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Depressive-Like Behavior, its Sensitization, Social Buffering and Altered Cytokine Responses in Rhesus Macaques Moved from Outdoor Social Groups to Indoor Housing

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Abstract

Psychosocial stressors appear to promote the onset of depressive illness through activation and sensitization of inflammatory mechanisms. Here, adult male rhesus monkeys brought from large outdoor social groups to indoor housing for 8 days reliably exhibited a hunched, depressive-like posture. When rehoused indoors a second 8 days about 2 weeks later, monkeys housed alone, but not those with an affiliative partner, showed sensitization of the depressive-like hunched posture. Housing indoors also affected circulating proinflammatory cytokines: IL-1β showed increased responsiveness to immune challenge, and IL-1β and TNF-α showed reduced suppression by dexamethasone. Sensitivity of the anti-inflammatory cytokine IL-10 to immune challenge exhibited a relative increase from the first to the second round of indoor housing in animals housed in pairs, and a relative decrease in animals housed alone. Cytokine levels during indoor housing were positively correlated with duration of depressive-like behavior. Plasma cortisol levels increased but did not differentiate housing conditions or rounds. Results demonstrate a rapid induction and sensitization of depressive-like behavior to indoor individual housing, social buffering of sensitization, and associated inflammatory responses. This paradigm may provide a practical nonhuman primate model for examining inflammatory-mediated consequences of psychosocial stressors on depression and possible social buffering of these effects.

Keywords

depression; depressive response; social stress; isolation; inflammation; glucocorticoid resistance; immune challenge; social buffering; nonhuman primate model; rhesus macaque

INTRODUCTION

Exposure to psychosocial stressors such as rejection or loss frequently precipitates the onset of a depressive episode (Kendler, Hettema, Butera, Gardner, & Prescott, 2003; Kendler, Karkowski, & Prescott, 1999; Slavich, O'Donovan, Epel, & Kemeny, 2010). Recent research

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has suggested a central role of inflammatory processes in mediating this effect (Slavich & Irwin, 2014). Stressors can elicit systemic inflammatory responses (Glaser & Kiecolt-Glaser, 2005; Maier & Watkins, 1998) and heightened inflammation commonly is associated with periods of depressive illness (Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Strawbridge et al., 2015). Moreover, episodes of depression can be induced with inflammatory stimuli (Bull et al., 2009; Capuron et al., 2002) and alleviated with anti-inflammatory medication (Köhler et al, 2014). It appears that a shift in the endogenous mediators of inflammation—with the action of proinflammatory cytokines increasing relative to that of opposing anti-inflammatory cytokines—may promote depression in stressful conditions (Miura et al., 2008; Roque, Correia-Neves, Mesquita, Palha, & Sousa).

Psychosocial stressors not only promote the onset of depression in the short-term, but can predispose individuals to depression in later life (Agid et al., 1999; Bernet & Stein, 1999; Brown, Harris, & Copeland, 1977; Reinherz, Giaconia, Hauf, Wasserman, & Silverman, 1999). It has been hypothesized that isolation and other stressful events sensitize neural and neurochemical, stress-related mechanisms (e.g., amygdala activity, corticotropin-releasing factor secretion) so that, in vulnerable individuals, relatively minor stressors at later times elicit disproportionate and uncontrolled reactions that bring on the depressive episode (Gold, Goodwin, & Chrousos, 1988; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Schulkin, McEwen, & Gold, 1994). Inflammatory mechanisms also may play a key role in these long-term effects (Ganguly & Brenhouse, 2015; Hennessy, Deak, & Schiml, 2010; Slavich & Irwin, 2014). Abuse or other adversity in childhood or adolescence has repeatedly been found to be positively related to elevated levels of proinflammatory cytokines and other markers of inflammation months to years later (Bertone-Johnson, Whitcomb, Missmer, Karlson, & Rich-Edwards, 2012; Carpenter et al., 2010; Coelho, Viola, Walss-Bass, Brietzke, & Grassi-Oliveira, 2014; Danese, Pariante, Caspi, Taylor, & Poulton, 2007; Gouin, Glaser, Malarkey, Beversdorf, & Kiecolt-Glaser, 2012; Miller & Chen, 2010; Miller, Rohleder, & Cole, 2009; Slopen, Kubzansky, McLaughlin, & Koenen, 2013). Further, depressive episodes during adolescence or adulthood have been shown to be accompanied by heightened measures of inflammation, which even has been found to predict the later onset of depression, but only for individuals who had experienced early-life maltreatment (Danese et al., 2008; Miller & Cole, 2012). Inflammatory markers continued to increase during adolescence in girls who had undergone early adversity (Miller & Chen, 2010), suggesting a continuing process of sensitization.

Sensitization of depressive-like behavior associated with inflammation has also been seen in laboratory rodents experiencing maternal separation. Guinea pig pups exhibit a depressive-like hunched posture when exposed to a several-hour period of isolation in a novel environment, and this response sensitizes with repeated separation. Both the initial and sensitized response can be attenuated with administration of anti-inflammatory compounds (Hennessy et al., 2007, 2015; Perkeybile, Schiml-Webb, O'Brien, Deak, & Hennessy, 2009; Schiml-Webb, Deak, Greenlee, Maken, & Hennessy, 2006). Although evidence indicates that social separation and negative social interactions can readily incite inflammatory-mediated depressive reactions, the ability of affiliative social partners to buffer these responses has not been systematically investigated.

