loss or infant loss suffered by subordinates (e.g., yellow baboons, *Papio cynocephalus*),[5,6] or (3) exclusion of subordinate females from resources crucial for successful reproduction, such as food (e.g., red deer, *Cervus elephas*)[7] or 'helpers' to raise offspring (e.g., saddle back tamarins, *Saguinus fuscicollis*).[8] Such dominance-driven harassment or exclusion exploits the generalized inhibitory reproductive responses that most vertebrate species show to chronic physiological stress, whether it is derived psychologically (i.e., harassment) or environmen-

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tally (i.e., food deprivation).[1] In addition to these three behavioral tactics exploiting the basic reproductive consequence of physiological stress, a fourth possible mechanism of rank-related reproductive suppression has been suggested: specialized neuroendocrine and behavioral responses by females to subordinate status may directly result in inhibition of sexual behavior (e.g., common marmoset, *Callithrix jacchus*),[9] ovulation (e.g., naked mole-rat, *Heterocephalus glaber*),[10] and implantation (e.g., white-footed mouse, *Peromyscus leucopus*)[11] without the engagement of generalized stress as a physiological mediator of reproductive inhibition.[12] Such specialized social mechanisms regulating reproductive success may well be the products of kin selection and therefore would be expected to be prevalent in highly cohesive societies with an extreme degree of female reproductive suppression and a high likelihood of genetic relatedness within groups (e.g., singular cooperatively breeding species).[13-15]

This paper is particularly concerned with the physiological, behavioral, and sensory mechanisms mediating such social contraception[16] and the specialized reproductive adaptations displayed by certain female mammals encountering inappropriate or suboptimal social environments in which to successfully rear offspring. We previously conceptualized inhibitory neuroendocrine mechanisms in cooperatively breeding species as "a controlled amount of . . . social stress" mediating anovulation in subordinate females (e.g., common marmosets and naked mole-rats).[17-19] However, specialized neuroendocrine responses inhibiting reproduction in subordinate females are manifest in a different fashion from those responses mediating stress-induced reproductive suppression. As exemplified in TABLE 1, studies of yellow and gelada baboons (*Theropithecus gelada*) exhibited findings consistent with the hypothesis that harassment-induced stress mediated reproductive suppression in subordinate females in established groups, whereas studies of cooperatively breeding common marmosets did not. Overt harassment of female subordinates in the former two species was associated with elevated circulating or urinary cortisol concentrations, and both were particularly pronounced during the subordinates' follicular phase of the ovarian cycle, perhaps reflecting concerted attempts by dominant females to disrupt or inhibit impending ovulation in subordinates[12,22] (TABLE 1). Decreased frequency of ovulation in subordinate females, as compared to dominant females, accompanied these behavioral and physiological events. In common marmosets, on the other hand, overt harassment of subordinate females and elevations in their circulating cortisol concentrations were not found in established groups (TABLE 1, FIGS. 1 and 4). Nevertheless, subordinate female marmosets exhibited far more extreme forms of reproductive inhibition than did subordinate female baboons, typically manifesting anovulation (FIG. 2) and absence of births. Clearly, the more pervasive inhibition of reproduction operative among subordinate female marmosets was not dependent on behavioral harassment and heightened adrenocortical activity. Interestingly, when subordinate female marmosets in wild and captive groups did occasionally give birth, the likelihood of infant survival was just as poor for subordinate female marmosets as for subordinate female baboons and was similarly linked to brutal intervention by dominant females.[24,25]

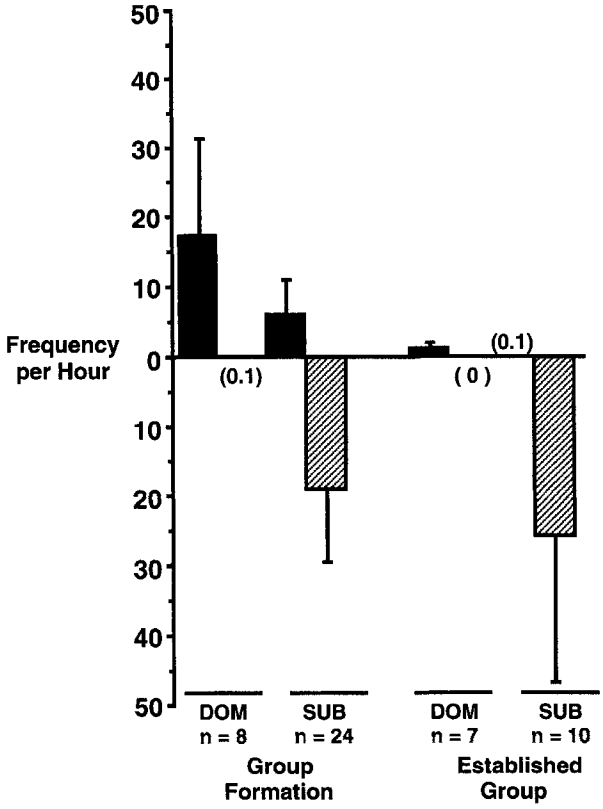

FIGURE 1. Mean (± 95% confidence interval) frequency of aggressive (*solid columns*) and submissive (*shaded columns*) behavior performed by dominant (rank 1) and subordinate (ranks 2 and below) female marmosets during the first 3 days after group formation and during an additional 3-day period, 5 weeks later (established groups). Data adapted from ref. 39.

