

Hypothalamic-pituitary-adrenal axis physiology and cognitive control of behavior in stress inoculated monkeys

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Abstract

Monkeys exposed to stress inoculation protocols early in life subsequently exhibit diminished neurobiological responses to moderate psychological stressors and enhanced cognitive control of behavior during juvenile development compared to non-inoculated monkeys. The present experiments extended these findings and revealed that stress inoculated monkeys: (a) mount neurobiological responses equivalent to non-inoculated monkeys when the stressor is of sufficient intensity, and (b) continue to exhibit enhanced cognitive control as young adults compared to non-inoculated monkeys. These results suggest that stress inoculation protocols alter the appraisal of and response to moderate stressors as less threatening and permanently enhance cognitive control, at least through early adulthood. These data therefore support the notion that the stress inoculation phenotype reflects stress resilience rather than stress pathology.

Keywords

cognitive control, cortisol, HPA axis, MHPG, monkey, resilience, response inhibition, stress inoculation

Introduction

Squirrel monkeys exposed to intermittent stressful experiences early in life (a laboratory manipulation called “stress inoculation”) subsequently exhibit diminished neurobiological responses to a variety of psychological stressors compared to non-inoculated control monkeys. Stress inoculated monkeys, for example, show diminished hypothalamic-pituitary-adrenal (HPA)-axis activation following exposure to a novel environment when accompanied by their mothers, and respond to the removal of mothers at weaning with smaller increases in plasma cortisol levels in the home cage compared to non-inoculated monkeys (Lyons, Martel, Levine, Risch, & Schatzberg, 1999; Parker, Buckmaster, Schatzberg, & Lyons, 2004; Parker, Buckmaster, Sundlass, Schatzberg, & Lyons, 2006). Stress inoculated monkeys likewise exhibit diminished adrenocortical activation in a repeated and familiar social-separation paradigm throughout adolescent development compared to non-inoculated monkeys (Levine & Mody, 2003). This diminished HPA-axis response may be due to lower brain noradrenergic tone (Plotsky, Cunningham, & Widmaier, 1989), as stress inoculated monkeys in this experiment concomitantly show smaller increases in cerebrospinal fluid (CSF) levels of 3-methoxy-4-hydroxyphenylethylene glycol (MHPG, a norepinephrine metabolite) compared to non-inoculated monkeys.

The stress inoculation phenotype appears to persist into adulthood, as stress inoculated monkeys exhibit diminished restraint stress-induced HPA-axis activation, and faster recovery to baseline values, compared to non-inoculated monkeys at 8 years of age (Parker et al., 2006). Interestingly, in some but not all experiments, stress inoculated compared to non-inoculated monkeys also exhibit lower circulating levels of CSF MHPG and plasma cortisol under undisturbed baseline conditions (Levine & Mody, 2003; Lyons et al., 1999; Parker et al., 2004, 2006, 2007).

When individuals are exposed to a stressor, and stress response systems react only modestly, the meaning of this blunted response is difficult to interpret in the absence of additional phenotypic information (Gunnar & Vazquez, 2001). Diminished neurobiological responses to stress, on one hand, can signify that a stressor is less challenging or threatening for a given individual. Such “neuroendocrine stress resistance” has been observed in adult rodents which received high levels of maternal care in infancy and psychologically resilient people compared, respectively, to adult rodents which received low levels of maternal care and less resilient people (Liu et al., 1997; Mikolajczak, Roy, Luminet, & de Timary, 2008). Alternatively, blunted neurobiological responses to stress can also reflect biological defects in the ability of stress response systems to generate robust neurobiological responses to stressors. Consistent with this possibility, hypocortisolism and blunted HPA-axis responses have been associated in some studies with psychiatric disorders such as posttraumatic stress disorder (PTSD) and conduct disorder (Cappadocia, Desrocher, Pepler, & Schroeder, 2009; Yehuda, 2009), although there have been inconsistencies (Lindley, Carlson, & Benoit, 2004; Meewisse, Reitsma, de Vries, Gersons, & Olf, 2007). The available data on stress neurobiology therefore do not help differentiate whether the stress

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inoculation phenotype reflects stress resilience or stress pathology.