We recently noted that adult male rhesus monkeys brought from large outdoor social groups to indoor housing exhibited a hunched, depressive-like posture (Hennessy, McCowan, Jiang, & Capitanio, 2014). Records of routine observations by Behavioral Management Staff at the California National Primate Research Center (CNPRC) suggested a prevalence of roughly 7% for all animals housed indoors, with adult males apparently most affected. On the other hand, records of opportunistic scans of behavior of animals maintained in large social groups in outdoor field cages indicated that the hunched posture was extremely rare under these conditions. Indoors, the rate was higher for animals housed individually (18.9%) than for those housed in pairs (0.9%), though a host of uncontrolled factors make any conclusion regarding these absolute or relative rates extremely tenuous. Adult males appeared to display the hunched posture much more-readily when not directly confronted by a human observer. That is, when humans were not in the room during filming, videos made of singly housed adult males revealed that 18 of the 26 males exhibited the hunched posture in just 10 min of observation per animal during the first week of individual indoor housing. In all, the observations suggest that a routine animal husbandry procedure—bringing monkeys from outdoor social groups to socially restricted indoor housing—may provide a practical nonhuman primate model for examining the effect of psychosocial stressors on the onset of depressive symptomatology.

These data, however, were gathered retrospectively from colony records and video tapes from experiments designed for other purposes. Here we present the results of a prospective study designed to confirm and expand our earlier observations as well as to assess whether the depressive-like response could be buffered by the presence of a single affiliative social companion. Adult male rhesus macaques were transferred from outdoor social groups to indoor housing either alone or with an affiliative partner. Monkeys were brought indoors for 8 days on two occasions, about 2 weeks apart, in order to determine if the behavioral response of adult rhesus, like that of young guinea pigs, would sensitize with repeated separations. We increased the duration of behavioral observations indoors compared to our earlier study and conducted supplemental observations to confirm the rarity of the hunched posture in the outdoor social groups. In addition to our primary measure of the hunched posture, we examined two behaviors that appeared associated with the hunched posture in our earlier study—day-time sleeping and lying down. These behaviors are unusual in healthy rhesus, particularly during active periods, but are common during times of heightened inflammation (Aubert, 1999; Hart, 1988) and are consistent with a depressive-like appearance. We also scored two behaviors typically reduced by proinflammatory activity (environmental exploration, general activity) and, in the monkeys housed with partners, several measures of social interaction. To begin to address potential underlying mechanisms, we examined plasma cortisol levels and activity of pro- and anti-inflammatory cytokines thought to contribute to depressive illness (i.e., IL-1β, TNF-α, and IL-10; Réus et al., 2015; Roque et al., 2009). In addition to baseline circulating cytokine levels, we assessed the response of cytokines to bacterial [i.e., lipopolysaccharide (LPS)] stimulation, as well as possible changes in resistance to glucocorticoid inhibition—two measures sensitive to earlier stress exposure in humans (Miller & Chen, 2010).

METHODS

Animals

The primary test subjects were 24, 4 to 6-year-old male rhesus macaques (*Macaca mulatta*) born and mother-reared at the California National Primate Research Center (CNPRC) and maintained in half-acre, outdoor field cages in large, mixed age and sex social groups. No animals with frequent or extended periods of rehousing indoors (e.g., recurrent hospitalizations) were included. In the interest of maintaining stability in the outdoor cages, we also excluded all alpha and most beta males from the field cages; otherwise, assignment to conditions was made without consideration of dominance rank. Because twelve of the 24 subjects were to be housed with an affiliative partner, we confirmed that all 24 exhibited positive social interactions with a juvenile conspecific (either a sibling or unrelated male) in its field cage. Younger siblings and unrelated males were chosen as potential partners to minimize the chances of aggression or sexual activity between a test subject and its partner. Identification of affiliative partners was based on routine surveys of the behavior of monkeys in the field cages by the Behavioral Management Unit at the CNPRC and supplemented by observations of social behavior by our team. Subjects were randomly assigned to either the Alone or With Partner condition. Four younger siblings (3 males, 1 female) and eight unrelated male juveniles served as partners. Twelve additional 2–6-year-old males were solely observed in the large outdoor social groups in order to determine the frequency of depressive-like behavior in that environment. All procedures were conducted according to CNPRC SOPs. The CNPRC is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Experimental protocols were approved prior to implementation by the University of California, Davis Institutional Animal Care and Use Committee.

Procedure

The 24 subjects were rehoused indoors for 8 days on two occasions at a 2-week interval (referred to as Round 1 and Round 2). Double-wide adult cages (1.7 × .7 × .8 m) were used for all subjects. For the With Partner condition, the two monkeys were initially placed on either side of the cage, separated by a metal partition. The partition was then retracted enough for the animals to see each other. When no fighting occurred (all cases), the partition was completely removed. The two monkeys then remained in constant contact, though were briefly separated during twice-a-day feedings and for periodic blood draws (see below).