STRESS-INDUCED REPRODUCTIVE SUPPRESSION

Harassment-induced stress is commonly invoked as the key psychological and physiological mediator of reproductive suppression in socially subordinate mammals.[1,4,12,17,23,26,27] Certainly, prolonged social strife induces persistent, pathological changes.[26,28–30] Glucocorticoid hormones, particularly cortisol or corticosterone, released from the adrenal cortex during stress, specifically inhibit reproductive function at the neuroendocrine hypothalamus, anterior pituitary gland, and gonad.[31–34] Other stress-related physiological changes may similarly inhibit reproductive function. However, the present consideration of physiological mechanisms mediating socially induced reproductive suppression focuses primarily on increased glucocorticoid levels. Elevated blood levels of glucocorticoids are commonly found in dominant and subordinate individuals during periods of social instability or hierarchy formation.[35–39] In

TABLE 1. Summarized Observations from Yellow Baboons, Gelada Baboons, and Common Marmosets Which Suggest That Social Subordination Inhibits Female Reproduction As a Result of Harassment-Induced Stress in Subordinate Females in the Former Two Species, But not in the Latter.

Observations	Yellow Baboons[6,5,20,21]	Gelada Baboons[22,23]	Common Marmoset
(1) Overt aggression towards subordinate females in established groups	+	+	–
(2) Intense harassment of subordinate females during follicular phase of the subordinates' ovarian cycle	+	+	–
(3) Elevated cortisol levels in subordinate females	+	+	–
(4) Inhibited ovarian function in subordinate females	+	+	++
(5) Births and successful infant rearing less frequent in subordinate females	+	+	++

stable, well-established social groups, measures of adrenocortical activity can be greater in subordinates than in dominants,[22,26,27,40–42] but reliable associations of elevated cortisol levels with stable low rank *per se,* rather than with aggression or wounding, are not always found, particularly among nonhuman primates.[26,38,39,43,44] In primates, differences in the dynamic physiological responses of dominant and subordinate individuals to stressful situations may also have physiologically relevant consequences for reproductive suppression (e.g., free-living subordinate male olive baboons, *Papio anubis*).[26] Together, such associations between glucocorticoid levels and social status have been taken to imply that the establishment or disruption of dominance relationships is frequently stressful and that socially subordinate individuals can experience greater psychosocial stress than can dominant ones. This would be consistent with the many studies linking reproductive impairments in subordinate females with the physical or psychosocial stress of subordination.[12] Cooperatively breeding species, however, present an exception to this pattern, because in such species, subordinate female status is not associated with elevated glucocorticoid levels.[10,39,45–48]

REPRODUCTIVE SUPPRESSION IN COOPERATIVELY BREEDING SPECIES

The most extreme examples of social suppression of reproduction in subordinate individuals are found in singular cooperatively breeding species, in which only the dominant female in a social group usually breeds and group members other than the genetic parents are needed to aid in the successful rearing of offspring.[49,50] In such cooperative breeding systems, the social environment provides the predominant proximate cues for timing reproductive effort.[10] This principle is exemplified by the rapid onset of functionally effective reproductive neuroendocrinology, gonadal physiology,

