

Prospective Investigation of Stress Inoculation in Young Monkeys

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Background: Retrospective studies in humans have identified characteristics that promote stress resistance, including childhood exposure to moderately stressful events (ie, stress inoculation).

Objective: Because of limited opportunities for prospective studies in children, we tested whether exposure to moderate stress early in life produces later stress resistance in a primate model.

Design and Main Outcome Measures: Twenty squirrel monkeys were randomized to intermittent stress inoculation (IS; n=11) or a nonstress control condition (NS; n=9) from postnatal weeks 17 to 27. At postnatal week 35, each mother-offspring dyad underwent testing in a moderately stressful novel environment for inferential measures of offspring anxiety (ie, maternal clinging, mother-offspring interactions, object exploration, and food consumption) and stress hormone concentrations (corticotropin [ACTH] and cortisol). At postnatal week 50, after acclimation to an initially stressful wire-mesh box attached to the home cage, independent young monkeys underwent testing for inferential measures of anxiety (ie, voluntary exploration and play) in the box.

Results: In the novel environment test, IS compared with NS offspring demonstrated diminished anxiety as measured by decreased maternal clinging ($P=.02$), enhanced exploratory behavior ($P=.005$), and increased food consumption ($P=.02$). Mothers of IS offspring accommodated offspring-initiated exploration ($P=.009$) and served as a secure base more often compared with NS mothers ($P=.047$). Compared with NS offspring, IS offspring had lower basal plasma ACTH ($P=.001$) and cortisol ($P=.001$) concentrations and lower corticotropin ($P=.04$) and cortisol ($P=.03$) concentrations after stress. In the subsequent home-cage wire-box test, IS offspring demonstrated enhanced exploratory ($P<.001$) and play ($P=.008$) behaviors compared with NS offspring.

Conclusions: These results provide the first prospective evidence that moderately stressful early experiences strengthen socioemotional and neuroendocrine resistance to subsequent stressors. This preclinical model offers essential opportunities to improve our understanding and enhance prevention of human stress-related psychiatric disorders by elucidating the etiology and neurobiology of stress resistance.

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EMOTIONAL AND NEUROBIOLOGICAL responses to psychosocial adversity show striking individual variation.¹⁻⁴ Some people who experience a stressful event develop mental health problems such as depressive or anxiety disorders, whereas others do not. Researchers have sought to identify attributes associated with resilient or stress-resistant individuals,^{5,6} with the expectation that understanding the etiology of resilience could increase prevention of stress-induced mental health problems by enhancing resistance to stress and adversity.^{7,8}

Garnezy et al⁹ formulated 3 conceptual frameworks (compensatory, protective factor, and challenge) to investigate the impact of stress. To date, research ef-

orts have focused almost exclusively on the characteristic-based compensatory and protective factor approaches. On the basis of these 2 frameworks, empirical studies have identified the following 3 categories of characteristics that ameliorate risk status: positive personality dispositions, a supportive family, and an extrafamilial support system that reinforces active and successful coping efforts.^{6,10}

Far less researched, but of equal importance, is the challenge approach. In this process-oriented framework, stress is viewed as a potential enhancer of future competence, provided the type and degree of stress are not excessive.⁹ Severe stress often leads to dysfunction,¹¹⁻¹⁴ whereas moderate stress provides a challenge that, when overcome, produces com-

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petence in the management of and increased resistance to future stressful circumstances.^{5,15} Various descriptions of inoculating,^{2,16} immunizing,^{15,17,18} steeling,^{7,19} toughening,^{20,21} and thriving,²² the notion that prior stressful experiences may strengthen an organism's resistance to subsequent stressors has long been recognized.

Although frequently noted in the resilience literature, empirical investigation of stress inoculation has lagged behind theoretical conjecture. Nevertheless, several studies have documented, often unexpectedly, evidence supporting stress inoculation in humans. In adults, experienced survivors of floods and earthquakes exhibit lower anxiety²³ and less depressed affect²⁴ after encounters with the same disaster compared with inexperienced survivors. Similarly, survivors of torture with prior psychological preparedness training and previous experience with similar traumatic stressors exhibit fewer symptoms of anxiety, depression, and posttraumatic stress disorder compared with unprepared, inexperienced torture survivors, despite the fact that individuals in the latter group experienced less severe trauma.²⁵ Although some sampling bias may occur (ie, individuals severely affected by the initial stressor may not be included in studies of repeated exposure), this documented effect can be described as specific inoculation because stress inoculation and manifest resistance are associated with the same, or similar, stressor.

Of greater interest are findings that indicate that stressful experiences may confer general inoculation or cross-immunization.²⁶ That is, exposure to one stressor may strengthen resistance to different stressors encountered later in life. In children and adolescents, prior stressful events are associated with diminished emotional distress associated with hospital admission,²⁷ attenuated fearfulness in a day-care setting,²⁸ and decreased cardiovascular responses to psychologically stressful laboratory tests (eg, mental arithmetic, video game performance, and hand submersion in ice water).¹⁶ In addition, in adults, women are found to better cope with stressful events (eg, spousal loss, major accident, and illness) if they previously experienced and successfully coped with stressful circumstances in childhood.²⁹ In contrast, unsuccessful coping efforts often lead to deleterious outcomes.¹

Despite their mostly retrospective and correlational nature, these reports provide important socioemotional evidence that documents the existence of stress inoculation in humans. Although the neurobiological underpinnings of this effect are largely unknown, data from developmental studies of primates by our group^{30,31} suggest that the hypothalamic-pituitary-adrenal (HPA) axis may provide a neural basis for programming stress resistance in the developing child. In human and non-human primates, psychosocial stressors are perceived by the brain, which activates hypothalamic release of corticotropin releasing factor, stimulates pituitary release of corticotropin (ACTH), and induces glucocorticoid (eg, cortisol) secretion from the adrenal cortex. During acute or manageable stress, glucocorticoids exert negative feedback on the brain and pituitary to turn off the HPA stress response.³² However, during chronic or unmanageable stress, the HPA axis frequently becomes dysregulated, and

this process has been implicated in the pathophysiology of depression and anxiety disorders.^{33,34}

It is now well established that chronic stress experienced during childhood often leads to impaired acquisition of appropriate coping skills, heightened HPA responsiveness to stress, and increased risk for the development of adult mental health problems.³⁵⁻³⁹ However, moderate exposure (ie, inoculation) to stressors early in life may protect against these deleterious effects. In particular, stress resistance may occur through manageable exposure to moderately stressful events that temporarily activate the HPA axis but permanently alter neuroendocrine sensitivity to subsequent stressors by fostering the acquisition of coping strategies that safeguard against the development of stress-related disorders.^{7,40} Because opportunities for prospective, controlled studies are limited in children, we prospectively tested the general inoculation theory of stress resistance in a primate model in the following experiments.