Studies of individuals with PTSD (Casada & Roache, 2005; Falconer et al., 2008; Wu et al., 2010) and conduct disorder (Raaijmakers et al., 2008; Young et al., 2009) report impaired response inhibition on paradigms (e.g., go/no go test) designed to assess cognitive impulsivity and the ability to inhibit or override prepotent behavioral responses. These findings have been interpreted as evidence that executive cognitive control networks are compromised in both PTSD and conduct disorder (Falconer et al., 2008; Young et al., 2009). Stress inoculated monkeys, in contrast, exhibit enhanced prefrontal-dependent cognitive response inhibition on a challenging detour-reaching test compared to non-inoculated monkeys during a developmental period that corresponds to late childhood in humans (Parker, Buckmaster, Justus, Schatzberg, & Lyons, 2005). Although we do not know whether this phenomenon persists in adulthood, these data from stress inoculated monkeys are similar to findings of enhanced cognitive response inhibition in resilient people (Nigg, Nikolas, Friderici, Park, & Zucker, 2007), and provisionally support the notion that early stress inoculation protocols create a stress resilient, rather than stress pathological, phenotype.

Two main hypotheses were tested in the present experiments. In Experiment 1, we exposed stress inoculated and non-inoculated monkeys to a social-isolation stressor of a comparatively more potent nature than those used in previous studies. The present paradigm did not afford social buffering or familiarity, unlike the aforementioned stress paradigms in which rearing-related differences were previously observed. This manipulation allowed us to test whether stress inoculated monkeys can mount a stress response equivalent to their non-inoculated counterparts when the stressor is of sufficient intensity. In Experiment 2, we sought to replicate as well as extend our previous cognitive response inhibition findings in a group of stress inoculated and non-inoculated monkeys in early adulthood. Because the 1.5-year-old monkeys in our previous experiment were well past the developmental age at which response inhibition is acquired (Diamond, 1990), here we tested, using a more difficult cognitive response inhibition paradigm, whether the same group of stress inoculated monkeys continues to exhibit superior cognitive response inhibition at 3.5 years of age.

General method

Subjects

Squirrel monkeys (*Samiri sciureus*) of Guyanese origin were housed at the AAALAC¹-accredited Stanford University Research Animal Facility and served as subjects. All monkeys wore identification necklaces. Subjects were typically reared in natal groups comprised of three to four mother–infant pairs. Group composition was primarily determined by infant birth dates to minimize developmental differences between cohabitating infants. Puberty occurs between 2 and 3 years of age, and the average maximum lifespan in captivity is ~ 21 years of age (Brady, 2000). All procedures were approved by Stanford University's Administrative Panel on Laboratory Animal Care and were carried out in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.

Animal husbandry and rearing protocols

Subjects were housed indoors in 1.8 × 1.2 × 1.8 m wire-mesh cages that were cleaned daily. Housing and testing occurred in climate-

controlled rooms with an ambient temperature of 26°C. Light/dark cycles were 12:12 hours with lights on at 7:00 a.m. All monkeys were provided unrestricted access to fresh drinking water and commercial monkey chow with daily fruit and vegetable supplements. Various toys, swinging perches, and simulated foraging activities were provided for environmental enrichment. To facilitate husbandry-related activities and experimental manipulations, monkeys were trained using vocal commands to quickly leave the home cage through a small sliding door connected to a stainless steel wire-mesh transport box used for capture and transportation.

Subjects remained undisturbed in their natal groups through 16 weeks of age. At 17 weeks of age, natal groups were randomly assigned to experimental conditions. In one condition, subjects were exposed to stress inoculation rearing protocols as previously described (Parker et al., 2004). From 17–27 weeks of age, each subject was removed from the natal group for a 1 h period once a week, placed in a familiar cage adjacent to other monkeys in a different room, and temporarily deprived 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, subjects remained undisturbed as non-inoculated controls. Mothers were removed from the premises and returned to the breeding colony when young monkeys were 1 year of age. Thereafter, monkeys were housed in peer groups except when experimental procedures necessitated otherwise as described below.

Experiment 1 method

Previous studies have shown that stress inoculated monkeys exhibit diminished CSF MHPG and plasma cortisol responses to psychological stressors compared to non-inoculated control monkeys (Levine & Mody, 2003; Lyons et al., 1999; Parker et al., 2004, 2006). There are two possible interpretations of these findings (Gunnar & Vazquez, 2001): (a) stressors used in these previous research paradigms were less challenging or threatening for stress inoculated compared to non-inoculated monkeys, or (b) the diminished responses of stress inoculated compared to non-inoculated monkeys reflect biological defects in the ability of their stress response systems to generate robust neurobiological responses to stressors. In order to differentiate between these two possibilities, in the present experiment we exposed stress inoculated and non-inoculated monkeys to a social-isolation stressor of a comparatively more severe nature (i.e., the present paradigm did not afford social buffering or familiarity, unlike previous stress paradigms in which rearing-related differences were observed).