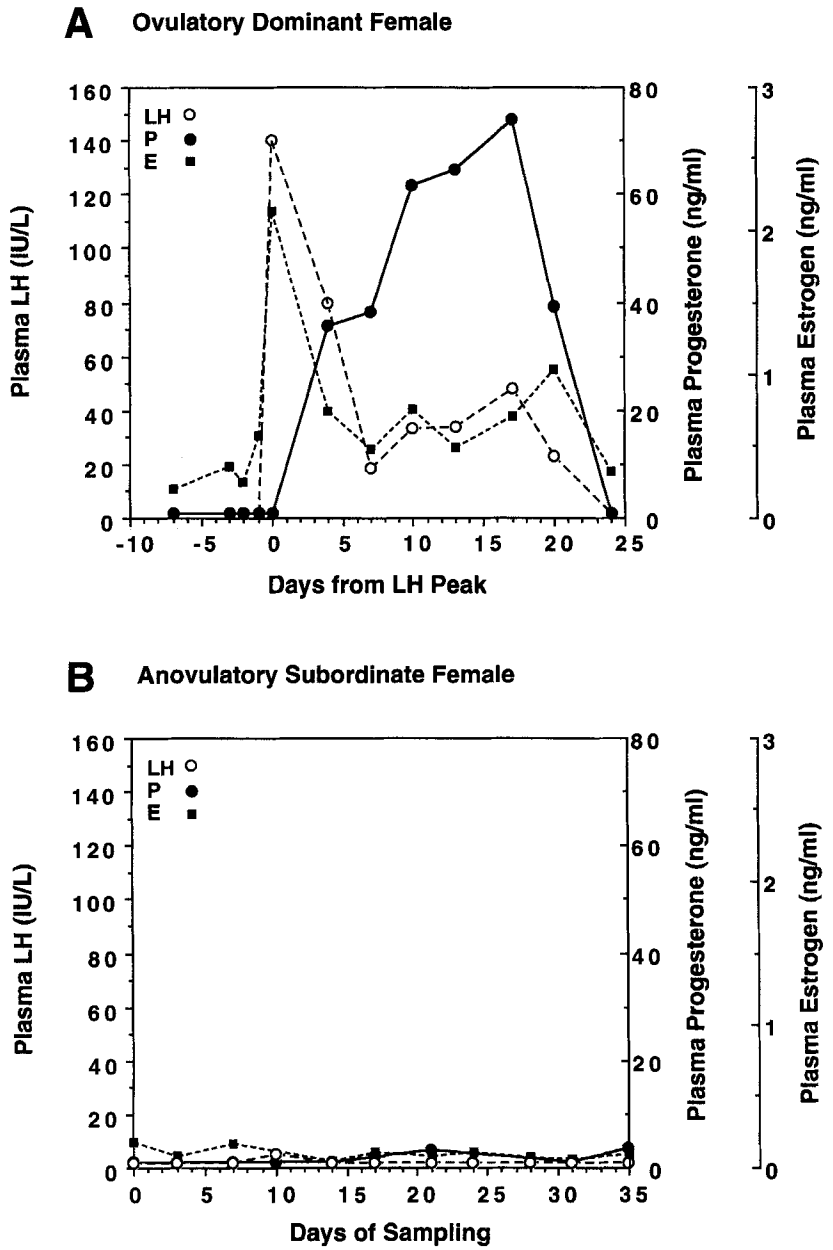

FIGURE 2. Plasma progesterone (*solid circles*), estrogen (*solid squares*), and luteinizing hormone (LH) (*open circles*) concentrations in (**A**) a typical ovarian cycle in a dominant female marmoset and (**B**) an anovulatory subordinate female.

and sexual behavior in individuals that were previously nonreproductive subordinates (originating from within the group or rapidly immigrating from outside the group) after the death or disappearance of the same-sexed dominant breeder (e.g., white-browed sparrow weaver, *Plocopasser mahali;*[45] naked mole-rat;[51,52] or common marmoset).[18] Such precise timing of reproductive effort enables subordinates to engage expeditiously in the intense, intrasexual competition for vacant breeding positions.

Singular cooperative breeders are most readily distinguished from "competitive" breeders, species in which competing females can raise their infants unaided (e.g., yellow baboons,[20] red deer,[53] and gelada baboons)[23] by (1) the frequent exclusion of all but one dominant female and one to two dominant males from successfully producing or raising offspring, (2) the critical role of individuals other than the breeding pair in rearing the young of a single breeding female, and (3) the prolonged retention of mature, but nonbreeding offspring in their natal groups.[12,49,54,55] On an ultimate level, reproductive failure among subordinate animals in cooperatively breeding species appears to occur because of competition for resources that limit subordinates' breeding opportunities and because their retention within a group increases the likelihood of their survival and the survival of the breeding female's offspring.[12,49,54,56,57] Nevertheless, there is no evidence to support the notion that harassment-induced elevations in glucocorticoid activity mediate social contraception in cooperatively breeding animals (e.g., (a) free-living groups: white-browed sparrow,[45] Florida scrub jay, *Aphelocoma c. coerulescens,*[56] dwarf mongoose, *Helogale parvula,*[47] and wild dog, *Lycaon pictus;*[47] (b) captive groups: common marmoset,[39] cotton-top tamarin, *Saguinus oedipus,*[48] and naked mole-rat).[10] The common marmoset, in particular, provides an excellent laboratory model in which to examine the mechanisms of socially induced reproductive inhibition in subordinate females of a cooperatively breeding species.

SOCIAL GROUPS OF COMMON MARMOSETS

Common marmosets and other members of the family Callitrichidae are small-bodied New World primates. In the wild, groups of common marmosets comprise 3-15 individuals and typically include 2-4 adults of each sex.[58,59] Groups seem to consist primarily of extended families, but they may also include unrelated immigrants. Offspring remain with their natal families into adulthood, and all group members contribute to infant care.[24,60] Both field[58,61,62] and laboratory[16,63,64] studies of common marmosets typically report that only a single, dominant female breeds in each social group. While infanticide by the dominant female and lack of "helpers" to raise offspring may partly explain the ineffective breeding of subordinate female marmosets in free-living groups,[23,65,66] field data as yet do not further address the proximate mechanisms of reproductive sovereignty held by dominant females.

To characterize the proximate (physiological, sensory, and behavioral) regulation of female reproductive success in common marmosets, we established a total of 66 standardized, mixed-sex groups of 4-7 unrelated adults or postpubertal animals at the Wisconsin Regional Primate Research Center in Madison, Wisconsin[39] or at the Institute of Zoology in London, UK,[67] with each group remaining together for 2 months to over 2 years, during an 11-year period. The social structure formed by

such groupings was typical for common marmosets. Clear, intrasexual hierarchies that were either linear or despotic were usually quantifiable within 3 days of group formation from the directionality of agonism displayed, and the dominant (rank 1) male and female developed the strongest affiliative relationship within each social group.[16,68] Although little overt aggression was exhibited within social groups established for 6 weeks or more (FIG. 1),[39] subordinate females were clearly distinguishable from the frequency and directionality of their submissive behavior. Infrequent overt aggression among females in established groups of common marmosets is fairly typical of cooperatively breeding societies (e.g., naked mole-rat,[69] *Saguinus* species of tamarins,[70] and dwarf mongoose)[71] and stands in marked contrast to the more frequent aggressive displays and interactions among females in established groups of "competitive" breeders (e.g., yellow baboons).[16]

To sustain unchanging composition of marmoset groups, we gave dominant females intramuscular injections of a synthetic prostaglandin F2α analog, cloprostenol, 14-30 days after each ovulation to terminate the luteal phase of the ovarian cycle or possible early pregnancies.[72] As female marmosets neither menstruate nor exhibit visually obvious cues indicative of ovarian function, blood samples were collected twice weekly from all females (a 4-5-minute procedure not involving anesthesia) to permit endocrine monitoring of ovarian activity.[39,67] Enzymeimmunoassay determination of plasma progesterone concentrations identified the follicular phase (≤ 10 ng/ml for 13 consecutive days or less: mean ± 1 standard deviation) and luteal phase (> 10 ng/ml for 11 consecutive days or more) of the ovarian cycle, ovulation (the day prior to plasma progesterone exceeding 10 ng/ml), and anovulation (≤ 10 ng/ml).[39,73] There were no indications that blood collection procedures resulted in obvious physiological disturbances. Radioimmunoassay of plasma cortisol concentrations in blood samples obtained at 11:45AM were not significantly altered by prior blood sampling at 9 AM on the same day,[39] suggesting that our routine handling and blood sampling did not disrupt the normal diurnal rhythm of plasma cortisol in the female marmosets under study. All plasma cortisol determinations were made from blood samples collected within 3 minutes of cage entry.