METHODS

SUBJECTS

Twenty squirrel monkeys (*Saimiri sciureus*) of Guyanese origin, born at Stanford University, Stanford, Calif, served as subjects. Monkeys received dye marks and number tags worn on necklaces to facilitate easy identification. Natal group composition was primarily determined by birth dates to minimize developmental differences between infants and genetic relatedness (ie, paternal half siblings were not assigned to the same natal group). Whenever possible, sex assignment was balanced across natal groups.

Subjects were housed in 1.8 × 1.2 × 1.8-m wire-mesh cages in groups of 3 or 4 mother-infant pairs. Animals were housed and tested in climate-controlled rooms on a light-dark cycle (12:12-hour ratio) with an ambient temperature of 26°C. Monkeys had ad libitum access to water, commercial monkey chow, fresh fruits, and vegetables. Monkey cages were cleaned daily. A sliding door in each home cage provided access to a small, portable capture cage. Monkeys were pretrained to enter the capture cage on voice commands to facilitate the experimental manipulations. All procedures were approved by the Stanford University Administrative Panel on Laboratory Animal Care.

STRESS INOCULATION PROTOCOL

In nature, squirrel monkeys locomote independently by postnatal week 5, forage successfully at postnatal week 7, and are weaned by postnatal week 16.⁴¹ By postnatal week 17, free-living monkeys are biologically independent,⁴¹ although they remain emotionally attached to their mothers as assessed by behavioral and pituitary-adrenal responses to maternal separation.^{42,43} In this experiment, subjects remained undisturbed in natal groups until postnatal week 17, at which time natal groups were randomly assigned to 1 of 2 experimental conditions. In one condition, 11 offspring (7 female and 4 male) from 4 natal groups were exposed to an intermittent stress inoculation (IS) protocol. Once every week from postnatal weeks 17 through 27, each young monkey was removed from the natal group for 1 hour, placed in a cage (46 × 46 × 46 cm) adjacent to unfamiliar adult monkeys in a different room, and temporarily deprived of all forms of contact with the natal group. Intermittent separations induce isolation calls, locomotor agitation, and acute increases in cortisol levels, which return to

baseline soon after reunion.⁴⁴⁻⁴⁶ No more than 1 monkey from each natal group was separated on a given day. In the other condition, 9 offspring (8 female and 1 male) from 3 natal groups remained undisturbed as nonstressed (NS) controls. Although sample sizes in this experiment are inadequate to detect the small effects typically found in clinical trials, samples of 9 to 11 monkeys per treatment condition are commonly used in preclinical studies to detect large effects on behavioral and neuroendocrine measures.^{45,47,48}

EXPERIMENT 1: NOVEL ENVIRONMENT TEST

Description

At postnatal week 35, each mother-offspring dyad was removed from the natal group and transported to a novel test cage (60 × 60 × 90 cm) in an unfamiliar room that did not contain other monkeys. The cage and room used for testing were different from those used for the IS protocol. The novel environment contained polyvinyl chloride perches and a variety of familiar and unfamiliar objects. Monkeys also had access to biscuits, cantaloupe, marshmallows, and water throughout testing. All food was replenished and the cages were cleaned between tests. Tests lasted 30 minutes, and each mother-offspring dyad underwent testing once a day for 5 consecutive days. All tests occurred between 3 and 6 PM, and testing occurred at the same time each day for a given dyad. Each week, 3 to 4 dyads underwent testing (no two from the same natal group), and the order of testing for IS and NS offspring was evenly distributed across daily and weekly schedules.

Behavior Scoring

Using a computer-aided recording program, one of us (K.J.P.) collected behavior. Offspring anxiety was inferentially assessed by dorsal contact (ie, the species-typical offspring riding posture on the mother's shoulders and upper back), exploration of unfamiliar objects, and food consumption. The following measures were analyzed: (1) latency to terminate dorsal contact, (2) latency to explore the first object, (3) total familiar and unfamiliar object exploration counts (scored each time a monkey mouthed or touched an object), and (4) total food consumption counts (scored each time a monkey placed food in its mouth). The number and quality of social transactions between mothers and offspring were also recorded as previously described.^{49,50} Briefly, transactions were initiated by attempts to change the immediate state of association between the mother and offspring by means of breaking or making dorsal contact initiatives, or making or breaking affiliative contact (ie, side-by-side huddling) initiatives. Successful attempts were scored whenever initiatives were accommodated by the target. Failed attempts were scored whenever initiatives were overtly resisted by the target (eg, an infant tried to move off the mother's back and the mother prevented this initiative by means of species-typical "shoulder scooping").

Blood Collection and Hormone Assays

Blood samples were collected from young monkeys 10 days before and 10 days after experimental manipulations to establish baseline levels of ACTH and cortisol. Blood samples were also collected immediately after testing at the first and last sessions to examine stress hormone concentrations after experimental manipulation. Blood sampling occurred between 3:30 and 6 PM to control for circadian variation.⁵¹ Samples were collected from manually restrained monkeys while blood (0.8 mL) was drawn by means of femoral venipuncture with single-use polypropylene syringes containing 20 μ L of EDTA. Sixty-eight (85%) of the

80 plasma samples were collected within 180 seconds from cage entry (median, 122 seconds; range, 63-403 seconds), and all but 4 samples (5%) were collected within 4 minutes. Each sample was immediately centrifuged at 4°C, and the plasma fraction was stored at -80°C. Hormone levels were measured in duplicate using commercially prepared ACTH (DiaSorin, Inc, Stillwater, Minn) and cortisol (Diagnostic Products Corporation, Los Angeles, Calif) radioimmunoassays as previously described.⁵² The intra-assay and interassay coefficients of variation were 9.8% and 11.5%, respectively, for ACTH, and 6.0% and 5.5%, respectively, for cortisol. Assay sensitivity was 7 pg/mL (1.5 pmol/L) for ACTH and 3 μ g/dL (82.8 nmol/L) for cortisol.

EXPERIMENT 2: HOME-CAGE TEST

Description

To assess the generality of rearing effects in a different test setting, a stainless steel wire-mesh box (46 × 46 × 46 cm) was attached at floor level to each group's home cage during postnatal week 50. Free access to the box was provided through an interconnecting opening (30 × 30 cm). On initial presentation, the box was anxiety provoking to all monkeys, as it instigated robust alarm calling. Before testing, monkeys were acclimated to the box for 1 h/d for 7 consecutive days, by which time alarm calling had ceased. Each natal group then underwent testing for 5 min/d for 4 consecutive days. Test sessions occurred between 2:45 and 4:30 PM, and the order of testing for the IS and NS natal groups was evenly distributed across daily schedules.