Subjects

Eleven stress inoculated ($N = 8$ females, 4 males) and nine non-inoculated ($N = 8$ females, 1 male) monkeys served as subjects in this experiment. Monkeys were an average of 2.25 years of age during testing. This age characterizes the late juvenile period for squirrel monkeys (Brady, 2000). It should be noted that these subjects previously demonstrated diminished adrenocortical responses to a novel environment in the presence of their mothers at 36 weeks of age (Parker et al., 2004).

Stress paradigm

Blood and CSF samples were collected from juvenile monkeys to establish baseline levels of cortisol and MHPG levels, respectively,

Table 1. Neurobiological responses to the 1-hour social-isolation stress paradigm for stress inoculated ($N = 11$) and non-inoculated ($N = 9$) monkeys

Measure	Experimental group	Baseline	Stressed		Stress effect	
CSF MHPG ng/ml	Stress inoculated	33 ± 1	45 ± 1	↑	$F(1, 18) = 176.66$	$P < 0.0001$
	Non-inoculated	32 ± 1	43 ± 1			
Plasma cortisol µg/dl	Stress inoculated	37 ± 5	171 ± 6	↑	$F(1, 18) = 235.43$	$P < 0.0001$
	Non-inoculated	62 ± 14	185 ± 15			

Note. Data are presented as mean ± SEM. MHPG is the abbreviation for 3-methoxy-4-hydroxyphenylethylene glycol. The arrow ↑ indicates a statistically significant increase in the concentration of a given neurobiological measure between assessment points.

under undisturbed conditions. Fourteen days after baseline sampling, monkeys were exposed to a novel 1-h social-isolation stressor in an unfamiliar cage (62 × 68 × 79 cm). Unlike previous stress paradigms, this cage had solid stainless steel side walls that prevented subjects from seeing or touching other monkeys. Blood and CSF samples were collected immediately afterward to examine the effects of stress exposure on the aforementioned neurobiological measures. Samples collected for each monkey were time-matched, and sampling occurred between 3:00 p.m. and 5:00 p.m. to control for circadian variation in cortisol and MHPG levels. Samples were collected from two to five monkeys per day during each sampling period. The sampling order for stress inoculated and non-inoculated monkeys was evenly distributed across daily schedules.

Blood and CSF collection procedures

Blood samples were collected from nonsedated manually restrained monkeys while blood (1 ml) was drawn from the femoral vein with a sterile 1 ml single-use syringe with a 25 gauge hypodermic needle containing 20 µL of ethylenediamine tetraacetic acid (EDTA). Each blood sample was transferred to a polypropylene tube and placed on ice. Blood samples were centrifuged at 4 °C and the plasma fraction was transferred to a chilled tube and flash-frozen on dry ice. Most blood samples were obtained within 3 minutes of capture, restraint, and collection (median latency to sample collection = 120 s, range = 58–287 s).

Anesthesia was then induced by intrasaphenous injection of 10 mg/kg ketamine hydrochloride and 0.5 mg/kg diazepam, and supplemented as needed (rarely) with intramuscular injection of 5 mg/kg ketamine hydrochloride. Using a sterile 1 ml single-use syringe with a 25 gauge hypodermic needle, 100–200 µl of CSF was drawn from the cisterna magna and immediately transferred to a siliconized tube and flash-frozen on dry ice. Most CSF samples were collected within 10 min of capture (median latency to sample collection = 496 s, range = 308–991 s). Immediately after completion of CSF sample collection, during recovery from sedation, each monkey was given a 12 ml subcutaneous injection (in the area between the scapulae) of Lactated Ringer's solution (Abbott Laboratories, Chicago, IL), continuously monitored until fully recovered from sedation, and then returned to the home cage. Samples were stored at –80°C for later determination of cortisol in plasma and MHPG in CSF.

Neurobiological measurement

Cortisol was measured in duplicate plasma samples using a commercially prepared radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). All samples were included in one assay run, and the intra-assay coefficient of variation was 3.7% for cortisol. Assay sensitivity was 3 µg/dl for cortisol. Concentrations of

MHPG in CSF were determined by high performance liquid chromatography (HPLC) with electrochemical detection. CSF samples were thawed, centrifuged for 2 min, and 5 µl was injected directly on a C18 reverse phase analytical column (5 mm, 250 × 4.6 mm; Biophase ODS, BAS, West Lafayette, IN) protected by a precolumn cartridge (5 mm, 30 × 4.6 mm, BAS) and a dual analytical electrode cell set at –0.02 V and +0.35 V, respectively (ESA, Bedford, MA), as previously described with modification (Lindley, Gunnet, Lookingland, & Moore, 1988). The mobile phase consisted of 0.05 M sodium phosphate, 0.03 M citric acid, 12.5% methanol, 0.03% octyl sodium sulfate, 0.1 mM EDTA adjusted to pH 2.3. Technicians were unaware of the experimental conditions while conducting the radioimmunoassay and HPLC.