REPRODUCTIVE CONSEQUENCES OF SOCIAL SUBORDINATION FOR FEMALE MARMOSETS

While subordinate females occasionally received mounts from dominant and subordinate males, only dominant females sexually solicited males, and only dominant females received ejaculations.[74] When removed from the group for 15-minute behavioral tests with unfamiliar males, however, subordinate females solicited and accepted mounts, illustrating that rapid onset of sexual behavior could be achieved in subordinate females in the absence of their dominant female and that inhibition of their sexual behavior might depend on the presence of their dominant female groupmate. Although reduced circulating levels of ovarian hormones in anovulatory subordinate female marmosets could, on their own, explain the reduced expression of sexual behavior shown by these females, comparable hormonal deficits in ovariectomized female marmosets did not abolish either proceptive or receptive behavior.[75]

Dominant female marmosets continued to undergo regular ovulatory cycles in established social groups, but subordinate females were commonly found to manifest

hypogonadotropic anovulation (FIG. 2). Determinations of bioactive luteinizing hormone (LH) levels in serial blood samples taken every 15 minutes for 4 hours from five dominant and five subordinate female marmosets revealed episodic fluctuations in LH values in the dominants in the midfollicular phase of the ovarian cycle, but only nonepisodic and low LH values in anovulatory subordinates.[76] This hypogonadotropic anovulatory condition of subordinates was rapidly reversible by removal of all higher ranking females or removal of the subordinate from her social group followed by subsequent single housing or pairing with a male.[18,67] Following the resumption of ovulatory cycles, reimposition of hypogonadotropic anovulation was readily achieved by returning females to subordinate status in their original group[77] or in a new social group.[67] Such reliable, repeatable, reversible, and rapid social manipulation of gonadotropic control of ovulation is displayed perhaps to a unique extent in female common marmosets and may belie adaptations to changeable ecological conditions, variable group composition, and an opportunistic lifestyle.[78–81]

The remainder of this paper concentrates on the mechanisms underlying reproductive suppression in anovulatory subordinate female common marmosets. It should be noted, however, that subordinate female marmosets exhibit marked variability in the degree and duration of hypogonadotropic anovulation related to rank, age, and group composition[18,82] which may provide important clues about the salient cues from the social environment that impinge on reproductive function in female marmosets.

PHYSICAL AND PHYSIOLOGICAL CORRELATES OF REPRODUCTIVE SUPPRESSION IN SOCIALLY SUBORDINATE FEMALE MARMOSETS

Reduced body weight, altered diurnal rhythms, hyperprolactinemia, and hypercortisolemia have all been associated with impaired ovarian function;[83] the latter two have also been linked with social stress and subordinate social status in females.[4,84] However, as FIGURE 3 illustrates, anovulatory subordinate female marmosets did not exhibit any of these changes. Body weight was not significantly lower in anovulatory subordinates than in dominants undergoing ovulatory cycles (FIG. 3A). Subordinates exhibited no perturbations in circulating levels, circadian patterning, or total exposure to melatonin over 24 hours (melatonin index; FIG. 3B).[85] Moreover, subordinates showed no evidence of hyperprolactinemia (FIG. 3C) or hypercortisolemia (FIG. 3D) in subordinates. Thus, female marmosets clearly showed no signs of a chronic, generalized stress response to established subordinate status, and elevated glucocorticoid levels could not be invoked as a key physiological mediator of anovulation. Even during the first 2 days after group formation, circulating cortisol concentrations failed to show any rise in subordinate females that were not wounded during hierarchy formation, in contrast to the elevated levels of plasma cortisol exhibited by dominant and subordinate females wounded during this time.[39] In both newly formed and established groups, subordinate status *per se* did not appear to increase glucocorticoid levels in female marmosets.

In further contrast to expectations based on a stress-related model, plasma cortisol concentrations in anovulatory subordinate female marmosets not only failed to exceed values in ovulatory dominant females, but also were significantly lower than those in

