Mild Early Life Stress Enhances Prefrontal-Dependent Response Inhibition in Monkeys

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Background: *Severely stressful early experiences have been implicated in the pathophysiology of psychiatric disorders. In contrast, exposure to mild early life stress (i.e., stress inoculation) strengthens emotional and neuroendocrine resistance to subsequent stressors. Herein we extend this research to examine the effects of mild early life stress on cognition.*

Methods: *Squirrel monkeys were randomized to a mild intermittent stress (IS; n = 11) or nonstress (NS; n = 9) condition from 17 to 27 weeks postpartum. At 1.5 years of age, monkeys were assessed for response inhibition on a test previously shown to reflect prefrontal-dependent cognitive function.*

Results: *IS monkeys demonstrated fewer response inhibition errors compared with NS monkeys. There were no rearing-related differences in aspects of performance that did not require inhibitory control. Compared with NS monkeys, IS monkeys had lower basal plasma pituitary-adrenal stress hormone levels. No rearing-related differences on neuroendocrine measures obtained 15 minutes after testing were found.*

Conclusions: *Results from this experiment provide the first evidence that exposure to mildly stressful early experiences improves prefrontal-dependent response inhibition in primates. Combined with our previous data, findings from this animal model suggest that exposure to mild early life stress may enhance the development of brain systems that regulate emotional, neuroendocrine, and cognitive control.*

Key Words: Cognitive, HPA axis, response inhibition, prefrontal cortex, primate, stress inoculation, stress resistance

In keeping with Selye's (1974) classical theory that stressors may serve a beneficial function provided that the type and degree of stress are not excessive, data have begun to accumulate which suggest that exposure to mild forms of early life stress provide later resistance to psychosocial adversity (Garmezy et al 1984; O'Leary 1998; Rutter 1987). In children, for example, prior mildly stressful life events are associated with diminished emotional distress during hospital admission (Stacey et al 1970) and attenuated fearfulness in a day care setting (Holmes 1935). In adolescents, prior childhood exposure to mildly stressful events is associated with decreased cardiovascular response to psychologically stressful laboratory tests (e.g., mental arithmetic, video game performance, hand submersion in ice water) (Boyce and Chesterman 1990). These protective effects conferred in childhood may be enduring, as women (Forest 1991) and men (Khoshaba and Maddi 1999) better cope with adverse events (e.g., death or divorce of spouse, major accident or illness, job stress) if they have previously experienced and successfully coped with stressful circumstances in childhood.

Although these retrospective, correlational studies provide important preliminary evidence that mild early life stress may protect against adverse stress-related health outcomes, until recently, little research had causally linked mildly stressful early experiences to the manifestation of stress resistance. Because opportunities for controlled studies of stress responsivity in children are uncommon, we previously tested whether exposure to mild stress early in life (i.e., stress inoculation) strengthens emotional and neuroendocrine resistance to subsequent stressors in primates (Parker et al 2004). Young squirrel monkeys in these experiments were randomized to a mild intermittent stress (IS) or nonstress (NS) postnatal rearing protocol. Results from these experiments revealed that later in life, IS monkeys demonstrate lower plasma adrenocorticotropic hormone (ACTH) and cortisol concentrations under undisturbed conditions and exhibit diminished anxiety and blunted pituitary-adrenal hormone responses to emotionally challenging circumstances compared with NS monkeys.

Exactly how pervasive the effects of mild early life stress are in organizing human and primate development is as yet unknown. However, insofar as mild early stress decreases the development of characteristics associated with psychiatric disorders (e.g., anxiety, stress sensitization), other functional domains impaired in patients with depressive and anxiety disorders merit investigation. One such domain is cognition. Patients with depressive and anxiety disorders, for example, exhibit significant prefrontal-dependent cognitive deficits (Austin et al 2001; Kuelz et al 2004). These include impaired response inhibition on a variety of tests such as the Stroop Color Word Test, the Go/Nogo Task, and the Wisconsin Card Sorting Test (Bannon et al 2002; Kaiser et al 2003; Malloy et al 1989; Merriam et al 1999; Schatzberg et al 2000; Trichard et al 1995). In addition to poor performance on these neuropsychological tests, the inability to inhibit inappropriate thoughts (e.g., negative ideation), actions (e.g., compulsive engagement in ritualistic behavior), and emotions (e.g., panic, anxiety, feelings of worthlessness) are generally considered to be defining features of these psychiatric disorders. Thus, in this study, we examine the effects of mild early life stress on prefrontal-dependent cognitive function.