For each round, testing was conducted in three waves of eight animals, four from each condition. During each wave, animals of the two conditions were housed in separate, though essentially identical adjacent rooms (the rooms were identically configured, though they were mirror images of each other). During each animal's second round, the assignment to rooms was switched, so that all monkeys were exposed to a different room on each of the two rounds. Which of the two rooms housed monkeys of which condition during which round was alternated across the three waves. Thus, each wave was divided into two cohorts, each consisting of four animals of each condition that were housed in separate rooms. The two cohorts were brought to indoor housing at a 24-h interval. This arrangement permitted

us to stagger behavioral observations and blood draws so as to facilitate scheduling and minimize disturbance on any one day.

Behavior observation

Behavior was observed for 20 min/day between 0830 and 1000 h on Days 2, 4, 6, and 8 of each round of indoor housing. Each day the four animals of the cohort were observed in turn for 5 min in a predetermined order for four repetitions. A webcam (Logitech c920 HD Pro) located in each of the two test rooms was used to record behavior on a laptop PC located in a third room, using The Observer (Noldus, 1991) software. The recordings were subsequently scored by an observer trained to 85% inter-observer reliability. Behaviors (definitions in Table 1) included the duration in s that the animals exhibited the hunched posture, lying down, sleeping, and activity, as well as the frequency of environmental exploration. As in our earlier paper (Hennessy et al., 2014), we also calculated an index of “total depressive-like responding” by summing the durations of the hunched posture, lying down, and sleeping. However, since analysis of this index yielded a pattern of results identical to that for the hunched posture, the index will not be considered further. In addition, in the With Partner condition we recorded the duration of physical contact between partners and grooming that was initiated and received by the subject. Unambiguous negative interactions (e.g., fighting) were never observed between a subject and partner.

Finally, for comparison with behavior during indoor housing, the same observer monitored the occurrence of the hunched posture in 12 males housed in the field cages. Each male was observed for 16, 5-min focal observations (four on each of 4 days) between 0830 and 1000 h. The observer sat quietly more than 3 m from the field cage, and assisted with binoculars, used the “HanDBase” application on an iPhone to record the total s that males were observed in the hunched posture.

Blood sample collection

Four blood samples were collected from each monkey during each round of testing between 1100 and 1145 h. The initial sample was taken while the male was in its social group in its field cage, 6 or 7 days prior to transfer indoors. The subsequent samples were collected on Days 1, 3, and 8 of indoor housing. Those collected in field cages and Day 8 were 7 ml in volume to allow for analysis of both cortisol and cytokines, while those taken on Days 1 and 3 indoors were 3 ml and used for assay of cortisol only. Samples were collected from the femoral vein without anesthesia. In the field cage, males were captured and held briefly in nets while blood was collected. Collected blood was placed on EDTA (for cortisol and baseline cytokine analysis) or heparin (for additional cytokine assessment). Blood collected for cortisol and baseline cytokine analysis was separated by centrifugation and stored at −80 °C until processing.

Cortisol assay

Cortisol analysis was with the ADVIA Centaur CP two site chemiluminescent immunoassay (Siemens). This procedure involves competitive binding of cortisol in unknown samples with acridinium ester-labeled cortisol to a polyclonal rabbit anti-cortisol antibody in the solid phase. The polyclonal anti-cortisol antibody is bound to monoclonal mouse anti-rabbit

antibody covalently coupled to paramagnetic particles for separation. Acid and base reagents initiate the chemiluminescent reaction and the intensity of the reaction is measured in relative light units (RLUs). An inverse relationship exists between the amount of cortisol present in the unknowns and the relative light units detected by system. Intra- and inter-assay variability were 1.3% and 2.4%, respectively.

Cytokine stimulation and glucocorticoid suppression

Whole blood samples obtained in field cages and on Day 8 were used to estimate effects of LPS stimulation as well as to assess glucocorticoid signaling sensitivity. To 3 ml of whole blood, 2.4 ml of DMEM culture media (Invitrogen, Carlsbad, CA) was added and 675 μl of diluted blood per well was incubated for 24 h following addition of 37.5 μl of concentrated LPS (Sigma-Aldrich, St. Louis, MO, USA) stock solution titrated to yield 50 ng/ml exposure dose in final culture volume. Stock solutions were supplemented with dexamethasone (Sigma-Aldrich, St. Louis, MO) to yield final culture concentrations of 0 (positive control), 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M dexamethasone.