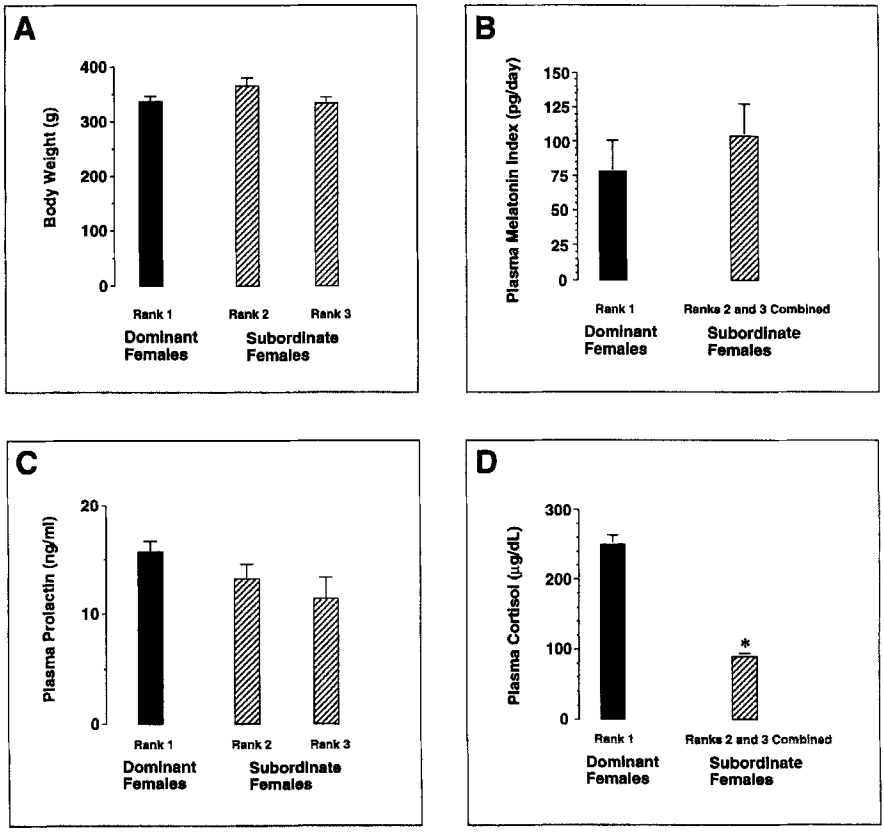

FIGURE 3. Mean ± SEM (**A**) body weight, (**B**) plasma melatonin index, (**C**) plasma prolactin concentrations, and (**D**) plasma cortisol concentrations in dominant (*solid columns*) and subordinate (*shaded columns*) female marmosets. Data for (**B**) adapted from ref. 85. *p <0.05 vs dominant females.

dominant females (FIG. 3D). Similarly, cortisol values were also lower in anovulatory subordinate female cotton top tamarins than in ovulating dominants, using noninvasive urinary measurements.[48] In marmosets, the difference in plasma cortisol levels between dominant and subordinate females reflected a decrease in cortisol values due to social subordination. As illustrated in FIGURE 4, plasma cortisol concentrations were only significantly altered, following group formation, in females that became anovulatory subordinates. The subordinates' cortisol values were significantly lower than those in the same females before group formation (when they were housed only with males and were undergoing regular ovarian cycles) and were also significantly lower than those in females that had become dominant. Interestingly, bilateral ovariectomy also resulted in a significant reduction in circulating cortisol concentrations in female marmosets 4 or more months after ovariectomy (FIG. 4). Ovariectomized females

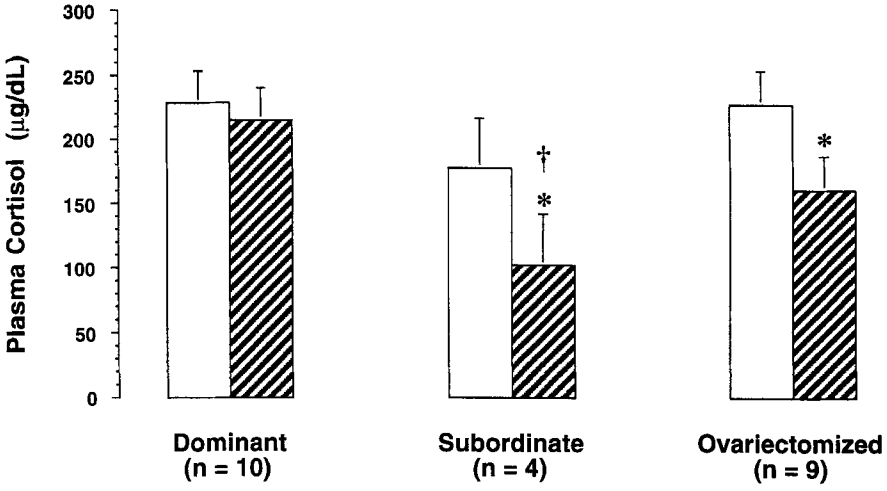

FIGURE 4. Mean ± SEM plasma cortisol concentrations in dominant, subordinate, and ovariectomized female marmosets before (*open columns*) and after (*shaded bars*) group formation or ovariectomy, respectively.* $p < 0.02$ vs before group formation/ovariectomy. †$p < 0.03$ vs dominant females after group formation.

were pair housed with males throughout. These results suggest that ovarian hormones may play as significant a role as subordinate social status in regulating circulating cortisol concentrations in female marmosets. The additional reduction in plasma cortisol levels in ovary-intact, anovulatory subordinate female marmosets compared to ovariectomized females (FIG. 4) might suggest a particular impairment of adrenocortical function due to social subordination *per se,* but this possibility remains to be clarified. Also unclear is the mechanism mediating the hypocortisolemic condition of subordinate female marmosets. In a preliminary study, we have not detected reduced circulating levels of adrenocorticortropic hormone (ACTH) in anovulatory subordinate female marmosets,[25] suggesting instead that alterations in ACTH-mediated cortisol secretion, inhibition of other glucocorticoid secretagogues (such as vasopressin), or increased metabolic clearance of cortisol may be causally involved in the reduction of circulating levels of cortisol. As plasma cortisol binding globulin levels are extremely low in this species and almost all plasma cortisol circulates unbound or loosely bound to albumin,[86-88] it is unlikely that alterations in plasma cortisol binding globulin levels play an important role in mediating the changes found in plasma cortisol in subordinate female marmosets.