Behavior Scoring

Behavior was collected by one of us (K.J.P.) using the computer-aided recording program. Offspring anxiety was inferentially assessed by means of voluntary exploration and play behavior in the wire-mesh box. The following behavior measures were analyzed for each monkey: (1) entry latency (scored when the monkey's entire body, excluding the tail, first entered the box), (2) total number of entries, and (3) duration of time spent in the box (the latter two scored when the monkey's entire body, excluding the tail, was in the box). The presence of play behavior was scored when monkeys wrestled, exhibited exaggerated leaping and chasing, and/or carried toys into the box.⁵³

DATA ANALYSIS

We assessed the effect of postnatal rearing experience (IS vs NS) on behavior and hormone measurements with repeated-measures analysis of variance using least squares estimates from general linear models in the MGLH module of Systat (Systat Software, Inc, Point Richmond, Calif). Postnatal rearing condition was considered a between-subjects factor, and test session was considered the repeated, within-subjects factor. We used the Geisser-Greenhouse correction to adjust for multiple comparisons across the repeated test-block factor.⁵⁴ For latency to terminate dorsal contact and total dorsal contact duration, the offspring's body weight, expressed as a percentage of its mother's, was used as a covariate to examine whether heavier offspring were carried for less time independent of rearing conditions. Hormone values were logarithmically transformed to stabilize the variance across groups and to satisfy the equal variance assumptions of parametric statistical tests. We also analyzed mother-offspring transactions (summed across sessions) using independent, 2-tailed *t* tests. The presence or absence of play behavior for each test session was analyzed using Pearson χ^2 tests, and α error was adjusted to protect against

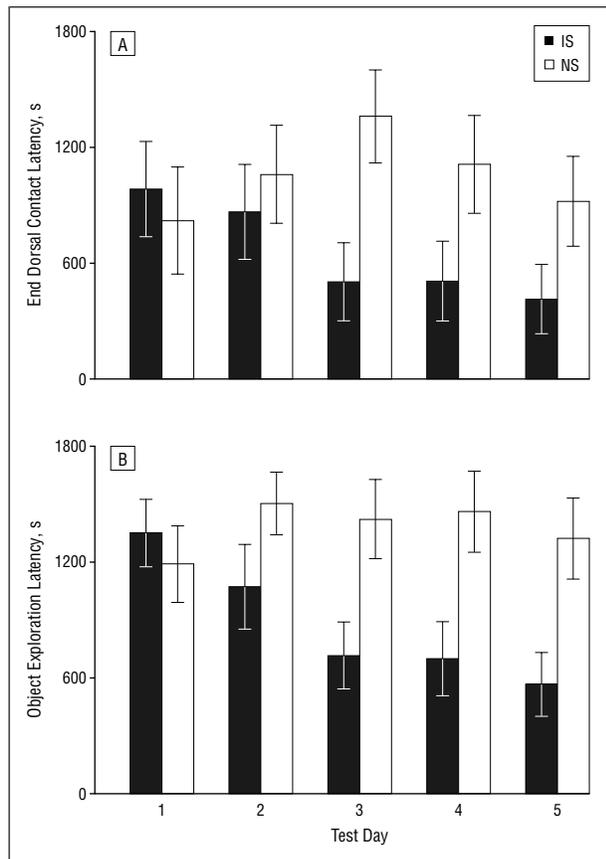


Figure 1. Rearing-related differences in mother-offspring dorsal contact and offspring exploratory behavior. Measures of latency to end dorsal contact (A) and latency to first explore an object (B) for each of the 5 consecutive test days of experiment 1 are presented for monkeys exposed to intermittent stress (IS; $n=11$) and nonstress (NS; $n=9$) protocols. Data are presented as mean \pm SEM.

multiple comparisons (ie, differences were considered significant when $P < .0125$). Descriptive statistics are presented as mean \pm SEM.

RESULTS

EXPERIMENT 1: NOVEL ENVIRONMENT TEST

Offspring Anxiety Measurements

Consistent with the stress inoculation effect, the novel environment was less anxiety provoking for IS offspring as rearing-related differences in dorsal contact termination latency, object exploration latency, unfamiliar but not familiar object exploration, and food consumption were found. During the first test session, 18 mother-offspring pairs (90%) entered the novel environment with the offspring clinging to the mother's back. At 35 weeks of age, young monkeys rarely cling to their mothers other than during times of significant emotional distress. However, across consecutive days, the novel environment was less anxiety provoking for IS mother-offspring pairs, as IS pairs more rapidly terminated dorsal contact ($F_{4,68}=4.56$ [$P=.02$]) (**Figure 1**). These observed differences in dorsal contact termination were not due to differences in offspring weight (IS and NS offspring weights did not dif-

fer), and heavier offspring did not hasten dorsal contact termination.

The IS offspring were also faster to explore a first object than NS offspring ($F_{1,18}=7.21$ [$P=.02$]), and this effect was time dependent ($F_{4,72}=3.21$ [$P=.03$]) (**Figure 1**). This rearing condition by test session interaction occurred because NS offspring demonstrated consistently high exploration latencies across test sessions, whereas IS offspring exhibited decreased first-object exploration latencies across consecutive test days ($F_{1,10}=20.02$ [$P=.001$]).

The IS and NS offspring exhibited similar amounts of familiar object exploration and did not differ on familiar object exploration counts (**Figure 2**). However, IS and NS offspring demonstrated an overall difference in exploration of unfamiliar objects ($F_{1,18}=5.59$ [$P=.03$]), and this effect was time dependent ($F_{4,72}=4.92$ [$P=.005$]) (**Figure 2**). Analysis within rearing condition showed that NS offspring remained reluctant to explore unfamiliar objects over time, whereas IS offspring demonstrated an increase in unfamiliar object exploration across consecutive test sessions ($F_{1,10}=14.47$ [$P=.003$]).

The IS and NS offspring also differed in food consumption counts overall ($F_{1,18}=4.59$ [$P=.046$]) and by test session ($F_{4,72}=3.77$ [$P=.02$]) (**Figure 2**). As observed previously with unfamiliar object exploration, NS offspring demonstrated consistently low food consumption across test sessions, whereas IS offspring showed an increase in food consumption over consecutive test sessions ($F_{1,10}=16.39$ [$P=.002$]).