In recent years, test paradigms designed to assess prefrontal-dependent response inhibition in children have been modified for use in primates (Diamond 1990; Lyons et al 2000; Wallis et al 2001). Studies of both Old World and New World monkey species generally confirm anatomical specificity for these functional tests, with some species differences and performance variation (Hauser 1999). Structural prefrontal cortical lesions in adult rhesus macaques and marmosets have been shown to

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impair response inhibition, whereas hippocampal ablations leave response inhibition intact (Diamond 1990; Diamond et al 1989; Dias et al 1996; Roberts and Wallis 2000; Wallis et al 2001). Similar response inhibition impairments are also evident in adult vervet monkeys treated with drugs that deplete prefrontal dopamine utilization (Jentsch et al 1997; Taylor et al 1990). Using one such test, we examine prefrontal-dependent response inhibition and plasma stress hormone concentrations in the previously studied IS and NS monkeys (Parker et al 2004). Specifically, we test whether exposure to mild early life stress 1) improves later performance on a cognitive task that requires response inhibition, 2) produces enduring differences in basal stress hormone concentrations as documented previously at 35 weeks of age, and 3) alters pituitary-adrenal hormone concentrations during cognitive testing.

Methods and Materials

Subjects

Twenty squirrel monkeys of Guyanese origin (*Samiri sciureus*) were born at the Stanford Research Animal Facility and served as subjects. All monkeys received unique dye marks and number tags worn on necklaces to facilitate easy identification. Subjects were housed in natal groups comprised of three to four mother-infant pairs. Group composition was determined primarily by infant birth dates, to minimize developmental differences between infants in the same natal group, and genetic relatedness (i.e., paternal half siblings were not assigned to the same natal group). Whenever possible, assignment of male and female infants was balanced across natal groups.

Subjects were housed indoors in 1.8 x 1.2 x 1.8-m wire-mesh cages that were cleaned daily. Housing and testing occurred in climate-controlled rooms on a 12:12 light/dark cycle with an ambient temperature of 26°C. Monkeys had ad libitum access to water, food (e.g., commercial New World monkey chow, fresh fruits, vegetables), and a variety of toys to provide environmental enrichment. A sliding door in each home cage provided access to a small, portable capture cage. Monkeys were trained to enter the capture cage on vocal command to facilitate experimental manipulations. All procedures were approved by Stanford University's Administrative Panel on Laboratory Animal Care.

Early Rearing Protocol

Subjects remained undisturbed in their natal groups through postnatal week 16. At postnatal week 17, natal groups were randomly assigned to one of two experimental conditions. In one condition, 11 offspring (7 female subjects and 4 male subjects) from four natal groups were exposed to a mild intermittent stress (IS) inoculation protocol. From postnatal weeks 17 to 27, each subject was removed from the natal group for a 1-hour period once a week, placed in a cage (46 x 46 x 46 cm) adjacent to unfamiliar adult monkeys in a different room, and temporarily deprived of all forms of contact with the natal group. No more than one monkey from each natal group was separated on a given day. In the other condition, nine offspring (eight female subjects and one male subject) from three natal groups remained undisturbed as NS control subjects. In nature, squirrel monkeys locomote independently by postnatal week 5, forage successfully at postnatal week 7, and are weaned by postnatal week 16 (Boinski and Fragaszy 1989). By postnatal week 17, free-living monkeys are physically independent (Boinski and Fragaszy 1989), although they remain emotionally attached to their mothers as assessed by behavioral and pituitary-adrenal responses to maternal separation (Coe et al 1978; Levine et al 1978).

Following completion of these early rearing protocols, all monkeys were tested at postnatal weeks 35 and 50 for rearing-related differences in anxiety and neuroendocrine stress responsivity (Parker et al 2004). Subsequently, all monkeys were housed under standard laboratory conditions until 52 weeks of age, at which time mothers were removed from the premises and returned to the breeding colony. With the exception of routine husbandry procedures, monkeys remained undisturbed in their peer groups for the next 6 months until the beginning of cognitive testing at 1.5 years of age.

Cognitive Training and Testing

Prior to the beginning of the study, monkeys were thoroughly acclimated to the experimental environment for 2 weeks. Acclimation entailed daily exposure to the test apparatus for 1 hour each morning while monkeys were in their home cages and for 6 hours each afternoon while monkeys were in their test cages. During acclimation, training, and testing, monkeys were transported to the experimental room at 1200 hours each day and housed individually in wire-mesh cages (60 x 60 x 90 cm) that allowed visual, auditory, olfactory, and limited tactile contact between adjacent animals. Procedures occurred between 1400 and 1800 hours, and the order of cognitive assessment for IS and NS monkeys was evenly distributed across daily and weekly schedules.

