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Social isolation affects partner-directed social behavior and cortisol during pair formation in marmosets, *Callithrix geoffroyi*

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ABSTRACT

Pair-bonded relationships form during periods of close spatial proximity and high sociosexual contact. Like other monogamous species, marmosets form new social pairs after emigration or ejection from their natal group resulting in periods of social isolation. Thus, pair formation often occurs following a period of social instability and a concomitant elevation in stress physiology. Research is needed to assess the effects that prolonged social isolation has on the behavioral and cortisol response to the formation of a new social pair. We examined the sociosexual behavior and cortisol during the first 90-days of cohabitation in male and female Geoffroy's tufted-ear marmosets (*Callithrix geoffroyi*) paired either directly from their natal group (Natal-P) or after a prolonged period of social isolation (ISO-P). Social isolation prior to pairing seemed to influence cortisol levels, social contact, and grooming behavior; however, sexual behavior was not affected. Cortisol levels were transiently elevated in all paired marmosets compared to natal-housed marmosets. However, ISO-P marmosets had higher cortisol levels throughout the observed pairing period compared to Natal-P marmoset. This suggests that the social instability of pair formation may lead to a transient increase in hypothalamic–pituitary–adrenal (HPA) axis activity while isolation results in a prolonged HPA axis dysregulation. In addition, female social contact behavior was associated with higher cortisol levels at the onset of pairing; however, this was not observed in males. Thus, isolation-induced social contact with a new social partner may be enhanced by HPA axis activation, or a moderating factor.

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1. Introduction

Adult social relationships are essential and beneficial for humans and monogamous animals [1], and long-term social isolation or separation can cause homeostatic dysfunction and detrimental health outcomes [e.g., [2–12]; reviewed in [13–15]]. Furthermore, stress has an intricate, reciprocal relationship to social bonds. In humans and monogamous species, disruption of the bond between the social pair evokes a significant biobehavioral stress response while maintenance of the pair bond buffers against the negative consequences of a stressful event [e.g., humans (*Homo sapiens*): [9,16–18]; marmosets (*Callithrix kuhlii* and *Callithrix jacchus*): [3,19,20]; titi monkeys (*Callicebus moloch*): [21]; prairie voles (*Microtus ochrogaster*): [22]; guinea pigs (*Cavia aperea f. porcellus*): [23]; dwarf hamsters (*Phodopus sungorus* and *Phodopus campbelli*): [24–26]; mice (*Peromyscus californicus* and *Peromyscus eremicus*): [5,27]]. This suggests that the maintenance of social bonds may result, in part, from the distress associated with bond disruption or loss and the anxiolytic benefits of social interactions. In addition, there are data that indicate an association between hypothalamic–pituitary–adrenal (HPA) axis activation following stressful experiences and the development of social attachment in childhood in humans [28] as well as adulthood in monogamous animals [29,30]. However, the biosocial mechanisms underlying such affiliative responses to stress remain largely unknown. The current research aims to test the effects of prior psychosocial stress on the formation and maintenance of a new social relationship in a monogamous primate utilizing an ethologically valid model of stress, social isolation in marmoset monkeys.

While cohesive same-sex social networks are the mode for primates, social monogamy between a single male and female as the primary social relationship occurs in a limited number of primate species [31–33]. Besides humans, the majority of monogamous primate groups are forest-dwelling arboreal species [34], which include marmoset monkeys of the genus *Callithrix*. Marmosets typically establish stable, long-term heterosexual relationships that are defined by high rates of sociosexual behavior and contact [35–37], preference for a familiar social partner [[38–40]; cf. [36]], and intruder-directed aggression [41–44]. Like other monogamous species, marmosets form new social pairs after emigration or ejection from their natal group typically resulting in periods of social isolation [[45,46]; reviewed in [47]] and often coinciding with an increase in

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stress physiology, particularly elevated plasma cortisol levels — an end-point of the HPA axis [48–51].

The periods of courtship and formation of heterosexual social relationships may regulate homeostatic HPA activity both transiently and in the long-term. In humans, both men and women have marked increases in salivary cortisol when presented with a sexually attractive confederate of the opposite-sex [52–54]. Men and women who report that they had recently fallen in love have significantly higher plasma cortisol levels than single, uncommitted participants [55]. In nonhuman primates, cortisol in male marmosets becomes elevated following a brief sexual encounter with a receptive female [56], but not after a brief encounter with a novel male [57]. In addition, cortisol is elevated during the initial weeks of pairing compared to several weeks or months after the onset of pairing in male and female marmosets [50,51,58]. As patterns of partner-directed social behaviors critical for the formation and quality of the social partnership are being established during the same period of HPA activity regulation [37], we suggest that social interaction with a partner may be influenced by HPA activity in marmosets, as previously suggested for other monogamous species [59].

The present study examines the hypothesis that stress associated with social isolation is capable of altering HPA axis function and promoting bond-related social behavior in marmosets. The goals were to (1) document the sociosexual behavior during pair formation, (2) determine if prior social isolation alters the behavioral and physiological responses to establishing new heterosexual pairs, and (3) compare cortisol levels during the initial pairing to the partner-directed behavior throughout the pairing period in male and female Geoffroy's tufted-ear marmosets (*C. geoffroyi*). Together, these data may provide some insight into the impact that social isolation and function of the HPA axis have on social bonding in marmosets.