Mother-Offspring Social Transactions

Mothers and offspring engaged in a total of 6195 social transactions across all test sessions. Transactions consisted of the following initiatives: breaking dorsal contact (10%), making dorsal contact (4%), making affiliative contact (49%), and breaking affiliative contact (37%).

Mothers and offspring did not differ on attempts to terminate dorsal contact, and there were no effects of rearing condition on offspring- or mother-initiated attempts. Although IS and NS offspring did not differ on the total number of times they attempted to leave their mothers, attempts by the IS offspring to break dorsal contact were more frequently accommodated by their mothers ($93\% \pm 5\%$) than were those of NS offspring ($45\% \pm 17\%$) ($t_{12}=3.10$ [$P=.009$]).

A total of 232 attempts to make dorsal contact were recorded after initial dorsal contact was broken. Most of these attempts (82%) were initiated by offspring, and there were no effects of rearing condition on offspring- or mother-initiated dorsal contact attempts. Of the offspring that attempted to regain the species-typical riding posture during testing, $37\% \pm 8\%$ of their initiatives to make dorsal contact were accommodated by their mothers. The IS and NS offspring did not differ in the proportion of initiatives that were accommodated.

As with dorsal contact attempts, offspring also made more affiliative overtures than did mothers, and offspring initiatives represented 82% of total affiliative overtures. Although IS and NS offspring did not differ on total affiliative overtures, they differed on the percentage of

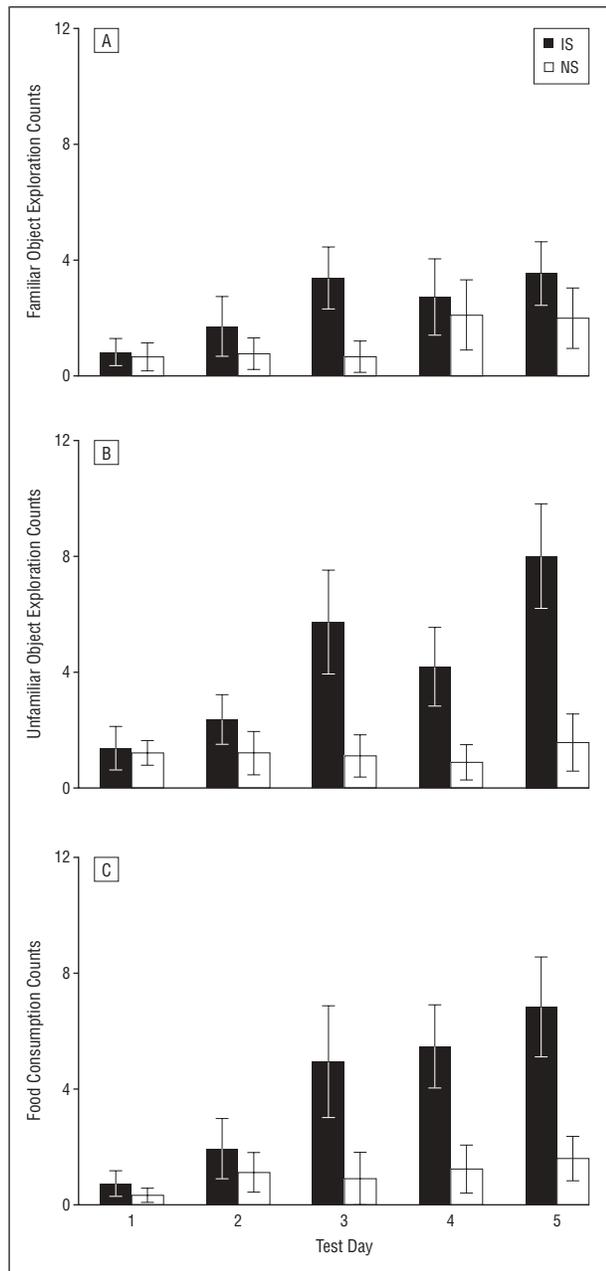


Figure 2. Rearing-related differences in offspring-initiated behavior. Measures of familiar objects exploration (A), unfamiliar objects exploration (B), and food consumption (C) for each of the 5 consecutive test days of experiment 1 are presented for monkeys exposed to intermittent stress (IS; $n=11$) and nonstress (NS; $n=9$) protocols. Data are presented as mean \pm SEM.

time their overtures were accepted ($78\% \pm 5\%$ for IS vs $61\% \pm 6\%$ for NS; $t_{15}=2.16$ [$P=.047$]). Offspring and mothers did not differ on who terminated side-by-side affiliative contact, and there were no rearing condition effects on offspring- or mother-initiated overtures. All targets accommodated termination of affiliative contact.

Offspring Stress Hormone Measurements

Main effects of rearing condition for ACTH ($F_{2,36}=5.76$ [$P=.01$]) and cortisol ($F_{2,36}=5.77$ [$P=.009$]) concentrations were found (**Figure 3**). At baseline, IS offspring

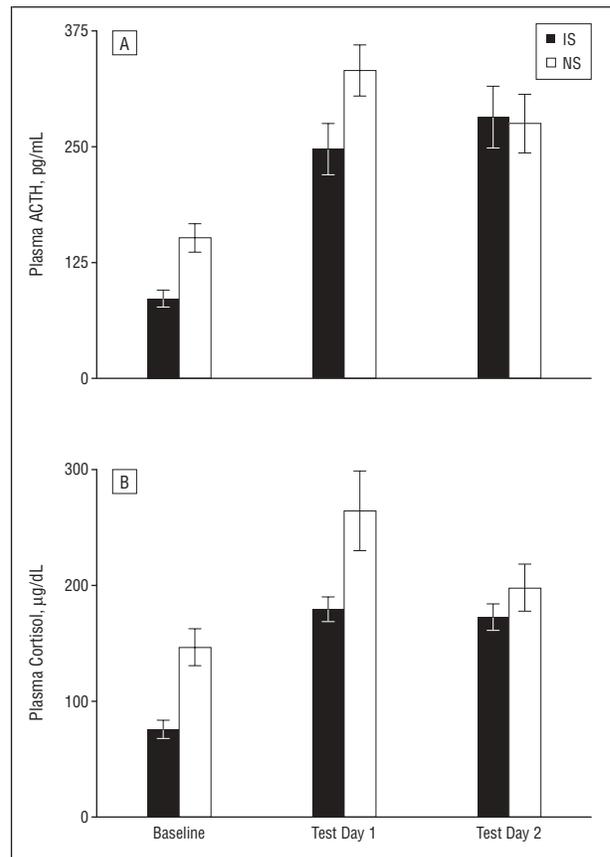


Figure 3. Rearing-related differences in offspring pituitary-adrenal hormone levels at baseline and after test days 1 and 5 of experiment 1. Plasma levels of corticotropin (ACTH) (A) and cortisol (B) are presented for monkeys exposed to intermittent stress (IS; $n=11$) and nonstress (NS; $n=9$) protocols. Data are presented as mean \pm SEM. To convert ACTH to picomoles per liter, multiply by 0.22; cortisol to nanomoles per liter, multiply by 27.59.