SPECIFIC NEUROENDOCRINE INHIBITION OF OVULATION IN SUBORDINATE FEMALE MARMOSETS

To determine whether inhibited or disrupted release of hypothalamic gonadotropin-releasing hormone (GnRH) was implicated in the neuroendocrine imposition

of hypogonadotropic anovulation, we administered GnRH replacement therapy to subordinate females living in their social groups.[17,89] Subordinates were fitted with a lightweight backpack housing a miniaturized infusion pump, programmed to infuse 1 μg GnRH hourly (in approximately 35 μl of saline solution) through an indwelling subcutaneous catheter. This treatment produced a rapid increase in plasma LH levels, and ovulation was induced within 2 weeks in six previously anovulatory subordinate female marmosets remaining in their social groups. Subordinate females quickly reverted to their hypogonadotropic anovulatory condition on removal of the GnRH pumps.[55] These findings did implicate inhibited or disrupted release of hypothalamic GnRH in the neuroendocrine imposition of hypogonadotropic anovulation in subordinate female marmosets.

To confirm this implied inhibition or disruption of hypothalamic GnRH release, we developed a push-pull perfusion method for direct measurement of dynamic GnRH release from the hypothalamus of conscious subordinate female marmosets. It is not possible to accurately determine hypothalamic GnRH release from measurements in the peripheral circulation.[90,91] Modifying a method employed to characterize the neuroendocrine control of GnRH release in female rhesus monkeys,[90] we implanted a cranial pedestal in five anovulatory subordinate and three ovulatory female marmosets.[92] At least 6 weeks later, a micromanipulator was attached to the pedestal and was used to lower a push-pull cannula (outer cannula: 20 ga; inner cannula: 28 ga) into the pituitary stalk-median eminence (S-ME), a hypothalamic area rich in GnRH-containing neuronal terminals.[93] Each female was then placed in a jacket/sling restraint beside its social group. Two days later we perfused the S-ME with artificial cerebrospinal fluid (aCSF, modified Krebs-Ringer phosphate buffer) at 23 μl/min and continually collected perfusate samples in 10-minute fractions for 3-7 hours. Concentrations of GnRH in aCSF perfusate samples were measured by RIA, and GnRH pulses were identified by the computer algorithm PULSAR.[90] Following perfusion, the cannula and micromanipulator were removed and each marmoset was returned to its social group.

In complete contrast to our expectations, GnRH release did not differ markedly between females in the midfollicular phase of the ovarian cycle and those that were anovulatory subordinates.[92] This is particularly well illustrated in FIGURE 5, which shows GnRH measurements in the S-ME over a 7-hour period from one female marmoset in the midfollicular phase of the ovarian cycle (FIG. 5A) and over a second 7-hour period, when the same female was an anovulatory subordinate (FIG. 5B). The GnRH concentrations and dynamic pattern of release were highly similar, while effective ovarian function was strikingly different during the two sampling periods. Considering data from all the females, baseline aCSF concentrations of GnRH were not significantly different between females in the midfollicular phase of the ovarian cycle (4.5 ± 3.3 ng/ml; mean ± sem) and anovulatory subordinates (2.0 ± 0.9 ng/ml). Cycling or subordinate females also demonstrated similar peak concentrations of GnRH (6.4 ± 4.7 vs 3.8 ± 1.8 ng/ml, respectively) and similar interpulse intervals of GnRH (40.9 ± 5.6 vs 38.6 ± 3.5 minutes, respectively).[92] These results suggested that GnRH release in the S-ME of anovulatory subordinate female marmosets was not notably altered from that in the midfollicular phase of the ovarian cycle of regularly ovulating females. Instead, other factors, such as reduced pituitary gonadotropic responsiveness to GnRH[67] or impaired ability to generate an ovulatory LH surge,[94]