The apparatus used in this experiment consisted of a clear Plexiglas box (8 x 8 x 8 cm) with one open side baited with a small marshmallow treat (Lyons et al 2000). The box was secured into a slot on a 61 x 13-cm horizontal tray that was attached to the front of each cage. For each trial, the box was manually advanced along the length of the tray at the same rate until it reached the front of each monkey's cage. Initially, all monkeys were administered 10 training trials per day for 7 consecutive days with the box opening always oriented straight toward the subject (Figure 1). Food retrieval was achieved by line-of-sight reaching into the center of the box. These 70 training trials served to reinforce as prepotent the straight-reaching response. Thereafter, monkeys were administered 10 test trials per day for 14 consecutive days with the orientation of the box opening varied systematically to assess inhibitory control of the straight-reaching response (Figure 1). On each day of testing, the box opening was oriented straight toward the subject on the 1st, 4th, 7th, and 10th trials. The box was rotated 90° so the opening was oriented to the monkey's right on the 2nd, 5th, and 8th daily trials. Finally, the box was rotated 90° so the opening was oriented to the monkey's left on the 3rd, 6th, and 9th daily trials. When the box was rotated 90° so the opening was oriented toward either side, the monkey was required to inhibit straight reaching and detour reach around the side of the box to retrieve the food treat. Two types of response inhibition errors were scored. Line-of-sight reaching errors were scored when a reach was aimed straight toward the center of the box when the opening was oriented toward either side. Detour-reaching errors were scored when a reach was aimed to the side of the box when the opening was oriented straight.

Each trial lasted 30 seconds or was terminated when the marshmallow was retrieved. Retrieval latency was measured from when the manually advanced box first abutted the front of the monkey's cage to when the monkey successfully grasped the marshmallow in its hand. Monkeys were not physically capable of retrieving the marshmallow unless the apparatus was fully advanced. During the ensuing 30-second intertrial interval, the

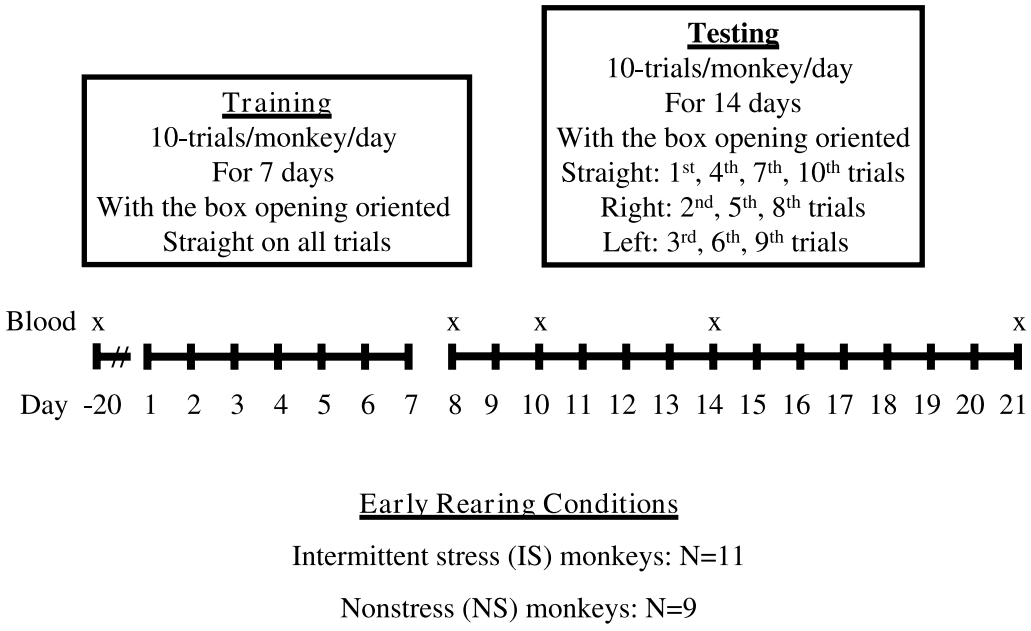

Figure 1. Schematic representation of the experimental design. Xs denote test days on which blood sampling occurred.

experimenter rebaited the box for the subsequent trial and recorded whether the marshmallow was successfully retrieved within 30 seconds, the latency to retrieve the marshmallow, and the number and direction of reach attempts. After completion of each daily test session, monkeys were returned to their home cages at 1830 hours. During acclimation, training, and testing, monkeys had ad libitum access to water and commercial New World monkey chow.