had significantly lower ACTH ($F_{1,18}=15.68$ [$P=.001$]) and cortisol ($F_{1,18}=16.47$ [$P=.001$]) levels than did NS offspring. Although the novel environment increased stress hormone levels in all monkeys, IS compared with NS offspring demonstrated lower stress hormone levels on the first day of testing (for ACTH, $F_{1,18}=4.94$ [$P=.04$]; for cortisol, $F_{1,18}=5.47$ [$P=.03$]), consistent with a stress inoculation effect. By the fifth day of testing, however, rearing-related differences in stress hormone levels were no longer discerned.

EXPERIMENT 2: HOME-CAGE TEST

Offspring Anxiety Measurements

Like the novel environment, the home-cage test was less anxiety-provoking for IS compared with NS offspring (**Figure 4**). Specifically, IS offspring exhibited decreased exploration latencies compared with NS offspring ($F_{1,18}=107.27$ [$P<.001$]). The IS monkeys also more frequently entered ($F_{1,18}=76.27$ [$P<.001$]) and spent more time in ($F_{1,18}=36.71$ [$P<.001$]) the wire-mesh box compared with NS offspring. The IS natal groups also exhibited more play behavior in the box than did NS offspring ($\chi^2_1=7.00$ [$P=.008$] for each test session), such that all IS but no NS natal groups played in the box during

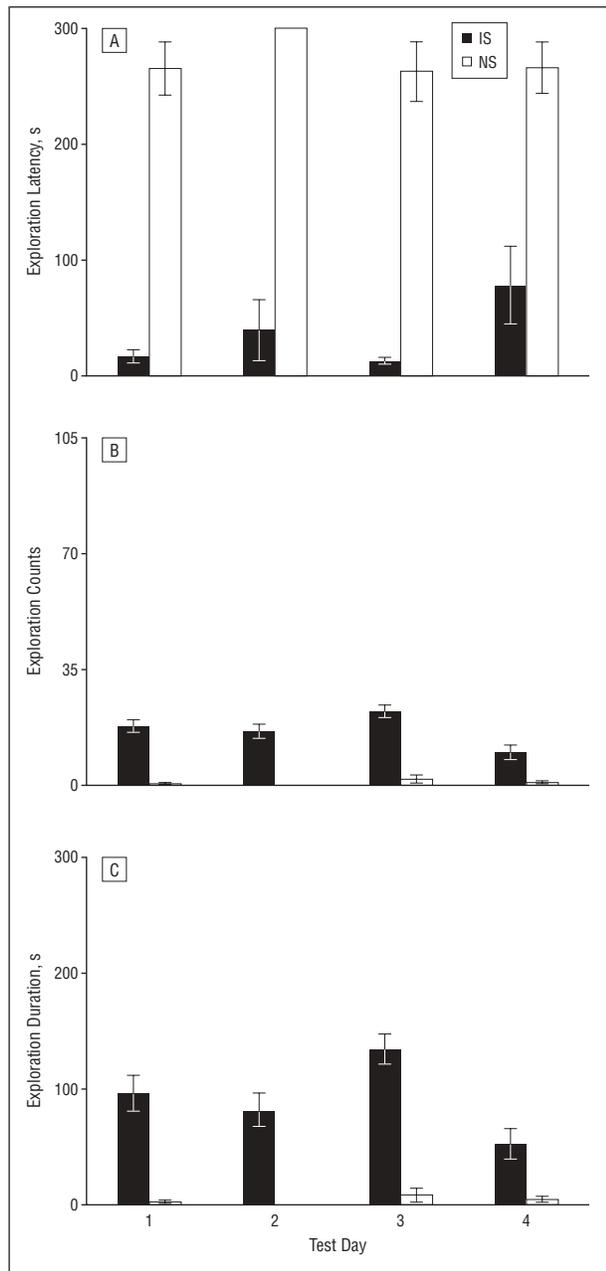


Figure 4. Rearing-related differences in offspring exploratory behavior. Measures of exploration latency (A), exploration occurrences (B), and exploration duration (C) for each of the 4 consecutive test days of experiment 2 are presented for monkeys exposed to intermittent stress (IS; $n=11$) and nonstress (NS; $n=9$) protocols. Data are presented as mean \pm SEM.

testing. Mothers did not enter the test box, and no mother was observed to physically or vocally restrict offspring entry into the box.

COMMENT

Results from these experiments provide the first prospective evidence of stress inoculation in primates. Specifically, squirrel monkeys previously inoculated with intermittent periods of moderate stress (IS condition) during early development demonstrated diminished anxiety in 2 different experimental paradigms compared with NS

control monkeys. These findings in monkeys parallel retrospective data in humans^{16,23-25,27-29} and suggest that controlled exposure to moderately stressful events early in life may produce stress resistance in developing humans and nonhuman primates.

By postnatal week 35, young squirrel monkeys in their home cage rarely cling to their mothers and increasingly initiate social and environmental exploration.⁵⁵ These developmental transitions from filial, mother-directed behavior (eg, clinging and nursing) to exploitative, other-directed behavior (eg, visual exploration, object and social play, investigatory activities) promote species-typical learning opportunities and support the young primate's transition to independence.^{56,57} Nevertheless, throughout development, mother-directed contact seeking occurs in response to highly stimulating events and typically leads to reduced arousal.⁵⁸ In experiment 1, IS and NS offspring remained in close dorsal contact with their mothers during the first 2 days of testing (Figure 1), which indicates that the novel test environment was a source of significant emotional distress for offspring from both rearing conditions.

During consecutive test days, however, the novel environment was less anxiety provoking for IS offspring, as they spent less time clinging to their mothers, initiated object exploration more rapidly, investigated more unfamiliar (but not familiar) objects, and consumed more food than did NS offspring (Figures 1 and 2). Similarly, in experiment 2, IS offspring at 1 year of age more rapidly acclimated to the anxiety-provoking test apparatus because they were faster to enter the wire-mesh box and spent more time exploring it (Figure 4). Moreover, IS but not NS monkeys played in the box throughout testing. Insofar as exploitative behavior typically occurs under conditions of low to moderate arousal,⁵⁶ these findings suggest that prior experience with moderately stressful events early in life results in more effective arousal regulation when emotionally challenging circumstances are again encountered.