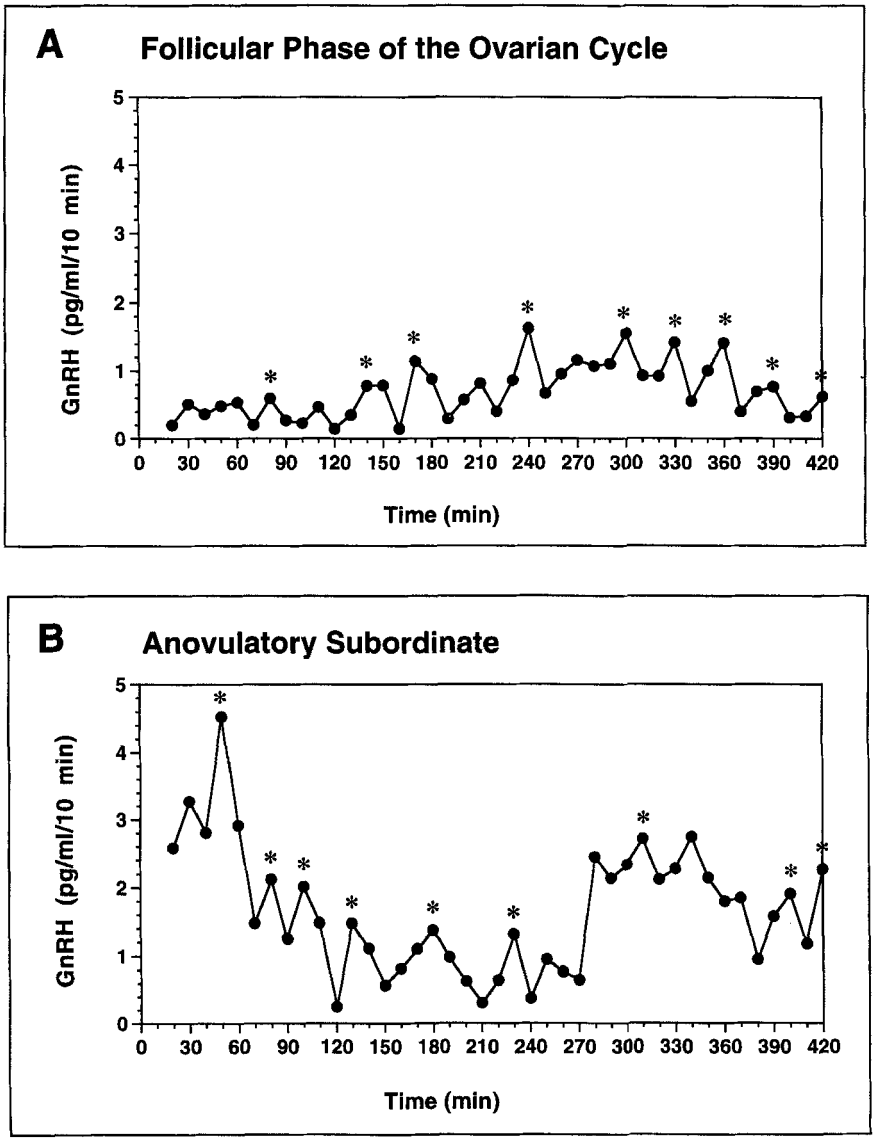

FIGURE 5. Push-pull perfusate concentrations of GnRH obtained from the hypothalamus of the same female marmoset (CJ0086) collected in 10-minute fractions over two separate 7-hour periods when (**A**) in the midfollicular phase of the ovarian cycle and (**B**) as an anovulatory subordinate. *GnRH pulse peak identified by PULSAR computer algorithm.

may be responsible for the hypogonadotropic anovulatory condition of subordinates. Certainly, minimal disruption of hypothalamic GnRH-pituitary gonadotropic function in long-term, anovulatory female marmosets would be consistent with the rapid ability of these females to ovulate within approximately the normal duration of a follicular phase, following removal from subordinate social status.[18,67]

Nevertheless, manipulation of social status and circulating levels of estradiol in subordinate female marmosets has produced clear evidence of specific ovarian hormone-dependent and ovarian hormone-independent mechanisms of LH suppression.[95] With respect to the former, subordinate female marmosets have been shown to exhibit an exquisite gonadotropic sensitivity to the inhibitory influences of estrogen feedback. With respect to the latter, an inhibitory influence of the endogenous opioid peptides on LH release was suggested from the elevated LH responses of ovariectomized subordinate females to the administration of the opiate receptor antagonist naloxone as compared to ovariectomized dominant females.[55] Intact subordinate females, in contrast, showed no such LH response to naloxone treatment. While the specific neuroendocrine nature of these ovarian hormone-dependent and hormone-independent mechanisms remains to be determined in the anovulatory subordinate female marmoset, a similar dichotomy of inhibitory neuroendocrine mechanisms has been implicated in the imposition of anovulation in seasonally anestrus ewes[96,97] and in lactational infertility in rats.[98,99] It is intriguing to speculate that in species that encounter environmentally determined reproductive constraints, there has been convergent evolution of adaptive neuroendocrine responses.

ASSOCIATIVE LEARNING OF CUES FROM FAMILIAR DOMINANT FEMALES MAY FORM AN IMPORTANT COMPONENT OF THE SPECIFIC MECHANISMS INHIBITING OVULATION IN SUBORDINATE FEMALE MARMOSETS

Olfactory, visual, and behavioral cues from dominant female marmosets have all been implicated in maintaining ovarian inhibition in subordinate female marmosets (FIG. 6).[100] Such redundancy in maintenance cues may partly explain the lack of overt aggressive maintenance of ovarian inhibition in subordinates by dominant female marmosets. Marmosets have developed a complex olfactory communication system, they have highly specialized sternal, suprapubic, and anogenital scent glands, and they have a fully functional vomeronasal organ (an accessory olfactory system) in addition to the main olfactory epithelium.[100-102] When anovulatory subordinate females were removed from their social groups to single housing, maintaining them in scent contact with their dominant female delayed the onset of ovulation from 10.8 ± 1.3 days in controls to 31 ± 6.4 days in the scent transfer females (FIG. 6).[77] Similar results, implicating olfactory cues from dominant females in the maintenance of ovarian inhibition in subordinate females, have been achieved using the closely related cotton top and saddleback tamarins.[103,104] Visual cues from dominant female marmosets also extended the period of ovulation suppression in subordinate females removed from their groups, suggesting that cues from the dominant female other than odor may play a role in maintaining anovulation in subordinates (FIG. 6).

However, in all instances the effectiveness of the olfactory or visual cues in inhibiting ovulation in subordinate females expired within a few weeks. Furthermore,

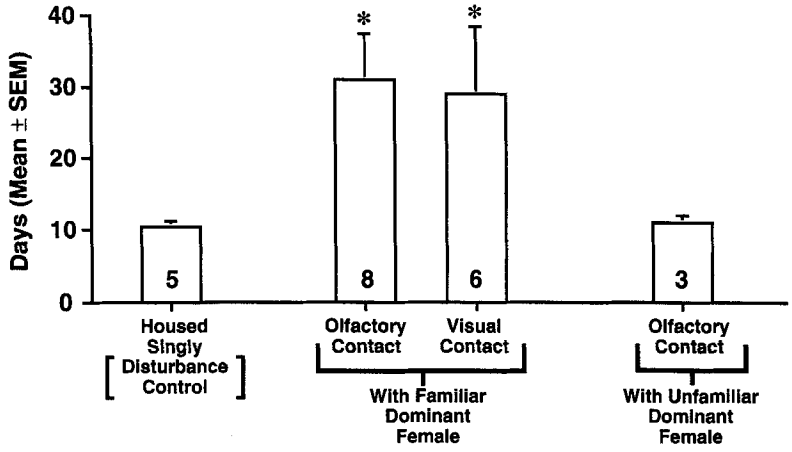

FIGURE 6. Mean ± SEM days until the onset of first ovulation following removal of subordinate female marmosets from their social groups and their subsequent single housing. Numbers of females are shown in each column. *$p < 0.05$ vs disturbance control. Data partially adapted from refs. 77 and 100.