Blood Collection and Hormone Assays

Blood samples were collected 2 weeks before the acclimation procedures to establish baseline measures of ACTH and cortisol in an undisturbed state. Subsequently, blood samples were collected on the 1st, 3rd, 7th, and 14th days of cognitive testing to examine stress hormone concentrations 15 minutes after the completion of cognitive testing. Hormone samples from each monkey were collected at the same time of day and occurred for all monkeys between 1430 and 1800 hours to control for circadian variation (Zeitzer et al 2003).

Blood samples were collected from manually restrained monkeys while blood (1 mL) was drawn by femoral venipuncture with single-use polypropylene syringes containing 20 μL of ethylenediamine tetraacetic acid (EDTA). Each blood sample was immediately centrifuged and the plasma fraction was transferred to a polypropylene tube for storage at -80°C. Hormones were measured in duplicate using commercially prepared ACTH (Diasorin, Inc., Stillwater, Minnesota) and cortisol (Diagnostic Products Corporation, Los Angeles, California) radioimmunoassays as previously described (Lyons et al 1995). The intra-assay and interassay coefficients of variation for ACTH were 2.8% and 16.1%, and 2.4% and 5.6%, respectively, for cortisol. Assay sensitivity was 5 pg/mL for ACTH and 7 μg/dL for cortisol.

Data Analysis

The effect of postnatal rearing condition (IS vs. NS) on cognitive performance and neuroendocrine measurements was assessed with repeated measures analysis of variance (ANOVA) using least squares estimates from general linear models in the Multivariate General Linear Hypothesis (MGLH) module of Systat (Evanston, Illinois). Postnatal rearing condition was considered a between-subjects factor, and test session was considered the repeated measures, within-subjects factor. Gender was also examined, and as it did not significantly affect any outcome measure, it is not discussed below. The Geisser-Greenhouse correction was used to adjust for multiple comparisons across the repeated test-block factor (Keppel 1982). Total errors and basal hormone concentrations were analyzed using unpaired t tests with postnatal rearing condition as the independent variable. For all analyses, test statistics were evaluated with two-tail probabilities ($p < .05$) and descriptive statistics are presented as mean ± SEM.

Results

Cognitive Performance with the Box Opening Oriented Straight

During the 7 consecutive days of training when the box opening was always oriented straight, all monkeys rapidly retrieved all marshmallow treats (retrieval latency per trial was .32 ± .02 seconds). Of the 1400 training trials, 99.6% were successfully completed on the first reach attempt, and the remaining trials (.4%) were successfully completed on the second reach attempt. IS and NS monkeys did not differ on the number of correct retrievals, the retrieval latency, or the number of reach attempts.

IS and NS monkeys successfully retrieved almost all marshmallow treats during straight presentation trials 1, 4, 7, and 10 of training (which corresponded to the four straight presentation trials during testing) (100%), week 1 of testing (99.8%), and week 2 of testing (99.3%). Although monkeys maintained a consistent success rate across weekly trials, retrieval latency differed across weeks [$F(2,36) = 12.79$; $p = .001$]. During training, monkeys successfully completed each trial in .33 ± .02 seconds. However, by the second week of testing, the latency to complete each trial was 1.75 ± .33 seconds, five times longer than observed during training ($t_{19} = 4.359$; $p < .0001$). Although possible, it is unlikely