Cytokine assay

Field cage and Day 8 baseline plasma samples, and supernatants of cytokine stimulation were recovered by centrifugation and assayed for concentrations of pro- and anti-inflammatory cytokines (proinflammatory: IL-1β, TNF-α; anti-inflammatory: IL-10) using the MILLIPLEX MAP Non-Human Primate Cytokine Magnetic Bead Panel Array Kit (Millipore, Billerica, MA, USA). Samples were assayed following manufacturer's instructions. Plates were read using a Bio-Plex HTF System with Luminex xMAP Technology (Bio-Rad, Hercules, CA, USA), and values were calculated using Bio-Plex Manager Software, version 4.1. Values of baseline cytokines sometimes fell below the sensitivity of the assay. In these cases (11 TNF-α, and 15 IL-10 samples—13% and 18% of the final sample, respectively) a score of "0" was entered as the baseline value.

Data analysis

Data were primarily analyzed with analysis of variance (ANOVA) procedures. Most behavioral measures were assessed with 2 (Condition—Alone vs. With Partner) × 2 (Round—1 vs. 2) ANOVAs with the last factor treated as a repeated measure. However, Lie and Sleep, for which more than half of the cells were scores of "zero", were analyzed with nonparametric tests (Mann-Whitney and Wilcoxon Paired-Comparisons for between and within subject measures, respectively). For cortisol, a 2 (Condition) × 2 (Round) × 4 (Sample—Field Cage, Days 1, 3, & 8) ANOVA with repeated measures for the last two factors was used.

Cytokine data were assessed with two separate sets of ANOVAs. A series of 2 (Condition) × 2 (Round) × 2 (Sample—Field Cage/Day 8) × 2 (Baseline/LPS) ANOVAs, with the last three factors treated as repeated measures, examined the effect of the manipulations on baseline circulating cytokines and estimated their response to LPS. We examined the percent suppression of cytokine levels by dexamethasone (cytokine concentration for a particular dose of dexamethasone divided by the level observed with just stimulation with LPS) with 2 (Condition) × 2 (Round) × 2 (Sample) × 4 (Dose) ANOVAs with the last three factors as

repeated measures. When sphericity was problematic as indicated by the Mauchly test, the Huynh-Feldt correction was employed, and corrected degrees of freedom are reported.

Post hoc analysis of significant interactions was done with tests for simple main and interaction effects. Paired *t*-tests compared social behavior in the two rounds for monkeys in the With Partner condition. A significance level of $p < 0.05$, 2-tailed was used throughout. Pearson Product Moment correlations were used for exploratory analysis of associations between the hunched posture and cytokine measures. Analyses were conducted with IBM SPSS versions 22 and 23 software. Three subjects in the With Partner condition were lost between rounds (two died, both with left ventricular hypertrophy; one had to be introduced to a new social group) resulting in a sample size of 9 for this condition.

RESULTS

Behavior

In the field cages, four of twelve monkeys occasionally exhibited hunching, though only while in physical contact with another monkey or engaging in social grooming. For these four animals, 15 to 83 s (out of a total of 4,800 s) were spent in this behavior. Indoors, the hunched posture was observed in all animals in both rounds. Individual animals spent from 96 to 4,778 s of the 4,800 s of observation each round in this posture. ANOVA of total time spent in the hunched posture yielded significant effects of Condition, $F(1, 19) = 4.37$, $p = 0.05$, Round, $F(1, 19) = 19.86$, $p < 0.001$, and the interaction of Condition x Round, $F(1, 19) = 17.32$, $p = 0.001$. Simple main effects tests showed that total time in the hunched posture increased (i.e., sensitized) from Round 1 to Round 2 for the Alone subjects ($p < 0.01$), but not for those in the With Partner condition. While there was no difference between conditions in the first round, the Alone subjects spent significantly more time in the hunched posture than did those in the With Partner condition in Round 2 ($p < 0.01$, Fig. 1a). [1]

The two measures of active behavior—environmental exploration and activity—exhibited complementary patterns with levels decreasing, rather than increasing, from Round 1 to 2 in monkeys tested alone. For environmental exploration, there was one significant effect, a Condition x Round interaction, $F(1, 19) = 10.08$, $p = 0.005$. The frequency of exploration declined from Round 1 to Round 2 for the Alone subjects ($p < 0.05$); the difference between rounds for monkeys in the With Partner condition only approached significance ($p < 0.1$). There was a significant difference between conditions for Round 2 ($p < 0.05$), but not for Round 1 (Fig. 1b). ANOVA for activity also yielded only a significant Condition x Round interaction, $F(1, 19) = 10.66$, $p < 0.005$. Simple main effects tests showed that activity declined from Round 1 to Round 2 for Alone subjects ($p < 0.05$), and significantly increased for monkeys in the With Partner condition ($p < 0.05$) so that the conditions again differed during Round 2 ($p < 0.05$), but not during Round 1 (Fig. 1c).

[1] A complication of the scoring system is that animals in the With Partner condition sometimes sat side by side in a manner that meets the definition for the hunched posture. In this case, it may be that animals were simply huddling with the partner and so exhibiting positive affiliative behavior. Although our primary analysis was of the more-conservative measure (all instances of the hunched posture included), we also calculated the time that this posture was exhibited by animals in the With Partner condition in the absence of additional measures of positive social interaction (i.e., contact and grooming initiated and received). For this “non-social hunched posture” measure, differences between groups were magnified and significant during the first as well as the second 8 weeks of testing. In the outdoor field cages, all of the limited instances of the hunched posture observed occurred in the context of social behavior.