2. Materials and methods

2.1. Subjects

Subjects were 21 adult-aged Geoffroy's tufted-ear marmosets from 12 natal groups living in their natal groups [Age: M = 3.1 years, SE = 0.3]. While controlling for genetic bias, marmosets were randomly divided into one of three groups: (1) adult-aged marmosets that remained in their natal group (Natal; 4 males and 4 females), (2) marmosets that were removed from their natal group and immediately paired with a novel, opposite-sex conspecific (Natal-P; 2 males and 3 females), and (3) marmosets that were removed from their natal group and paired with a novel, opposite-sex conspecific after a period of long-term social isolation (ISO-P; 4 males and 4 females). All marmosets were housed in colony rooms at the Callitrichid Research Center (CRC) at the University of Nebraska at Omaha (UNO). Colony rooms at the CRC were maintained at a temperature range of 19.0–22.0 °C and a 12 h:12 h light–dark cycle. Natal-, single-, and paired-housing enclosures were wire-meshed cages (minimum 0.8 m^3 per animal) and equipped with branches, nest boxes, other assorted enrichment items, and opaque panels to prevent any visual contact between groups. All other dietary and husbandry information were consistent with CRC protocol and can be reviewed in Schaffner et al. [37]. All animal use procedures were approved by the Institutional Animal Care and Use Committee (Protocol#: 07-033-FC). The CRC is a registered research facility with the U.S.D.A., and is accredited by the Association of Zoos and Aquaria (AZA). All appropriate guidelines for housing and conducting research with animals were followed.

2.2. Pairing and behavioral observations

Marmosets from the Natal-P (n = 5) and ISO-P (n = 8) groups were selected to create male–female pairs that were unrelated and unfamiliar to each other. For the ISO-P group, adult marmosets were removed from their natal group due to either scheduled removal or following a disruption in the natal group that lead to the ejection of the adult offspring from the group. These subjects were placed in a single-housing enclosure, isolated from visual and social contact with conspecifics with limited olfactory and vocal communication for long-term periods that ranged from 6 to 20 weeks (M = 13.7, SE = 1.9). The variance in the isolation period was a result of the unscheduled ejections that lead to changes in the pairing schedule for subjects. These pairs were formed by simultaneously introducing males and females into a novel cage in the morning (0900–1000 h). In one case, one experimental female was introduced into the home cage of a male; however, only the female in this pair was included into the analysis. Twenty minute behavioral observations were conducted between 0900 and 1500 h approximately 3 to 5 days a week through the first 90 days of pairing. Before each observation period, animals were allowed several minutes to acclimate to the presence of the observer. The interactions between the male and female were recorded to observe social, sexual, aggressive, and territorial/communicative behaviors and maintenance of spatial proximity between the pair, previously described in Smith et al. [60]. The establishment and maintenance of the social partnership was observed using the social and sexual behaviors and social proximity between the pair as described in the literature [35–37,60]. Aggressive and territorial/communicative behaviors were selected to assess distress between the pair and territorial intergroup communication, respectively [43,61,62].

2.3. Urine collection

Urine samples (2–5 samples per 10-day block) were collected during the first 90 days post-pairing from the adult marmosets in the Natal-P and ISO-P conditions. Samples were also collected from the Natal group every second or third day during a 10-day block to serve as controls for comparing urinary cortisol levels. Urine collections were done under stress-free conditions, and a more detailed description of this procedure can be found in French et al. [63]. Briefly, all urine samples used in this study were first void samples collected in the morning between 0600 and 0830 h. Animals were trained to urinate into hand-held pans for a preferred food item in their home cages with their partner present. Urine was transferred from the pan with a glass pipette to a microcentrifuge vial and centrifuged at 2500 rpm for 3 min. The supernatant portion was transferred to a clean vial and frozen at −20 °C until the time of assaying.

2.4. Cortisol assay

Urine samples were analyzed for cortisol concentrations using a cortisol enzyme immunoassay (EIA). Assay standard sensitivity was set at 7.8–1000 pg Cort/50 µl. Samples were diluted 1:6400 in distilled-deionized water and 50 µl of this solution was taken to assay. The inter-assay coefficient of variation, calculated from pooled urine analyzed on each plate, was 15.79%. The average intra-assay coefficient of variation, also calculated from pooled urine analyzed on each plate, was 2.77%. To minimize the possible confounding effects of inter-assay variation, samples for each animal were run on the same day and in a single assay when applicable. To control for variations in fluid intake and urine solute concentration, we divided the concentration of Cort by the concentration of creatinine (Cr), a muscle metabolite that is excreted at near-constant rates. Cr concentrations were determined using a modified Jaffe endpoint assay [64]. Further details of the assay validation can be found in Smith and French [65].

2.5. Statistical analyses

The sociosexual behavior and excreted urinary cortisol levels for male and females during pairing were averaged across 10-day blocks