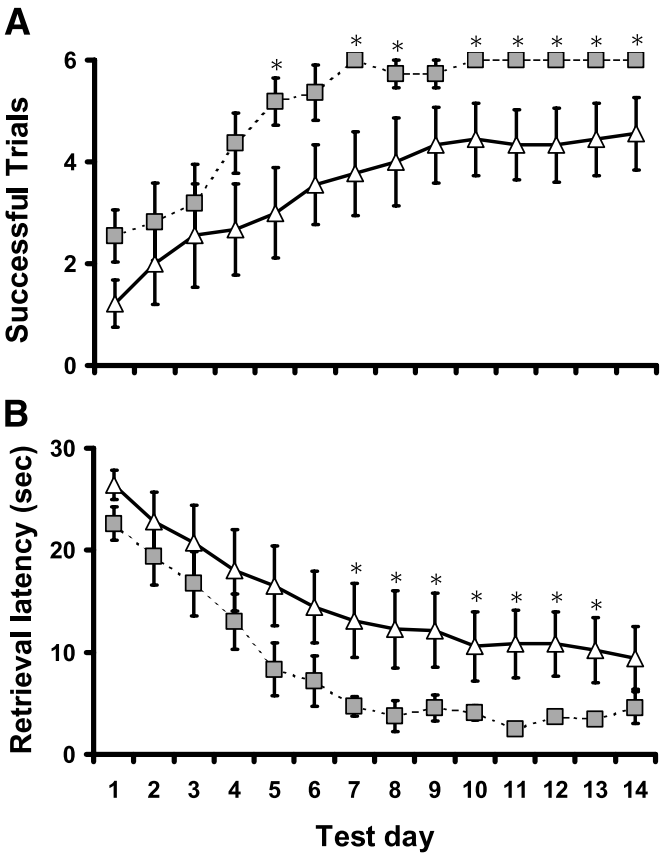

Figure 2. Rearing-related differences in cognitive performance when the box opening was oriented left or right. Measures of **(A)** total successful trials and **(B)** average retrieval latency for the six side-facing trials for each of the 14 consecutive days of testing are presented for monkeys previously exposed to intermittent stress (gray bars; $n = 11$) and nonstress (open bars; $n = 9$) protocols. Data are presented as mean ± SEM. Asterisks indicate days on which intermittently stressed monkeys differ significantly from nonstressed monkeys ($p < .05$).

that increased retrieval latency reflected decreased motivation, because monkeys also differed on the total number of reach attempts required to successfully retrieve the marshmallow across weeks [$F(2,36) = 14.48$; $p < .0001$]. Specifically, by the second week of testing, monkeys made more detour-reaching errors (i.e., they directed more reaches toward the sides of the box) before successfully retrieving the marshmallow treat than during training. These data suggest that the testing portion of this experiment increased cognitive demand in monkeys from both rearing conditions. No rearing-related differences in correct retrievals, retrieval latencies, or reach attempts were found within or across weeks for the straight-facing trials.

Cognitive Performance with the Box Opening Oriented Left or Right

IS monkeys successfully completed more daily side-facing trials during testing than NS monkeys [$F(1,18) = 5.17$; $p = .036$]. Although both IS and NS performance improved over time [$F(13,234) = 20.26$; $p < .0001$], IS monkeys nevertheless retrieved significantly more marshmallow treats than NS monkeys on 8 of the 14 test days (Figure 2A). Moreover, during the last 5 days of testing, all IS monkeys successfully completed 100% of their daily trials, whereas by the last day of testing, only 44% of NS monkeys did.

During the first day of testing, subjects required an average of 24.33 ± 1.16 seconds to complete each trial when the box opening was oriented left or right. This was 76 times longer than observed during matched trials 2, 3, 5, 6, 8, and 9 of training ($t_{19} = 20.761$; $p < .0001$). Over the 14 consecutive days of testing, retrieval latency decreased dramatically for both groups [$F(13,234) = 33.421$; $p < .0001$]. Although IS and NS monkeys did not differ on retrieval latencies during the first test week, IS monkeys exhibited faster retrieval latencies than NS monkeys during the second week of testing [$F(1,18) = 5.65$; $p = .029$] (Figure 2B).

On the first day of testing, all monkeys made an average of 11.77 ± 4.63 reach attempts per trial when the box was oriented to the side. Over time, both IS and NS monkeys made fewer reach attempts [$F(13,234) = 18.37$; $p < .0001$]. Nevertheless, during the last week of testing, when rearing-related differences were most clearly evident, IS monkeys more successfully inhibited inappropriate straight-reaching attempts compared with NS monkeys. In particular, IS monkeys made fewer line-of-sight straight-reaching response inhibition errors on all reach attempts (total number of reaches straight/total number of reaches = % reaches straight) ($t_{18} = 2.73$; $p = .014$) and on all first reach attempts (total number of first reach attempts/total number of first reaches = % first reaches straight) ($t_{18} = 2.10$; $p = .050$) compared with NS monkeys (Figure 3).