Positive social interaction was common in the With Partner condition. Subjects spent an average of more than half of observation periods in physical contact with their cage-mate. They often were observed grooming and, especially, being groomed by their partner. The duration of these behaviors did not differ between Rounds 1 and 2 (Table 2).

For the measures of lie and sleep, Mann-Whitney *U* tests compared the Alone and With Partner conditions during each round, and Wilcoxon tests assessed changes from Round 1 to 2 for subjects in each condition. These analyses yielded two significant differences between conditions: With Partner monkeys showed longer duration of sleeping during Round 1 ($p < 0.01$) and longer duration of lying down during Round 2 ($p < 0.05$) than did monkeys tested alone (Table 3).

Cortisol

ANOVA resulted in one significant effect, a main effect of Sample, $F(3, 57) = 115.62$, $p < 0.001$. As is clear in Figure 2, removal to indoor housing sharply elevated cortisol levels of monkeys in both conditions, but only on the first day indoors. We note that this effect was not associated with a longer duration of time to collect Day 1 samples. Mean s from initiation of disturbance until blood collection at the various time points was as follows: field cage—521 s, Day 1—508 s, Day 3—492 s, and Day 8—637 s.

Baseline and stimulated cytokine levels

For IL-10, ANOVA yielded a significant main effect of Baseline/LPS, $F(1, 19) = 112.38$, $p < 0.001$, with a 200-fold increase in mean IL-10 values following LPS stimulation (*Mean*: baseline = 52; LPS-stimulated = 10,431). There also were significant interactions of Condition x Round, $F(1, 19) = 5.38$, $p < 0.05$, and Condition x Round x Baseline/LPS, $F(1, 19) = 5.33$, $p < 0.05$. This 3-way interaction was examined further with simple Condition x Round interactions for both the baseline and LPS-stimulated values. The simple interaction was significant for LPS-stimulated ($p < 0.05$) but not baseline values. There was a relative increase in sensitivity of IL-10 to LPS from Round 1 to 2 for With Partner monkeys and a relative decrease in sensitivity of this anti-inflammatory cytokine to LPS across rounds for Alone animals (Fig. 3).

For IL-1β, ANOVA resulted in significant main effects of Sample, $F(1, 19) = 8.34$, $p < 0.005$, and Baseline/LPS, $F(1, 19) = 95.44$, $p < 0.001$, as well as a significant interaction of these two variables $F(1, 19) = 8.34$, $p < 0.01$. Tests for simple main effects showed that LPS stimulation resulted in greater IL-1β levels when animals were in the field cage than when they were indoors ($p < 0.01$), but that there was no difference between field cage and indoor baseline values. Additional tests for simple main effects confirmed that IL-1β values were greater following LPS stimulation than at baseline for both field cage and Day 8 samples (p's < 0.001). For TNF-α, there only was a main effect of Baseline/LPS, $F(1, 19) = 143.40$, $p < 0.001$, with the expected higher levels following LPS stimulation (Table 4).

Cytokine sensitivity to glucocorticoid inhibition

ANOVA of percent suppression of IL-10 by dexamethasone yielded only main effects for Round, $F(1, 19) = 4.42$, $p < 0.05$ (percent suppression greater during Round 1 than Round

2) and Dose, $F(2.45, 46.53) = 17.18$, $p < 0.001$ (greater suppression with higher doses, Fig. 4a). Dexamethasone suppression of the two proinflammatory cytokines, IL-1β and TNF-α, varied depending on whether samples were collected from monkeys when in the field cages or following 8 days indoors. For both cytokines, there were main effects of Sample, $F(1, 19) = 8.52$, $p < 0.001$ for IL-1β, and $F(1,19) = 7.12$, $p < 0.05$ for TNF-α, and Dose, $F(2.04, 38.75) = 42.00$, $p < 0.001$for IL-1β, and $F(3, 57) = 68.67$, $p < 0.001$ for TNF- α, as well as the interaction of Sample x Dose, $F(3, 57) = 18.23$, $p < 0.01$ for IL-1β, and $F(2.03, 38.58) = 13.32$, $p < 0.001$ for TNF-α. As can be seen in Figure 4b and c, there was less suppression of these proinflammatory cytokines by the higher doses of dexamethasone in samples collected from monkeys on Day 8 indoors than in samples collected when the monkeys were in field cages. While the highest dose of dexamethasone was sufficient to reduce cytokine levels to nearly 50% of the LPS-stimulated value in samples collected from monkeys in the field cages (50.1% and 51.9% for IL-1β and TNFα, respectively), these values remained at 84.3% and 73.3% of the stimulated value in samples collected following 8 days of indoor housing.

Exploratory behavior/cytokine correlations

To explore possible associations of immune activity and behavior, correlation coefficients were calculated between the duration of the hunched posture and various cytokine measures. Although a number of significant associations were detected, the one consistent finding was a significant positive correlation between the hunched posture in each round and circulating levels of each of the three cytokines on the last day of separation for that round for monkeys tested in the Alone condition (Table 5). In contrast, for animals in the With Partner condition, these correlation coefficients were uniformly negative (−.476 to −.592) and nonsignificant.