in the marmoset experiments, the length of time a subordinate female remained in her group tended to be positively associated with the latency to ovulate during scent transfer.[77] A similar association was not found in controls. These results implied that an element of associative learning of olfactory cues (and possibly visual cues) from dominant females might play an important role in the neuroendocrine maintenance of anovulation. This hypothesis was supported by recent scent transfer experiments employing odor from unfamiliar dominant females outside the subordinates' groups. In these scent transfer experiments, odor from unfamiliar dominant females failed to produce the delayed onset of ovulation achieved by using the odor from familiar dominant females (FIG. 6).[105] Thus, classical conditioning rather than pheromonal induction might provide the neural basis for reproductive inhibition in subordinate female marmosets. Such a mechanism, founded on recognition of cues from known individuals, is certainly a viable possibility as individual females could readily be identified from extracts of their naturally deposited anogenital scent marks during either behavioral bioassays or quantified chemical analyses.[106] This type of mechanism would mean that cues from only the dominant female groupmate and not those from dominant females in surrounding groups would carry reproductive salience for subordinates and that changes in the social environment, such as disappearance of the dominant female, emigration of the subordinate to a new group, or immigration of new animals to the group could readily extinguish the conditioned reproductive inhibition. A diagrammatic form of our present conceptualization of the conditioning process in subordinate female marmosets is illustrated in FIGURE 7. Initial harassment and intimidation by the dominant female (unconditioned stimulus) result in anovulation (unconditioned response). The association of the dominant female's individualistic olfactory and visual cues with her harassment and intimidation may then result

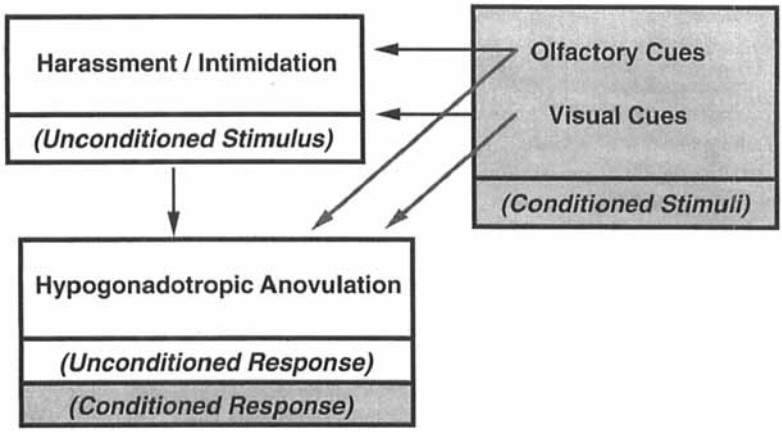

FIGURE 7. Diagrammatic representation of proposed classical conditioning of anovulation in subordinate female marmosets.

in the olfactory and visual cues becoming conditioned stimuli. Such cues would then effectively maintain anovulation as a conditioned response in subordinate female marmosets in established groups.

BEHAVIORAL PREDICTORS OF DOMINANCE IN FEMALE COMMON MARMOSETS

Attainment of dominant or subordinate status by female marmosets in our captive groups was closely related to preexisting individual differences in their agonistic behavior.[68] Prior to group formation, 32 females underwent stranger-encounter testing in which individual females were confronted with female strangers in controlled 15-minute tests, and their agonistic responses were recorded. In six of eight groups, the pre-group formation agonistic behavioral patterns predicted whether a female would subsequently become dominant or subordinate. Once the groups were established, either the behavioral patterns of individual females during the stranger-encounter tests did not change or the females became less responsive.[68] Thus, a female marmoset's likelihood of attaining dominance in a group could be accurately assessed from her agonistic behavior displayed prior to group formation. What was particularly surprising, given that dominance is especially important for female reproductive success in marmosets, was that almost half the females tested before group formation showed submissive responses during stranger-encounter tests and that many of these submissive females did not appear to contend for dominance in their newly formed groups, submitting freely to their female groupmates.[68] It is interesting to speculate on an ultimate level that such interindividual behavioral differences reflect different social/reproductive strategies among female marmosets, with a large proportion of females opting to curtail reproduction until more favorable conditions prevail.[12,68]

SUMMARY

1. Specific neuroendocrine, behavioral, and sensory mechanisms not mediated by generalized stress inhibited ovulation in subordinate female common marmosets.

2. Increased sensitivity to estradiol negative feedback and ovarian hormone-independent mechanisms were identified as the neuroendocrine mediators of hypogonadotropic anovulation in subordinate female marmosets.

3. Direct hypothalamic measurement of GnRH in anovulatory subordinate females provided no clear evidence of reduced or disrupted GnRH release from the hypothalamus.

4. Associative learning of olfactory (and visual) cues from dominant females may provide a psychological conditioning component to the neural mechanisms regulating the anovulatory response to social subordination in female marmosets.

5. Behavioral characteristics of individual female marmosets may play important roles in the attainment of social and reproductive status by each individual.

6. Such specialized behavioral and physiological responses to the social environment contribute to the common marmoset's adaptation to a cooperative breeding strategy.

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