Data analysis

The effects of rearing condition (stress inoculated vs. non-inoculated) on MHPG and cortisol levels were assessed with repeated measures analysis of variance (ANOVA). Rearing condition was considered the between-subjects factor, while stress exposure (baseline and poststress) were considered within-subjects factors. Gender was excluded from analysis because this study was not powered to examine interaction effects between this variable and rearing condition on neurobiological measures of interest. Test statistics were evaluated with two-tail probabilities ($p < 0.05$).

Results

Neurobiological responses to a novel social-isolation stressor. Following the novel social-isolation stressor, significant increases in CSF MHPG $F(1, 18) = 176.656$; $p < 0.0001$, and plasma cortisol $F(1, 18) = 235.429$; $p < 0.0001$, measures were observed for both stress inoculated and non-inoculated monkeys (Table 1). Specifically, there was a 35% and 268% increase from baseline values for CSF MHPG and plasma cortisol, respectively. No significant rearing differences in baseline or stress responsivity measures were observed for CSF MHPG or plasma cortisol levels, in contrast to previous studies.

Experiment 2 method

Stress inoculated monkeys exhibit enhanced prefrontal-dependent cognitive response inhibition on a detour-reaching task compared to non-inoculated control monkeys at 1.5 years of age (Parker et al., 2005). The ability to inhibit prepotent straight reaching is typically acquired by 3–4 months of age in Rhesus macaque infants and 11–12 months of age in human infants (Diamond, 1990). Because the monkeys in our previous experiment were well past the developmental age at which response inhibition is acquired, it is unlikely that rearing-related differences were due

Table 2. Overview of the cognitive-test paradigm used in Experiment 2

Week	Acclimation period		Training trial		Test trials
	Monday	Tuesday	Wednesday	Thursday	Friday
1	Acclimation to test environment each day		3 blocks per day; 10 straight-facing trials per block		3 blocks; alternating 5 straight- and 5 side-facing trials per block
2	Acclimation to test environment each day		3 blocks per day; 10 straight-facing trials per block		3 blocks; alternating 5 straight- and 5 side-facing trials per block
3	Acclimation to test environment each day		3 blocks per day; 10 straight-facing trials per block		3 blocks; alternating 5 straight- and 5 side-facing trials per block

to a developmental delay in acquiring the ability to control prepotent reaching. Data from young humans indicate that inhibitory proficiency improves with development but that cognitive performance is highly stable across time (Kochanska & Knaack, 2003; Kochanska, Murray, Jacques, Koenig, & Vandegest, 1996). Using a more difficult cognitive response inhibition paradigm than that used at 1.5 years of age, we tested in the present experiment whether rearing-related differences in response inhibition reflect transient developmental differences in cognitive task proficiency (i.e., non-inoculated monkeys eventually “catch up” to stress inoculated monkeys), or whether exposure to early life stress inoculation protocols permanently alters inhibitory control of behavior, such that cognitive performance is stable across assessment points for both stress inoculated and non-inoculated monkeys.

Subjects

Eleven stress inoculated ($N = 8$ females, 4 males) and nine non-inoculated ($N = 8$ females, 1 male) monkeys served as subjects in this experiment. Monkeys were an average of 3.5 years of age during testing. This age characterizes the beginning of early adulthood in squirrel monkeys (Brady, 2000). It should be noted that monkeys in this experiment previously served as subjects in the cognitive test at 1.5 years of age (Parker et al., 2005).

Cognitive-test paradigm

The cognitive-test paradigm occurred every day Monday through Friday for three consecutive weeks (Table 2). Monkeys were acclimated to the test environment and test apparatus on Mondays and Tuesdays for 6.5 hr. Monkeys underwent training trials on Wednesdays and Thursdays, and testing trials on Fridays, of each week. During acclimation, training, and testing, monkeys were transported to the test room at 9:30 a.m. each day and housed individually in wire-mesh cages ($60 \times 60 \times 90$ cm) that allowed visual, auditory, olfactory, and limited tactile contact between adjacent animals. Training and testing occurred between 12:00 p.m. and 4:00 p.m. After completion of acclimation, training, or testing, monkeys were returned to their home cages.