DISCUSSION

The results of this prospective study confirm our earlier observation from retrospective data that adult male rhesus monkeys brought from large outdoor social groups to socially restricted, indoor housing frequently exhibit a depressive-like hunched posture that is very rarely observed in the outdoor social groups (Hennessy et al., 2014). All monkeys of both conditions brought indoors displayed the hunched posture in each round. For those males housed alone indoors, time spent in the posture increased, i.e., sensitized, during the second round of indoor housing about two weeks after the first. There also was evidence of social buffering in that the presence of an affiliative social partner prevented this sensitization. If one considers only those instances of hunching that were clearly not associated with social behavior in the With Partner condition, buffering of the depressive-like response was apparent during both Rounds 1 and 2.

A hunched posture like that seen here has also been observed in rhesus macaques following injection with Interferon-α, a proinflammatory cytokine (Felger et al., 2007), and analogous postures have been associated with increased inflammatory activity in other species (Dantzer, 2004; Hart, 1988; Hennessy et al., 2009). Administration of proinflammatory cytokines also has been shown to reduce measures of activity and exploration (Barak et al.,

2002; Dantzer, 2004; Friedman, Boinski, & Coe, 1995). In the current study, activity and exploration both declined from the first to the second round of separation in Alone monkeys as time spent in the hunched posture increased. In sum, behavioral results observed here are consistent with the possibility of mediation by inflammatory mechanisms.

In addition, we found direct evidence of changes in cytokine activity induced by the indoor housing procedure. Eight days of indoor housing reduced responsiveness of IL-1β to LPS stimulation, but at the same time also reduced the sensitivity of both IL-1β and the other proinflammatory cytokine measured, TNF-α, to the suppressive action of the higher doses of dexamethasone. A reduction in sensitivity to glucocorticoid suppression (i.e., increased glucocorticoid resistance) would have the opposite effect of reduced responsiveness to LPS; that is, it would tend to increase levels of both IL-1β and TNF-α. Increased glucocorticoid resistance has been observed in adolescent girls who have undergone early-life stress (Miller & Chen, 2010) and has been suggested to account for the simultaneously high levels of circulating cortisol and cytokines observed in depressed patients (Horowitz & Zunszain, 2015).

IL-10 activity varied with the social housing conditions. There was a relative increase in sensitivity of this anti-inflammatory cytokine to LPS across rounds for animals tested in pairs, and a relative decrease across rounds for those tested in isolation. This finding is in agreement with the interpretation of the sensitization of depressive-like behavior being due to a relative shift by Alone animals toward a proinflammatory phenotype. Although this study was not designed to assess a causative role of cytokine action on behavior, additional indication that the cytokine and behavioral effects we observed may be functionally related comes from the finding of the exploratory correlations: each of the cytokines measured was positively correlated with the duration that the hunched posture was displayed by Alone monkeys in each of the two rounds.

Social buffering of hypothalamic-pituitary-adrenal (HPA) and other physiological and behavioral responses to stressors has frequently been observed. (e.g., Edgar et al., 2015; Hennessy et al., 2009; Kiyokawa, 2015). In the current study, plasma cortisol sharply increased the first day indoors, but the effect was no greater for monkeys tested alone than for those with an affiliative partner. Nonetheless, sensitization of the depressive-like response was completely prevented by the presence of the companion and a potential underlying mechanism—a decline in levels of the anti-inflammatory cytokine IL-10 from the first to the second round of indoor housing—was reversed when a companion was present. These findings suggest the concept of social buffering might be extended to inflammatory-mediated depressive-like behavior elicited by stressors, and that this effect may be independent of buffering of HPA activity.

Males were selected for the current experiment because retrospective observations from our previous study suggested that males may be more-sensitive than females to rehousing indoors (Hennessy et al, 2014). Nonetheless, how males and females would differ under the conditions tested here remains unknown and certainly warrants future investigation. Furthermore, in our previous paper we suggested that lying on the substrate and sleeping during the active daylight period (i.e., mornings) may be mediated by proinflammatory

activity. The current findings do little to confirm this possibility. Both behaviors occurred infrequently. Neither behavior showed patterns that paralleled that of the hunched posture nor showed sensitization in the Alone condition, and in fact, both occurred more frequently in the With Partner than the Alone condition in one of the two rounds.