In bonnet macaque⁴⁸ and human⁵⁹ infants, behavioral responses to emotionally challenging circumstances are also influenced by the quality of the mother-infant relationship. In experiment 1, mothers of IS and NS offspring differed on several measures of maternal accommodation of offspring-initiated overtures. First, IS mothers were more permissive of offspring-initiated termination of dorsal contact than were NS mothers. In Japanese macaques⁴⁷ and vervet monkeys,⁶⁰ a less permissive (ie, restrictive) maternal style is associated with decreased offspring exploration. Consequently, by more frequently accommodating exploitative tendencies in their offspring, IS mothers may have better facilitated offspring exploration of the novel environment compared with more restrictive and less accommodating NS mothers. Second, similar to human mothers of securely attached infants,^{61,62} IS mothers more frequently accommodated offspring-initiated bouts of brief side-by-side huddling that may have buffered offspring from overarousal by providing a secure base from which to explore. Why IS and NS mothers differ in measures of maternal responsivity is unclear, but one possible explanation is that repeated exposure to highly aroused offspring af-

ter each separation period facilitated the development of increasingly effective maternal means by which to assess and reduce offspring arousal. Over time, repeated exposure to manageable stressors combined with the presence of a reassuring and accommodating mother at reunion may have strengthened emotional self-regulation and promoted exploitative tendencies in developing IS monkeys. Because exploitative actions broaden the range and complexity of situations that offspring tolerate and approach freely,^{56,57} such cumulative experience may provide a socioemotional foundation for the development of stress resistance.

Maternal differences also present a potential confound, as it is unclear whether rearing-related differences in offspring anxiety are the product of stress inoculation or reflect maternally driven changes in offspring behavior. In experiment 2, when there was little direct maternal influence on offspring behavior because of offspring maturity, IS offspring nevertheless continued to demonstrate diminished anxiety compared with NS offspring. Moreover, additional data from these monkeys support the notion that rearing-related differences are sustained well after removal of the mothers, as juvenile IS monkeys demonstrate enhanced cognitive and emotional control compared with NS monkeys on preliminary tests of prefrontal cortical function.⁶³

Another issue that warrants comment is whether these findings reflect a specific or a general stress inoculation effect. Experiment 1 and the inoculation procedure shared several similarities, including separation from the natal group and confinement in an unfamiliar environment. Our experimental results may thus reflect increased familiarization by IS monkeys with these common procedures. However, there were considerable differences between experiment 2 and the inoculation procedure, including voluntary vs involuntary participation, present vs absent bnatal group, and home- vs novel-cage environment. Despite these differences, the home-cage test was less anxiety provoking for IS than NS offspring, which suggests a general, rather than specific, stress inoculation effect.

In addition to rearing-related differences in socioemotional behavior, IS monkeys exhibited lower basal and stress-induced increases in plasma concentrations of ACTH and cortisol (Figure 3). These results parallel findings of studies of other monkey cohorts that examined the effects of early maternal availability on offspring development.^{30,31} In these previous experiments from our group, we unexpectedly found that intermittently separated offspring responded to the removal of mothers at weaning with smaller elevations in plasma cortisol concentrations, fewer distress calls, and more time spent near peers relative to offspring raised in high- or low-demand foraging conditions.³⁰ Moreover, in early adulthood, intermittently separated monkeys demonstrated enhanced glucocorticoid negative-feedback sensitivity compared with monkeys from other rearing conditions.³¹

Data from these previous studies^{30,31} and those presented herein support a role for the HPA axis as a potential neurobiological mediator of stress resistance in monkeys. However, the process by which early environmental events program stress resistance remains unknown. In rodents, increased maternal care received dur-

ing infancy is associated with blunted pituitary-adrenal hormone responses to stressful circumstances in adulthood. This is mediated, in part, by enhanced glucocorticoid negative-feedback regulation resulting from increased glucocorticoid receptor expression in the hippocampus.⁶⁴ As ACTH and cortisol levels are diminished in IS monkeys, it is likely that, as in rodents, brain mechanisms above the pituitary enhance the inoculated monkey's ability to more efficiently regulate stress hormone responses to emotionally arousing events.

Similar to securely attached children,⁶⁵ IS offspring demonstrate low levels of circulating basal cortisol. In contrast, hypercortisolism is often observed in chronically stressed individuals (eg, former Romanian orphans,⁶⁶ children of emotionally unavailable mothers⁶⁷). Although cortisol plays a critical role in an organism's ability to cope with stressors, the deleterious consequences of sustained cortisol overproduction are well documented.⁶⁸ Insofar as high basal cortisol levels associated with adverse early experiences permanently alter stress biology and confer vulnerability to develop adult-onset depressive and anxiety disorders,^{37,39,69} broadly speaking, comparatively low basal cortisol levels may serve a protective function during development.

Because encounters with stress and adversity are unavoidable, theorists have argued that stress resistance cannot reasonably reside in the avoidance of risk experiences, but rather, in successful engagement with and mastery of them.^{4,7} However, it is important to remember that stressful events, even comparatively mild ones, may still increase vulnerability to the effects of subsequent stressors if they supersede the developing organism's ability to cope with them. For example, in young marmoset monkeys,⁷⁰ initiation of a brief, repeated parental separation protocol during postnatal week 1 has stress-sensitizing effects, whereas a similar protocol initiated during postnatal week 17 in squirrel monkeys, as described herein, has stress-inoculating properties. 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.

As with all studies, potential limitations should be considered. First, it was impossible for the observer who recorded behavioral data to be blind to the treatment conditions. However, hormone levels for experiment 1 were determined blindly, and robust group differences in the same direction predicted a priori were found. Thus, it seems unlikely that experimenter bias produced the behavioral differences observed in these experiments. Second, 11 primary outcome measures were analyzed in experiment 1, and although almost all treatment-related differences were highly statistically significant, the possibility of false-positive results due to compounded α error from multiple analyses should be noted. Last, these studies should be replicated by other researchers studying this and other species to assess the generalizability of our findings.

Although no known studies have examined pituitary-adrenal responsivity in stress-inoculated humans, prospective evidence from our experiments in monkeys sup-

ports the notion that the HPA axis may provide a neural basis for programming stress resistance in the developing child. Moreover, these findings raise important clinical questions concerning the role of stress management in therapeutic interventions. However, as with primates, the effects of stress inoculation in developing children are likely to be complex and context dependent. With continued investigation of stress inoculation in primate models, a comprehensive understanding of the neurobiology of stress resistance may ultimately provide a foundation for new approaches to the successful treatment and prevention of stress-induced depressive and anxiety disorders.