The experimental apparatus consisted of two clear Plexiglas boxes ($8 \times 8 \times 8$ cm), one with one open side, and the other with two open sides. Only one box was used for a given trial. For each trial, the box was baited with a minimarshmallow treat. The box was then secured into a slot on a 61×13 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.

On training days, monkeys were administered three blocks of 10 trials per day (for a total of 30 trials per monkey per day) with the box with one open side always oriented straight toward the subject. Food retrieval was achieved by line-of-sight reaching into the center of the box. These two weekly training days served to reinforce as prepotent the straight-reaching response.

Immediately following the second day of training, monkeys were administered three blocks of 10 trials per day (for a total of 30 trials per monkey), with the orientation of the box opening varied systematically to assess inhibitory control of the prepotent straight-reaching response. On each testing day, the box with one open side was oriented straight toward the subject on the 1st, 3rd, 5th, 7th, and 9th trials. On the 2nd, 4th, 6th, 8th, and 10th trials, the box with two open sides oriented to the monkey's right and left was used. When this latter box was rotated so the openings were oriented toward the sides, the monkey was required to inhibit straight reaching and detour reach around either 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 two openings were oriented towards the sides. Detour-reaching errors were scored when a reach was aimed to the side of the box when the opening was oriented straight.

For all aspects of training and testing, each trial lasted 30 sec 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-sec intertrial interval, the experimenter rebaited the box for the subsequent trial, and recorded whether the marshmallow was successfully retrieved within 30 sec, the latency to retrieve the marshmallow, and the number and direction of reach attempts. The interblock interval was 30 min.

Data analysis

The effect of rearing condition (stress inoculated vs. non-inoculated) on cognitive performance was assessed with repeated-measures ANOVA. Rearing condition was considered the between-subjects factor and block, training day/week, and/or test day were variously considered the repeated-measures, within-subjects factors. Gender was excluded from analysis as in Experiment 1. The Geisser-Greenhouse correction was used to adjust for multiple comparisons across the repeated test-block factor (Keppel, 1982). Pearson product-moment correlations (r) were used to examine the relationship between performance on the cognitive test at 1.5 years of age and performance on the cognitive test at 3.5 years of age. For all

analyses, test statistics were evaluated with two-tail probabilities, $p < 0.05$, and descriptive statistics are presented as mean \pm SEM.

Results

Cognitive performance with the box opening oriented straight: Training trials. During prepotent training, when the box opening was always oriented straight, monkeys from both rearing conditions successfully completed nearly all trials. Specifically, of the 3,600 prepotent training trials, 94% were successfully completed on the first-reach attempt, 98.86% were successfully completed on the second-reach attempt, and 99.97% of the retrievals were ultimately successful (i.e., only one trial out of 3,600 was not successfully completed). The number of correct retrievals did not differ by block, day, or week.

In general, all monkeys rapidly retrieved all marshmallow treats (retrieval latency per trial was 1.1 ± 0.03 sec) on all six prepotent training days. Monkeys only made more reach attempts, $F(10, 180) = 8.389$; $p < 0.0001$, and took longer to retrieve the treat, $F(10, 180) = 3.913$; $p < 0.017$, on the first block of trials on the first day of Week 2 training, which was preceded by the first day of cognitive testing the first week. These data suggest that the testing portion of this paradigm transiently increased cognitive demand in the subsequent week's first training block. Stress inoculated and non-inoculated monkeys did not differ on the number of correct retrievals, the retrieval latency, or the number of reach attempts.

Cognitive performance with the box opening oriented straight: Test trials. Every stress inoculated and non-inoculated monkey successfully retrieved all marshmallow treats (45 trials/monkey) during the straight-presentation trials on test days. Retrieval latencies during the straight-presentation trials of testing did not differ from matched straight-presentation trials (1, 3, 5, 7, and 9) during training, $F(3, 54) = 2.409$; $p = 0.125$. A main effect of block on successful first-reach attempts was observed for all subjects, $F(2, 36) = 3.813$; $p = 0.039$. Specifically, monkeys successfully completed more straight-facing trials on their first reach attempt during the first (97%) compared to the third (89%) block of trials, ($p = 0.032$) for all test days. Successful first-reach attempts on the first and third blocks did not differ statistically from those assessed in the second block of trials (91%). These findings likewise suggest that the testing portion of this experiment increased cognitive demand. No rearing-related differences in correct retrievals, retrieval latencies, or reach attempts were found for straight-facing trials.