Animals brought indoors faced a combination of potential stressors that have been observed to increase inflammatory and depressive responses in other species. These include novelty, absence of natural daylight and exercise, social disruption, and for the Alone animals, lack of any social partner with which to directly interact (Bogdanova, Kanekar, D'Anci, & Renshaw, 2013; Eyre & Baune, 2012, Harb, Hidalgo, & Martau, 2015; LeMay, Vander, & Kluger, 1990). A better understanding of the relative contribution of the various factors in producing the outcomes observed here, and of means for mitigating their effects, would seem to be of practical value for implementing housing changes in captive primate colonies. The results also appear to have implications for many experimental protocols. It is commonplace to house monkeys individually indoors for some time prior to an experiment to allow for habituation to the new surroundings. Because our earlier observations suggested that monkeys are unlikely to exhibit the hunched posture if a human is physically present in the room, and because the cortisol response dissipates after several days, reliance on simple behavioral scans or assessment of circulating cortisol may lead to erroneous conclusions regarding the acclimation of a monkey to its new housing environment prior to experimentation. In fact, earlier (Capitanio et al., 2006), we suggested that a period of up to three months may be required for adaptation to a relocation from outdoor social groups to individual housing indoors; the dexamethasone suppression data from the present study clearly suggest that, despite rapid normalization of the cortisol response, eight days of indoor housing are insufficient to return the HPA-immune relationship to pre-relocation, outdoor levels.

Although many aspects of the procedures differ, the results with adult rhesus observed here parallel earlier findings in young guinea pigs (Hennessy, 2014). In both cases, a period of isolation in novel surroundings reliably elicits a depressive-like behavioral response, the behavioral response sensitizes upon a second separation, and markers of inflammation are increased during separation. In the guinea pigs, studies with anti-inflammatories have provided strong evidence for inflammatory mechanisms underlying behavior, whereas in the monkeys, the evidence is suggestive at present. The results with both species also may parallel processes in humans. Mounting evidence indicates that inflammatory mechanisms are critical mediators of the strong link between psychosocial stress and the immediate or delayed development of depressive illness (Anisman, 2009; Ganguly & Brenhouse, 2015; Iwata, Ota, & Duman, 2013; Slavich & Irwin, 2014). The present findings suggest that the common animal husbandry procedure of bringing adult monkeys from outdoor social groups to individual, indoor housing offers promise as a practical nonhuman primate model to complement rodent models in testing aspects of this hypothesis.

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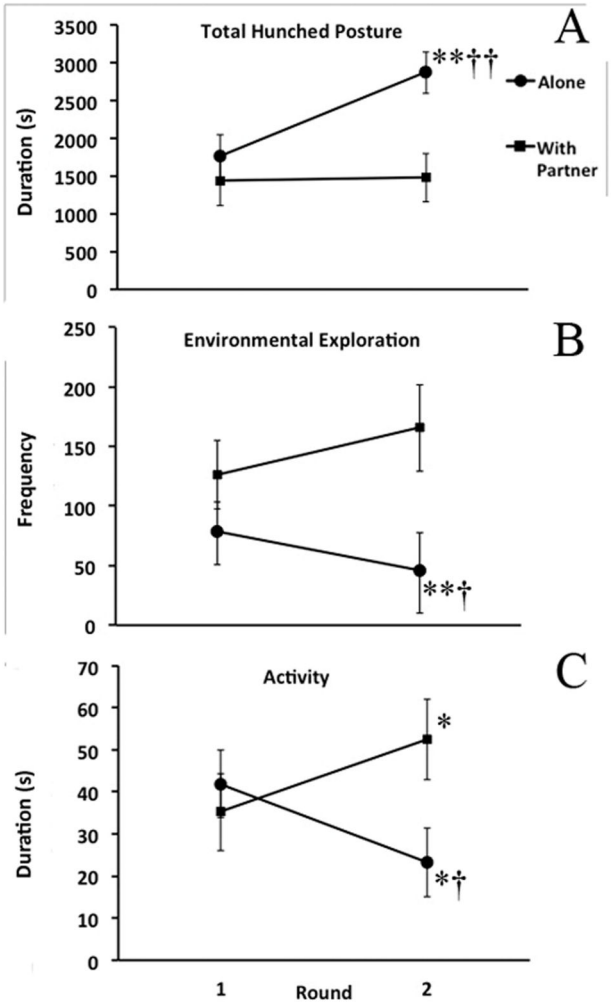

Figure 1.

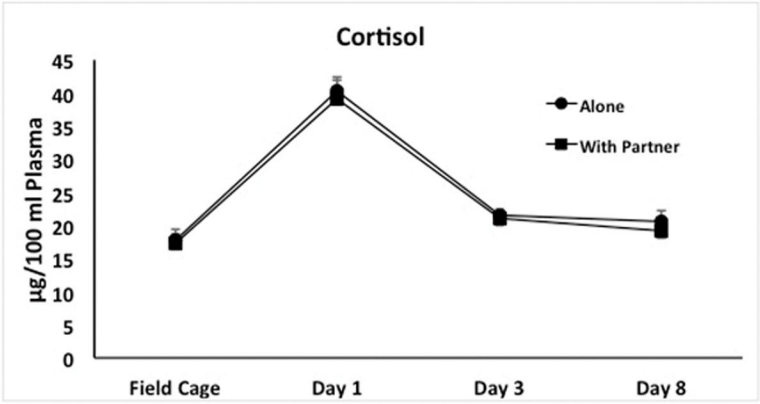

Figure 2.