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REFERENCES

- Rutter M. Stress, coping, and development: some issues and some questions. In: Garnezy N, Rutter M, eds. *Stress, Coping, and Development in Children*. New York, NY: McGraw-Hill Co; 1983:1-41.
- Eysenck H. Stress, disease, and personality: the "inoculation effect." In: Cooper CL, ed. *Stress Research: Issues for the Eighties*. New York, NY: John Wiley & Sons Inc; 1983:121-146.
- Norris FH, Friedman MJ, Watson PJ, Byrne CM, Diaz E, Kaniasty K. 60,000 disaster victims speak, I: an empirical review of the empirical literature, 1981-2001. *Psychiatry*. 2002;65:207-239.
- Selye H. The stress concept: past, present, and future. In: Cooper CL, ed. *Stress Research: Issues for the Eighties*. New York, NY: John Wiley & Sons Inc; 1983:1-20.
- O'Leary VE. Strength in the face of adversity: individual and social thriving. *J Soc Issues*. 1998;54:425-446.
- Olsson CA, Bond L, Burns JM, Vella-Brodrick DA, Sawyer SM. Adolescent resilience: a concept analysis. *J Adolesc*. 2003;26:1-11.
- Rutter M. Resilience: some conceptual considerations. *J Adolesc Health*. 1993; 14:626-631, 690-696.
- Masten AS, Hubbard JJ, Gest SD, Tellegen A, Garnezy N, Ramirez M. Competence in the context of adversity: pathways to resilience and maladaptation from childhood to late adolescence. *Dev Psychopathol*. 1999;11:143-169.
- Garnezy N, Masten AS, Tellegen A. The study of stress and competence in children: a building block for developmental psychopathology. *Child Dev*. 1984;55: 97-111.
- Garnezy N. Stressors of childhood. In: Garnezy N, Rutter M, eds. *Stress, Coping, and Development in Children*. New York, NY: McGraw-Hill Co; 1983:43-84.
- Frank E, Anderson B, Reynolds CF III, Ritenour A, Kupfer DJ. Life events and the research diagnostic criteria endogenous subtype: a confirmation of the distinction using the Bedford College methods. *Arch Gen Psychiatry*. 1994;51:519-524.
- Brown GW, Harris TO, Hepworth C, Robinson R. Clinical and psychosocial origins of chronic depressive episodes, II: a patient enquiry. *Br J Psychiatry*. 1994; 165:457-465.
- Bebbington P, Der G, MacCarthy B, et al. Stress incubation and the onset of affective disorders. *Br J Psychiatry*. 1993;162:358-362.
- Paykel ES. Contribution of life events to causation of psychiatric illness. *Psychol Med*. 1978;8:245-253.
- Rutter M. Psychosocial resilience and protective mechanisms. *Am J Orthopsychiatry*. 1987;57:316-331.
- Boyce WT, Chesterman E. Life events, social support, and cardiovascular reactivity in adolescence. *J Dev Behav Pediatr*. 1990;11:105-111.
- Seligman ME, Rosellini RA, Kozak MJ. Learned helplessness in the rat: time course, immunization, and reversibility. *J Comp Psychol*. 1975;88:542-547.
- Levine S, Weiner S, Coe C. The psychoneuroendocrinology of stress: a psychological perspective. In: Brush FR, Levine S, eds. *Psychoneuroendocrinology*. Orlando, Fla: Academic Press Inc; 1989: 341-377.
- Rutter M. Resilience in the face of adversity: protective factors and resistance to psychiatric disorder. *Br J Psychiatry*. 1985;147:598-611.
- Dienstbier RA. Arousal and physiological toughness: implications for mental and physical health. *Psychol Rev*. 1989;96:84-100.
- Miller NE. A perspective on the effects of stress and coping on disease and health. In: Levine S, Ursin H, eds. *Coping and Health*. New York, NY: Plenum Publishing Corp; 1980:323-354.
- O'Leary VE, Ickovics JR. Resilience and thriving in response to challenge: an opportunity for a paradigm shift in women's health. *Womens Health*. 1995;1:121-142.
- Norris FH, Murrell SA. Prior experience as a moderator of disaster impact on anxiety symptoms in older adults. *Am J Community Psychol*. 1988;16:665-683.
- Knight BG, Gatz M, Heller K, Bengtson VL. Age and emotional response to the Northridge earthquake: a longitudinal analysis. *Psychol Aging*. 2000;15:627-634.
- Basoglu M, Mineka S, Paker M, Aker T, Livanou M, Gok S. Psychological preparedness for trauma as a protective factor in survivors of torture. *Psychol Med*. 1997;27:1421-1433.
- Anisman H, Irwin J, Beauchamp C, Zacharko RM. Cross-stressor immunization against the behavioral deficits introduced by uncontrollable shock. *Behav Neurosci*. 1983;97:452-461.
- Stacey M, Dearnden R, Pill R, Robinson D. *Hospitals, Children and Their Families: The Report of a Pilot Study*. London, England: Routledge & Kegan Paul; 1970.
- Holmes FB. Experimental study of the fears of young children. In: Jersild AT, Holmes FB, eds. *Children's Fears*. New York, NY: Teacher's College, Columbia University; 1935:167-296. Child development monograph 20.
- Forest KB. The interplay of childhood stress and adult life events on women's symptoms of depression. *Diss Abstr Int*. 1991;51:3237.
- Lyons DM, Martel FL, Levine S, Risch NJ, Schatzberg AF. Postnatal experiences and genetic effects on squirrel monkey social affinities and emotional distress. *Horm Behav*. 1999;36:266-275.
- Lyons DM, Yang C, Mobley BW, Nickerson JT, Schatzberg AF. Early environmental regulation of glucocorticoid feedback sensitivity in young adult monkeys. *J Neuroendocrinol*. 2000;12:723-728.
- Hadley ME. *Endocrinology*. Upper Saddle River, NJ: Simon & Schuster; 1996.
- Schatzberg AF, Nemeroff CB. *The American Psychiatric Press Textbook of Psychopharmacology*. Washington, DC: American Psychiatric Association; 1998: xxii, 1095.
- Parker KJ, Schatzberg AF, Lyons DM. Neuroendocrine aspects of hypercortisolism in major depression. *Horm Behav*. 2003;43:60-66.
- Repetti RL, Taylor SE, Seeman TE. Risky families: family social environments and the mental and physical health of offspring. *Psychol Bull*. 2002;128:330-366.
- Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP. Developmental neurobiology of childhood stress and trauma. *Psychiatr Clin North Am*. 2002; 25:397-426, vii-viii.
- Kaufman J, Plotsky PM, Nemeroff CB, Charney DS. Effects of early adverse experiences on brain structure and function: clinical implications. *Biol Psychiatry*. 2000;48:778-790.
- Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, Koss MP, Marks JS. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: the Adverse Childhood Experiences (ACE) Study. *Am J Prev Med*. 1998;14:245-258.