Cognitive performance with the box openings oriented to the sides. Stress inoculated and non-inoculated monkeys completed a similar number of side-facing trials in this test paradigm, $F(1, 18) = 0.02$; $p = 0.968$. A Test Day \times Trial Block interaction was observed, $F(4, 72) = 4.062$; $p = 0.044$, such that monkeys' performance improved over time, with both stress inoculated and non-inoculated monkeys successfully completing 100% of their test trials by the second block of testing on Day 2. Finer grained analyses on the first day of testing revealed that stress inoculated monkeys retrieved more marshmallow treats on their first-reach attempts for the three trial blocks of Test Day 1 compared to non-inoculated monkeys, $F(1, 18) = 5.912$; $p = 0.026$. Specifically, stress inoculated monkeys successfully completed 68% of these 15 side-facing trials on their first-reach attempt versus 28% of non-inoculated monkeys. Stress inoculated monkeys also successfully

completed more first-block side-facing trials on their first-reach attempt for all three test days compared to non-inoculated monkeys, $F(1, 18) = 5.562$; $p = 0.030$ (see Figure 1).

Consistent with the rearing-related difference in successful first-reach attempts, analysis of retrieval latencies revealed a Rearing Condition \times Test Day \times Trial Block interaction. Decomposing the interaction, we found that stress inoculated monkeys were already completing their Block 1 trials on Test Day 1 as fast as they subsequently did on the first block of trials on Test Days 2 and 3, $F(2, 20) = 1.905$; $p = 0.194$ (Figure 1). Non-inoculated monkeys, in contrast, demonstrated an 84.96% decrease in retrieval latencies on Trial Block 1 from Test Day 1 to Test Day 3, $F(2, 16) = 8.372$; $p = 0.019$ (Figure 1).

In the first block of trials on Test Day 1, stress inoculated monkeys made a total of 8.5 ± 1.7 straight-reach attempt errors compared to 31.7 ± 5.8 errors for non-inoculated monkeys when the box openings were oriented to the sides. Over time, both stress inoculated and non-inoculated monkeys made fewer straight-reach attempt errors, $F(2, 36) = 14.643$; $p < 0.0001$. Nevertheless, across the first three blocks of testing on Day 1, $F(2, 36) = 13.970$; $p < 0.0001$, and for Block 1 across the three test days, $F(2, 36) = 11.899$; $p = 0.001$ (Figure 1), stress inoculated monkeys more successfully inhibited inappropriate straight-reaching attempts compared to non-inoculated monkeys.

Relationship between cognitive performances at 1.5 and 3.5 years of age. In our earlier experiment at 1.5 years of age (Parker et al., 2005), cognitive performance was assessed in the same monkeys using a test paradigm that consisted of 1 week of training (10 straight-facing trials per day) followed by 2 weeks of testing (10 trials per day, alternating between straight- and side-facing trials). Individual differences in performance were most pronounced on side-facing trials during the second week of testing. The relationship between the number of correct first-reach attempts on side-facing trials during the second week of testing in this earlier experiment and the number of correct first-reach attempts on side-facing trials for the first day of testing (when individual differences were most pronounced) in the present experiment was therefore examined. Similar to the human literature, cognitive performance was remarkably consistent across the two assessment points which spanned juvenile development to early adulthood, $r_{18} = 0.477$; $p = 0.034$.

Discussion

These experiments sought to clarify and extend our understanding of the stress inoculation phenotype in two functional domains: stress responsivity and cognitive control. These studies demonstrated that stress inoculated monkeys: (a) mount neurobiological responses equivalent to non-inoculated monkeys when the stressor is of sufficient intensity, and (b) continue to exhibit enhanced cognitive control as young adults compared to non-inoculated monkeys. These data support the notion that the stress inoculation phenotype reflects stress resilience rather than stress pathology. Stress inoculated monkeys therefore more closely resemble adult rodents which received high levels of maternal care in infancy and psychologically resilient people than individuals with PTSD or conduct disorder.