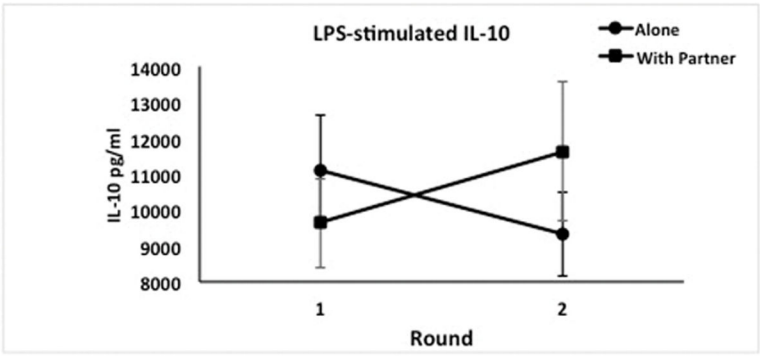

Figure 3.

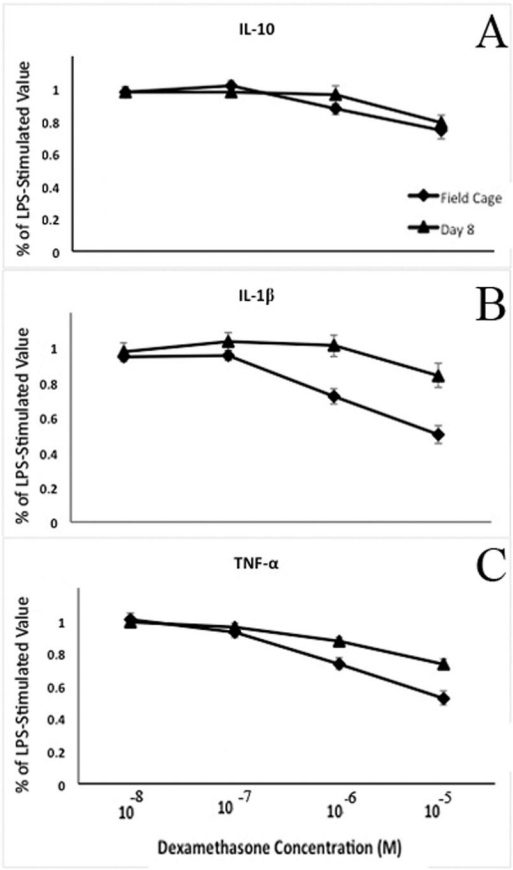

Figure 4.

Table 1

Behavior Definitions.

Behavior	Definition
Hunched posture	Sitting with head the same level or lower than the shoulders; arms and limbs huddle to the center of the body; no movement of the body or the four limbs; eyes open or unable to determine if the eyes are open or not. When in hunched posture, the animal can yawn or scratch (D)
Activity	Directed whole body movement (D)
Environmental Exploration	Tactile manipulation of the physical environment; the animal must be actively manipulating object (F)
Lie	Relaxed posture with body resting on a horizontal surface. Weight is not supported by limbs; eyes are open (D)
Sleep	Sitting or lying with eyes closed (D)
Contact	Monkey makes tactile contact with partner (D)
Groom Initiate	Monkey uses hands to pick through fur or pick at skin of partner; licking of fur or skin of partner also counted (D)
Groom Receive	Partner uses hands to pick through fur or pick at skin of subject monkey; licking of fur or skin of monkey also counted (D)

Note: D = duration; F = frequency

Table 2

Mean (se) Duration of Social Interactions.

	Round 1	Round 2
Contact	2,986.5 (191.0)	2,655.4 (241.8)
Groom Initiate	88.5 (56.5)	85.4 (34.1)
Groom Receive	313.1(82.4)	382.6 (164.0)

Table 3

Median (Interquartile Range) Number of S Spent Lying Down and Sleeping

	Round 1	Round 2
Lie		
Alone	0.0 (0.0–57.9)	0.0 (0.0–0.0) *
With Partner	31.9 (0.0–80.7)	35.6 (0.0–165.1)
Sleep		
Alone	1.0 (0.0–19.9) **	1.8 (0.0–97.9)
With Partner	90.2 (30.6–226.8)	18.5 (0.0–97.4)

Note: Differs from With Partner

* $p < 0.05$,

** $p < 0.01$

Table 4

Mean (se) Baseline and LPS-Stimulated IL-1β and TNF-α values (pg/ml).

	Field Cage	Day 8
IL-1β		
Baseline ***	1.9 (0.3)	2.0 (0.3)
LPS-stimulated	13,493.4 (1,562.3) **	10,017.5 (1,085.5)
TNF-α		
Baseline ***	115.2 (28.0)	125.7 (27.8)
LPS-Stimulated	15,552.3 (1,554.4)	18,172.7 (1,552.7)

Note:

** Differs from Day 8, $p < 0.01$;

*** differs from LPS-stimulated, $p < 0.001$

Table 5

Correlations between duration of the hunched posture and circulating cytokine levels on last day of separation.

	Round 1	Round 2
IL-10	.754***	.628*
IL-1β	.768***	.649*
TNF-α	.735**	.671*

Note:

* $p < 0.05$,

** $p < 0.01$,

*** $p \leq 0.005$