Neuroendocrine Assessment

Most blood samples (88%) were collected within 180 seconds from cage entry (median latency to sample collection = 122 seconds, range 61–404 seconds), and all but three samples (97%) were collected within 240 seconds. In keeping with reports that squirrel monkey plasma measures of ACTH and cortisol obtained within these time limits and using these procedures do not reflect disturbance effects from sampling per se (Lyons et al 1995, 1999b), sample collection latencies accounted for less than 1% of the variance in plasma levels of both ACTH and cortisol.

As reported previously (Parker et al 2004), IS monkeys demonstrated lower basal concentrations of ACTH ($t_{18} = 5.951$; $p < .0001$) and showed a similar trend for cortisol ($t_{18} = 2.008$;

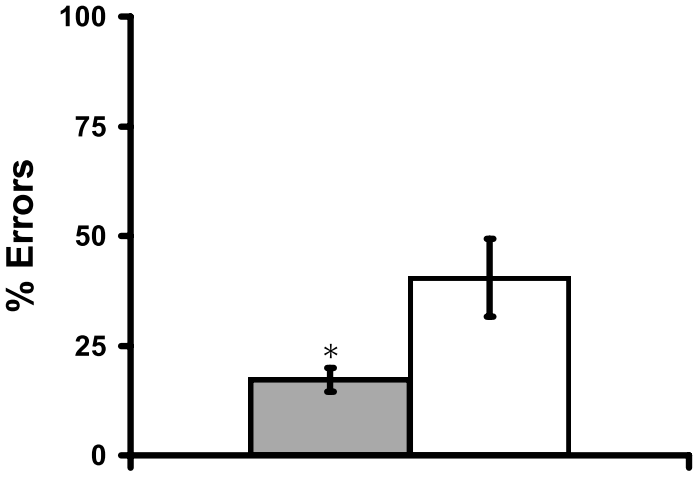

Figure 3. Rearing-related differences in line-of-sight response inhibition errors on all reach attempts when the box opening was oriented left or right are presented for monkeys previously exposed to intermittent stress (gray bars; $n = 11$) and nonstress (open bars; $n = 9$) protocols. Data are presented as mean ± SEM. The asterisk indicates that intermittently stressed monkeys differ significantly from nonstressed monkeys ($p < .05$).

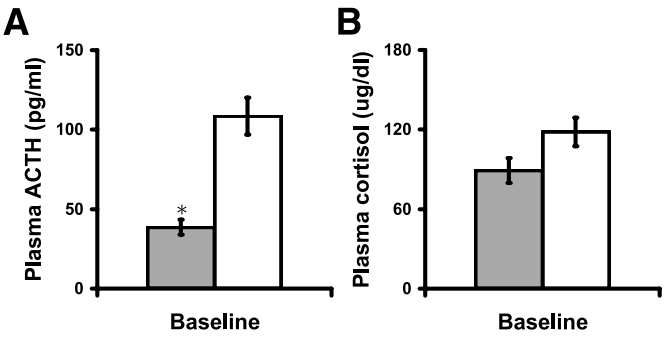

Figure 4. Rearing-related differences in pituitary-adrenal hormone levels at baseline. Plasma levels of **(A)** ACTH and **(B)** cortisol are presented for monkeys previously exposed to intermittent stress (gray bars; $n = 11$) and nonstress (open bars; $n = 9$) protocols. Data are presented as mean ± SEM. The asterisk indicates that intermittently stressed monkeys differ significantly from nonstressed monkeys ($p < .05$). The pound sign indicates a trend for intermittently stressed monkeys to differ from nonstressed monkeys ($p = .06$). ACTH, adrenocorticotropic hormone.

$p = .06$) compared with NS monkeys (Figure 4). Repeated measures analysis of variance determined that there were no rearing-related differences on neuroendocrine measures obtained 15 minutes after completion of cognitive testing, nor were there any differences in hormone levels across test days. No interaction effects of rearing condition and test day were found for either ACTH or cortisol.

Discussion

Juvenile squirrel monkeys previously exposed to mild IS during postnatal development subsequently demonstrate enhanced response inhibition compared with monkeys from a NS rearing condition. No other rearing-related differences in aspects of cognitive performance that did not require response inhibition were found (e.g., performance on straight-facing trials; motivation as indexed by latency to initiate marshmallow retrieval). These results are consistent with the notion that mild early life stress affects response inhibition, rather than nonspecific aspects of performance. Combined with our previous findings (Levine and Mody 2003; Lyons et al 1999a; Parker et al 2004), these data suggest the intriguing possibility that exposure to mild early stress may protect against the development of a diverse set of characteristics (e.g., anxiety, stress sensitization, impaired cognitive control) which are promoted by severely stressful early experiences (Heim and Nemeroff 2002; Kaufman et al 2000; Sanchez et al 1998) and associated with depressive and anxiety disorders (Austin et al 2001; Davidson 2002; Drevets 2001; Kuelz et al 2004; Parker et al 2003).