In Experiment 1, both stress inoculated and non-inoculated monkeys exhibited robust increases in CSF MHPG and plasma

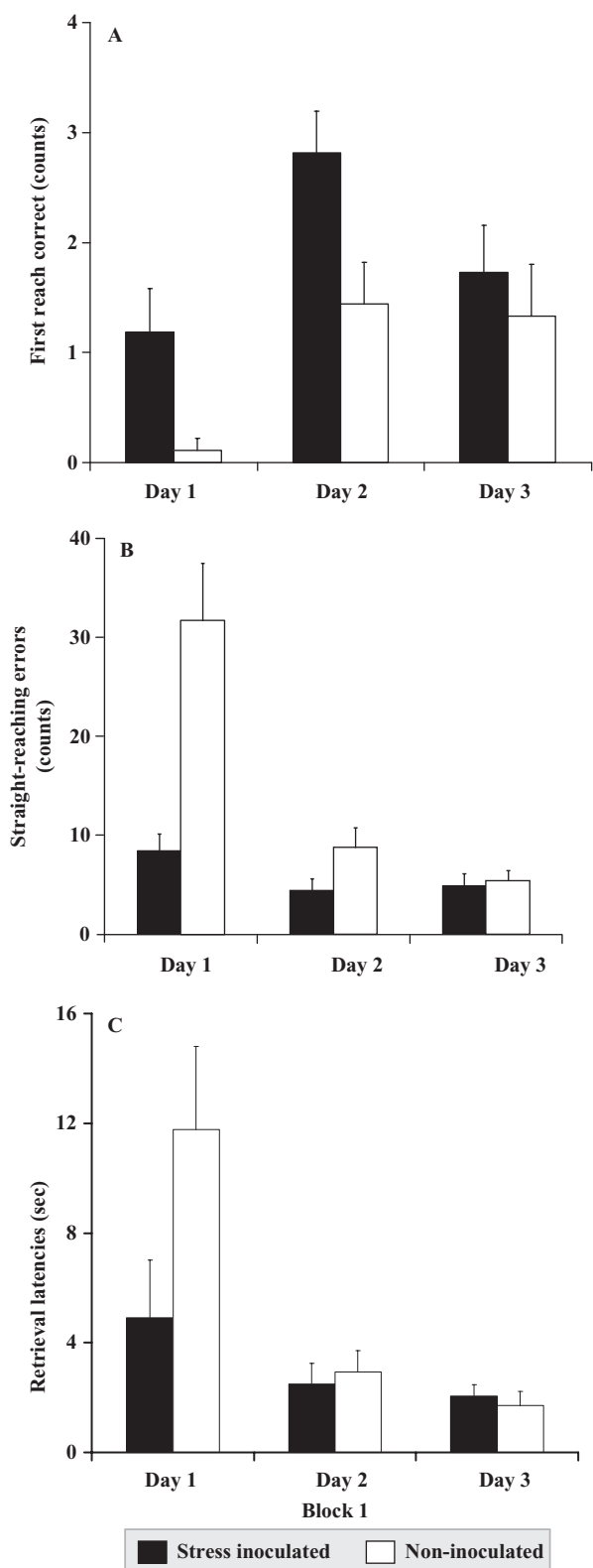


Figure 1. Rearing-related differences in cognitive performance on side-facing trials. Measures of (A) total correct first-reach attempts, (B) total straight-reach attempt errors, and (C) average retrieval latency for the five side-facing trials for Block 1 of each of the three days of testing are presented for stress inoculated ($N = 11$) and non-inoculated ($N = 9$) monkeys. Data are presented as mean \pm SEM.

cortisol concentrations following exposure to a 1-hr social-isolation stress paradigm. No rearing-related differences were observed. The present results stand in contrast to previous reports that stress inoculated monkeys exhibit diminished neurobiological responses to a variety of psychological stressors across infancy into midlife adulthood (Levine & Mody, 2003; Lyons et al., 1999; Parker et al., 2004, 2006). A possible explanation for these discrepant findings is that stress inoculated monkeys perceive lower grade stressors as less threatening than non-inoculated monkeys, but above a certain threshold, all monkeys respond with equally robust noradrenergic and HPA-axis activation. This hypothesis is supported by evidence that lack of either social support or stressor familiarity—both of which were absent in Experiment 1 but variously present in prior experiments—increases emotional distress and noradrenergic and HPA-axis responses during stress exposure compared to manipulations that afford social buffering or to which the subject is familiar (DeVries, 2002; Dienstbier, 1989; Levine & Wiener, 1988).

Enhanced cognitive-response inhibition is observed in resilient people (Nigg et al., 2007), and findings from Experiment 2 confirmed that a similar phenomenon persists at least through early adulthood in stress inoculated compared to non-inoculated monkeys. As in our previous study (Parker et al., 2005), no other rearing-related differences in aspects of cognitive performance that did not require response inhibition were found (e.g., correct retrievals on straight-facing trials; motivation as indexed by retrieval latencies on straight-facing trials). These results are consistent with the notion that early stress inoculation protocols affect response inhibition, rather than nonspecific aspects of cognitive performance.