39. Glaser D. Child abuse and neglect and the brain: a review. *J Child Psychol Psychiatry*. 2000;41:97-116.
40. Huether G, Doering S, Ruger U, Ruther E, Schussler G. The stress-reaction process and the adaptive modification and reorganization of neuronal networks. *Psychiatry Res*. 1999;87:83-95.
41. Boinski S, Fragaszy DM. The ontogeny of foraging in squirrel monkeys, *Saimiri oerstedii*. *Anim Behav*. 1989;37:415-428.
42. Coe CL, Mendoza SP, Smotherman WP, Levine S. Mother-infant attachment in the squirrel monkey: adrenal response to separation. *Behav Neural Biol*. 1978;22:256-263.
43. Levine S, Coe CL, Smotherman WP, Kaplan JN. Prolonged cortisol elevation in the infant squirrel monkey after reunion with mother. *Physiol Behav*. 1978;20:7-10.
44. Coe CL, Glass JC, Wiener SG, Levine S. Behavioral, but not physiological, adaptation to repeated separation in mother and infant primates. *Psychoneuroendocrinology*. 1983;8:401-409.
45. Hennessy MB. Multiple, brief maternal separations in the squirrel monkey: changes in hormonal and behavioral responsiveness. *Physiol Behav*. 1986;36:245-250.
46. Stanton M, Levine S. Brief separation elevates cortisol in mother and infant squirrel monkeys. *Physiol Behav*. 1985;34:1007-1008.
47. Bardi M, Huffman MA. Effects of maternal style on infant behavior in Japanese macaques (*Macaca fuscata*). *Dev Psychobiol*. 2002;41:364-372.
48. Andrews MW, Rosenblum LA. Attachment in monkey infants raised in variable- and low-demand environments. *Child Dev*. 1991;62:686-693.
49. Lyons DM, Kim S, Schatzberg AF, Levine S. Postnatal foraging demands alter adrenocortical activity and psychosocial development. *Dev Psychobiol*. 1998;32:285-291.
50. Lyons DM, Mendoza SP, Mason WA. Sexual segregation in squirrel monkeys (*Saimiri sciureus*): a transactional analysis of adult social dynamics. *J Comp Psychol*. 1992;106:323-330.
51. Zeitzer JM, Buckmaster CL, Parker KJ, Hauck CM, Lyons DM, Mignot E. Circadian and homeostatic regulation of hypocretin in a primate model: implications for the consolidation of wakefulness. *J Neurosci*. 2003;23:3555-3560.
52. Lyons DM, Ha CM, Levine S. Social effects and circadian rhythms in squirrel monkey pituitary-adrenal activity. *Horm Behav*. 1995;29:177-190.
53. Baldwin JD. The behavior of squirrel monkeys (*Saimiri*) in natural environments. In: Rosenblum LA, Coe CL, eds. *Handbook of Squirrel Monkey Research*. New York, NY: Plenum Publishing Corp; 1985:35-53.
54. Keppel G. *Design and Analysis: A Researcher's Handbook*. Englewood Cliffs, NJ: Prentice Hall International Inc; 1982.
55. Rosenblum LA. Mother-infant relations and early behavioral development in the squirrel monkey. In: Rosenblum LA, Cooper RW, eds. *The Squirrel Monkey*. Orlando, Fla: Academic Press Inc; 1968:207-233.
56. Mason WA. Motivational factors in psychosocial development. In: Arnold WJ, Page MM, eds. *Nebraska Symposium on Motivation*. Lincoln: University of Nebraska Press; 1971:35-67.
57. Lyons DM. Conflict as a constructive force in social life. In: Mason WA, Mendoza SP, eds. *Primate Social Conflict*. Albany: State University of New York Press; 1993:387-408.
58. Mendoza SP, Smotherman WP, Miner MT, Kaplan J, Levine S. Pituitary-adrenal response to separation in mother and infant squirrel monkeys. *Dev Psychobiol*. 1978;11:169-175.
59. Ainsworth MD, Bell SM. Attachment, exploration, and separation: illustrated by the behavior of one-year-olds in a strange situation. *Child Dev*. 1970;41:49-67.
60. Fairbanks LA, McGuire MT. Long-term effects of early mothering behavior on responsiveness to the environment in vervet monkeys. *Dev Psychobiol*. 1988;21:711-724.
61. Ainsworth MD, Blehar MC, Waters E, Wall S. *Patterns of Attachment: A Psychological Study of the Strange Situation*. Hillsdale, NJ: Lawrence A Erlbaum Assoc; 1978.
62. Ainsworth MD, Wittig BA. Attachment and exploratory behavior of one-year-olds in a strange situation. In: Foss BM, ed. *Determinants of Infant Behaviour*. Vol 4. London, England: Methuen; 1969:111-136.
63. Parker KJ, Buckmaster CL, Schatzberg AF, Lyons DM. Rearing-related differences in primate HPA physiology, socioemotional behavior, and cognitive performance [abstract]. *Abstr Soc Neurosci*. 2003;660:5.
64. Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci*. 2001;24:1161-1192.
65. Gunnar MR, Brodersen L, Nachmias M, Buss K, Rigatuso J. Stress reactivity and attachment security. *Dev Psychobiol*. 1996;29:191-204.
66. Gunnar MR, Morison SJ, Chisholm K, Schuder M. Salivary cortisol levels in children adopted from Romanian orphanages. *Dev Psychopathol*. 2001;13:611-628.
67. Bugental DB, Martorell GA, Barraza V. The hormonal costs of subtle forms of infant maltreatment. *Horm Behav*. 2003;43:237-244.
68. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med*. 1998;338:171-179.
69. Newport DJ, Stowe ZN, Nemeroff CB. Parental depression: animal models of an adverse life event. *Am J Psychiatry*. 2002;159:1265-1283.
70. Dettlign AC, Feldon J, Pryce CR. Repeated parental deprivation in the infant common marmoset (*Callithrix jacchus*, primates) and analysis of its effects on early development. *Biol Psychiatry*. 2002;52:1037-1046.