Although rearing-related differences in response inhibition emerged later in testing, the cognitive task proved challenging for all monkeys. On the first day of testing, for example, monkeys made an average of 11.77 ± 4.63 reach attempts per side-facing trial. Monkeys also took longer and made more attempts before successfully completing the straight-facing trials during the testing portion of the experiment. This latter finding suggests that the behavioral changes noted on the straight-facing trials were affected by "interference" from the side-facing trials and that monkeys were not relying solely on visual perception. Taken together, these data are consistent with the notion that the testing portion of this experiment increased cognitive demand in monkeys from both rearing conditions. Over consecutive test days, success rates improved in both groups. However, at peak levels of test performance, all of the IS monkeys completed all relevant test trials on the last 5 test days, whereas fewer than half of the NS monkeys achieved similar levels of success. This impaired performance by NS monkeys was not due to slower first reach initiation or decreased motivation but rather persistent straight-reaching attempts on the side-facing trials that precluded successful trial completion.

The ability to inhibit prepotent straight reaching is typically acquired by 3 to 4 months of age in rhesus macaque infants and 11 to 12 months of age in human infants (Diamond 1990). Because the 1.5-year-old monkeys in this experiment were well past the developmental age at which response inhibition is acquired, it is unlikely that rearing-related differences are due to a developmental delay in acquiring the ability to control prepotent reaching. Data from young humans indicate that inhibitory proficiency improves with development (Kochanska et al 1996). Comparison of juvenile and adult squirrel monkey performance on the response inhibition test supports this notion, as adult monkeys make fewer line-of-sight straight reaches on the side-facing trials and fewer detour reaches on the straight-facing trials compared with juvenile monkeys in this experiment (Lyons et al 2000). The possibility that these rearing-related differences in response inhibition reflect transient differences in task proficiency (i.e., NS monkeys eventually "catch up" to IS monkeys) should thus be considered. However, performance on tests of response inhibition is highly stable across time, such that children who perform worse than peers at one age score lower than peers at later ages (Kochanska and Knaack 2003; Kochanska et al 1996). Follow-up studies are required to determine whether rearing-related differences reflect transient developmental differences in task proficiency or whether exposure to mild early life stress permanently alters inhibitory control of behavior.

As previously demonstrated at 35 weeks of age (Parker et al 2004), IS monkeys at 1.5 years of age continue to exhibit lower basal levels of pituitary-adrenal stress hormones compared with NS monkeys. Similar to IS monkeys, securely attached children demonstrate lower levels of circulating basal cortisol compared with insecurely attached children (Gunnar et al 1996). In contrast, hypercortisolism has been observed in young humans previously exposed to severely stressful early experiences. These reports include infants of emotionally unavailable mothers (Bugental et al 2003), multiply (i.e., sexually, physically, and emotionally) abused school-aged children (Cicchetti and Rogosch 2001), former Romanian orphans (Gunnar et al 2001), and adolescents who experienced early postnatal maternal depression (Halligan et al 2004). Neuropsychological performance was not assessed in these pediatric studies, and it remains unknown whether hypercortisolemic children exhibit prefrontal-dependent cognitive impairments similar to those reported for hypercortisolemic adults (Belanoff et al 2001; Erickson et al 2003). However, it is well established that severely stressful early experiences lead to pediatric cognitive deficits, and reports include decreased intelligence quotient (IQ), impaired reading comprehension, and delayed language development (Castle et al 1999; Delaney-Black et al 2002; Hoffman-Plotkin and Twentyman 1984; Koenen et al 2003; Oates et al 1984). Thus, data from these various studies suggest that insofar as sustained exposure to glucocorticoids increases the risk for cognitive and emotional deficits, broadly speaking, comparatively low basal ACTH and cortisol concentrations in healthy young humans and monkeys may serve a protective function during development.