Unlike our previous study, in which juvenile monkeys from both rearing conditions performed poorly during the initial presentation of side-facing trials, we knew that adult monkeys in the present study would be proficient at response inhibition (Lyons, Lopez, Yang, & Schatzberg, 2000). To increase task difficulty for these adult monkeys, we designed a different paradigm in which subjects completed not one but three blocks of trials per day (i.e., 10 trials vs. 30 trials) and which featured 2 training days prior to each weekly test day to maximally reinforce as prepotent the straight-reaching response. Behavioral data showed that on the first day of Week 2 training (which was directly preceded by the first test day), monkeys made more reach attempts and took longer to retrieve marshmallows on the 30 straight-facing trials. Monkeys also successfully completed more straight-facing trials on their first-reach attempt during the first (97%) compared to the third (89%) block of trials across all test days. These findings suggest that the behavioral changes noted on the straight-facing trials were affected by “interference” from the side-facing trials, and that the testing portion of this experiment indeed increased cognitive demand in monkeys from both rearing conditions.

Rearing-related differences in Experiment 2 were most evident on the first test day and on the first block of trials across test days (each of which was preceded by 60 straight-facing trials). On the first day of testing, stress inoculated monkeys successfully completed 68% of the 15 side-facing trials on their first-reach attempt versus 28% of non-inoculated monkeys. Monkeys’ performance steadily improved over time, but stress inoculated monkeys continued to successfully complete more first-block side-facing trials on their first-reach attempt for all three test days compared to non-inoculated monkeys. This rearing-related difference was not attributable to slower first-reach initiation or decreased motivation. Rather, non-inoculated compared to stress inoculated monkeys

were less able to inhibit inappropriate straight-reaching attempts on side-facing trials (e.g., 31.7 ± 5.8 vs. 8.5 ± 1.7 errors on Block 1 of Test Day 1) immediately following prepotent training days.

Data from young humans indicate that inhibitory cognitive control improves with development (Kochanska et al., 1996). Informal comparison of juvenile and adult squirrel monkey performance on the response inhibition test supports this notion, as adult monkeys make fewer straight-reach attempts on the side-facing trials and fewer detour reaches on the straight-facing trials compared to juvenile monkeys (Lyons et al., 2000; Parker et al., 2005). Performance on tests of response inhibition is highly stable across time, such that children who perform better than peers at one age score higher than peers at later ages (Kochanska & Knaack, 2003; Kochanska et al., 1996). This was likewise the case for monkeys in our experiment, as we observed a positive correlation between cognitive performance at 1.5 years of age and cognitive performance at 3.5 years of age. These data support the notion that the rearing-related differences we previously observed when subjects were juveniles did not reflect transient developmental differences in task proficiency, but rather, that exposure to early stress inoculation protocols alters inhibitory control of behavior in an enduring way.

As with all studies, several limitations must be considered. First, we did not evaluate rearing-related differences in neurobiological responsivity to stressors of varying intensities in the same experiment. This design would have allowed us to more directly evaluate whether rearing-related differences are observed only when stressors are “milder” but not “more severe” in nature. We do have indirect proof for this hypothesis, however, as rearing-related differences were previously observed in this monkey cohort when stress levels of cortisol were 80% increased above baseline levels (Parker et al., 2004), but no rearing-related differences were observed when stress levels of cortisol were 268% increased above baseline levels in the present experiment. Second, we did not assess gender differences in either of our experiments, both of which had more female than male subjects. The generalizability of our findings is thus constrained by a skewed sex ratio, and additional research is needed to assess the effects of early stress inoculation protocols on gender differences in stress responsivity and cognitive control.

In conclusion, these results indicate that stress inoculation protocols do not create biological defects in the stress response or induce transient developmental differences in cognitive control. Rather, stress inoculation protocols appear to alter the appraisal of and response to “milder” stressors as less threatening and permanently enhance inhibitory cognitive control of behavior, at least through early adulthood. Studies of elderly rodents have shown that an early life manipulation (i.e., postnatal handling) that creates a phenotype similar to that created by stress inoculation protects against the development of age-related cognitive impairments observed in control animals (Meaney, Aitken, Bhatnagar, & Sapolsky, 1991; Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988). Additional research is required to examine whether exposure to early stress inoculation protocols likewise protects against various age-related cognitive impairments, particularly the decline of inhibitory cognitive control documented in older human subjects (Christ, White, Mandernach, & Keys, 2001; Williams, Ponsse, Schachar, Logan, & Tannock, 1999).

Note

1. Association for Assessment and Accreditation of Laboratory Animal Care International.

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