In contrast to basal measures of plasma ACTH and cortisol, no rearing-related differences in neuroendocrine measures obtained 15

minutes following completion of daily testing were found. This is also in contrast to previous findings that IS monkeys demonstrate blunted stress hormone responses to an emotionally challenging novel environment compared with NS monkeys (Levine and Mody 2003; Parker et al 2004). Previous research has shown that performance on prefrontal-dependent cognitive tests is influenced by stress, as cognitive performance in primates is impaired by noise stress (Arnsten and Goldman-Rakic 1998) and stress-level glucocorticoid administration (Lyons et al 2000). However, the extensive acclimation period prior to testing in this experiment evidently eliminated any rearing-related differences in pituitary-adrenal hormone responses to the testing procedures. Because the only experimental difference between IS and NS monkeys was postnatal exposure to mild early life stress, the biology which underlies these cognitive differences is likely derived from these early experiences.

Studies in rodents have long supported the notion that early experiences alter developing brain systems and permanently enhance cognitive function. One of the first reports of this kind indicated that neonatal electric shock treatment improves adult performance on a multiple-unit maze (Griffiths and Stringer 1952). Since then, maternal separations ranging from 3 minutes to 24 hours (Lehmann et al 1998, 1999; Tang et al 2003) and "handling" (Levine 1956; Meaney et al 1988) have been shown to enhance conditioned avoidance learning, spatial learning, and social memory compared with undisturbed animals. In rodents, these permanent alterations in cognitive performance have been attributed to enduring increases in maternal care induced by repeated pup manipulation (Meaney 2001). Whether or not a similar phenomenon occurs in mother squirrel monkeys as a consequence of early rearing protocols merits investigation.

The possibility that mildly stressful early experiences exert their effects directly on young monkeys should also be considered. Specifically, mild stress may have directly altered the neural substrates involved in cognitive function or indirectly influenced cognitive performance by primarily changing emotion regulation. The available data do not help to distinguish between these two possibilities. Nevertheless, it should be noted that our rearing protocol was initiated during a period of increasing offspring physical and emotional independence (Boinski and Fragaszy 1989). Far from exceeding the young monkeys' coping abilities, the intermittent and mildly stressful separation experiences may have provided important opportunities for IS monkeys to develop the capacity for emotion regulation essential for autonomous functioning. NS monkeys, deprived of these critical emotional challenges, failed to adequately develop this capacity. Thus, when NS monkeys were faced with the demanding cognitive test, they engaged in more impulsive and perseverative behavior than IS monkeys, who were better equipped to deal with such challenges.

A final aspect of this study that merits comment is that early stressful experiences–whether mild or severe–have been found in rodents (Kaufman et al 2000; Meaney et al 1991), primates (Parker et al 2004; Suomi 1997), and humans (Forest 1991; Glaser 2000; Kaler and Freeman 1994) to alter emotional, cognitive, and hypothalamic-pituitary-adrenal (HPA) axis function. A better understanding of how early life stress modifies the development of brain systems involved in what has been termed in humans as effortful control (Kochanska et al 2000), cognitive control (Botvinick et al 2001), or self-regulation (Posner and Rothbart 2000) will more clearly elucidate the relationships between emotion, cognition, and HPA axis physiology. Thus far, lesion and imaging studies have linked the regulation of emotional and cognitive control to specific subdivisions of the anterior cingulate cortex (ACC) and the prefrontal cortex (PFC) (Aron et al 2004; Bush et al 2000; Carter et al 1999; Davidson 2002; Ochsner et al 2002; Roberts and Wallis 2000). These brain regions have also been implicated in HPA axis regulation, as fronto-cingulate transitional cortex, and medial PFC play a role in attenuating HPA responses to stress and in "turning off" the stress response (Bonne et al 2003; Diorio et al 1993; Meaney et al 1996; Sullivan and Gratton 2002). Recently, our laboratory reported that early experiences alter prefrontal cortical volumes in monkeys (Lyons et al 2002), and experiments are underway to longitudinally examine rearing-related differences in structural brain morphology as they relate to emotional, cognitive, and neuroendocrine control in IS and NS monkeys.

In summary, prospective evidence from this experiment supports the notion that mild early life stress enhances response inhibition in primates. We emphasize, however, that stressful events–even mild ones–may exert deleterious consequences if they exceed the developing organism's ability to cope with them. Thus, as with any developmental event, the type, timing, duration, and severity of a given stressor, within a given species, are likely to be important factors in determining whether early experiences ultimately produce a protective or deleterious outcome. With continued investigation using prospective, controlled studies in monkeys and retrospective studies in humans, a comprehensive understanding of the effects of mild early life stress may provide a foundation for developing novel approaches for treatment and prevention of depressive and anxiety disorders.

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