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Transgenerational Effects of Early Variable Foraging Demand Stress in Female Bonnet Macaques

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Abstract

Stress coping is an important part of mammalian life, influencing somatic and mental health, social integration, and reproductive success. The experience of early psychological stress helps shape lifelong stress coping strategies. Recent studies have shown that the effects of early stress may not be restricted to the affected generation, but may also be transmitted to offspring. Understanding whether early stress influences development in subsequent generations may help us understand somewhat why many stress-related traits and diseases, for which little genetic basis has been discovered, run in families. Experimental early life “variable foraging demand” (VFD) stress has been associated with behavioral hypo-responsiveness to stress in infant and adolescent bonnet macaques. The present study examined the behavioral effects of experimental early VFD stress in adult bonnet macaques, and further investigated whether non-exposed adolescent offspring of VFD macaques were also affected. Thirty female bonnet macaques from four rearing histories were observed for behavioral response during stress: adults which had been VFD reared as infants ($n = 11$), adults which had been Control reared as infants ($n = 9$), and foraging demand naïve adolescents whose mothers were VFD ($n = 4$) or Control reared ($n = 6$). Subjects were observed for behavioral response during two experimental stressor conditions, including: (1) relocation to a novel environment; and (2) relocation with exposure to a “human intruder” making eye contact. Factor analysis yielded five factors that described categories of behavior across stress conditions. While adult VFD and Control reared females unexpectedly did not differ significantly, non-exposed adolescent offspring of VFD reared mothers displayed significant hypo-responsiveness in all behavioral categories compared with non-exposed adolescent offspring of Control females. We suggest that stress hypo-responsiveness previously observed in adolescent VFD reared animals may abate with age, but is nonetheless observed in the next generation. We conclude that VFD stress affects behavioral development of subsequent generations in non-human primates.

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Keywords

transgenerational effects; stress; bonnet macaque; maternal; variable foraging demand

INTRODUCTION

Individual stress reactivity (or stress coping) strategies impact many aspects of mammalian life, including somatic and mental health, social integration, and reproductive success [Wegman and Stetler, 2009]. Because trait-like stress adaptation strategies affect many areas of social life, understanding how these strategies develop may provide insight into the psychobiological and evolutionary consequences of stress. Across species, the experience of an early life stressor is one of the most potent influences in development [humans: Bowlby, 1951; rhesus macaques: Harlow and Zimmerman, 1959; rats: Denenberg, 1964]. The experience of an early psychological stressor affects different individuals in different ways, but in general, may reprogram stress adaptation across the lifespan [Denenberg, 1964; Hofer, 1970; Levine, 2001; Young et al., 1973]. The effects of early stress on non-human primate development have been demonstrated using an ecologically relevant “variable foraging demand” (VFD) manipulation. The VFD paradigm first described by Rosenblum and Pauly [1984] mimics the unpredictability of resources that may occur naturally in macaque life history. VFD stress is energetically challenging, strains the mother–infant relationship and disrupts infant social relationships [Rosenblum and Pauly, 1984]. First applied in bonnet macaques (*Macaca radiata*), the VFD paradigm, like early maternal deprivation, influences trait stress response in developing bonnet macaques. The impact on the infant during VFD conditions is observable: infant separation distress is higher, and social play is lower, than Control infants during the period in life when the foraging paradigm is administered [Andrews and Rosenblum, 1994; Rosenblum and Pauly, 1984]. These effects persist after the foraging demand paradigm has ceased, as VFD juveniles show reduced behavioral response (vocalizing, self-directed behavior, and locomotion) to a human intruder wearing a mask, and exhibit decreased affiliative behavior in newly formed social groups (less grooming, approaches, proximity, more avoidance) compared with Controls [Andrews and Rosenblum, 1994]. These effects may shift over development, however, as young adult VFD macaque males show greater emotional responsiveness to a fear-inducing stimulus than control males [human intruder wearing a mask; Jackowski et al., 2011]. Longitudinal stability of these strategies in female macaques has not yet been studied.

It is possible that the effects of these early experiences may not be limited to the affected individual: they may be passed on to the next generation, as has been demonstrated in other mammals [rats: Champagne and Meaney, 2007; Francis et al., 1999; mice: Franklin et al., 2010; humans: Yehuda & Bierer, 2008]. The intergenerational transmission of the effects of early stress on stress adaptation in rodent models has been relatively well established. Infant rats that experience early stress in the form of poor maternal care tend to develop hyperactive responses to stress and poor mothering skills. This cycle is perpetuated from mother to daughter [Champagne and Meaney, 2007; Francis et al., 1999; Levine, 2001]. The mode of transmission in rats may be partly environmental and partly molecular: the experience of poor mothering is associated with epigenetically driven changes in the

regulation the glucocorticoid receptor gene, which partly regulates hypothalamic–pituitary–adrenal axis function [Weaver et al., 2004]. The transmissibility of the effects of early stress is less well understood in primates. Some experiences are transmitted across generations in non-human primates, as maternal behavior is correlated between biological mothers and daughters in vervets [Fairbanks, 1989] and even between foster mothers and daughters in rhesus macaques [Maestriperi, 2005]. If the effects of an experimental stressor are perpetuated across generations, it would mean that the effects of even short-term early stress may have important long-term evolutionary and developmental psychobiological consequences.

In the present study, we first sought to determine whether early life VFD stress influenced stress adaptation in adulthood. We also investigated whether the effects of maternal VFD were evident in non-exposed offspring. Methods for eliciting individual differences in stress response often include separating the individual animal from its social group and familiar housing and observing the magnitude of an individual's response to stressful stimuli. During separation, test animals are often exposed to one or more novel and potentially stressful situations, including a novel environment, novel objects, and/or novel conspecifics or experimenters [Golub et al., 2008; Higley et al., 1992; Schneider, 1992]. Non-human primates are often consistent in how they respond to these challenges, displaying either behavioral activation or inhibition during stress, indicating that these strategies may be trait-like [Kalin et al., 1991; Kinnally et al., 2008; Suomi, 1983]. We therefore compared behavioral stress responsiveness during a series of two stressful experimental conditions among four groups of female bonnet macaques. These groups included adult females that had been VFD or control-reared and adolescent foraging demand naïve females that had been raised by mothers exposed to VFD or control conditions early in life. We included only females in the present study because the greatest opportunity for environmental transmission between generations in wild matrilineal macaque species is between mothers and daughters, who remain together in social groups while male offspring may emigrate. If the effects of VFD stress are stable into adulthood, we predicted that adult VFD reared females would show behavioral hypo-responsiveness within each stressful condition compared with adult Control reared females. Further, if the effects of VFD stress are transmitted to subsequent generations, we predicted that adolescent offspring of VFD reared females would also show a diminished behavioral response to stress compared with offspring of Control reared females.

METHODS

Subjects

Thirty female bonnet macaques (*M. radiata*), 3–13 years of age (mean 8.4 years), were included in this study. All were housed at SUNY Downstate Medical Center in one of 17 indoor social enclosures (2 m × 3 m × 2.5 m) with 1–5 other adult females and juveniles. Animals were maintained on 12:12 light dark cycle and fed monkey chow once per day at 1,400 hr. Adult subjects had been raised in either variable foraging demand (VFD-reared, $n = 11$), or Control low foraging demand/foraging demand naïve conditions (never exposed to the foraging device; Control-reared, $n = 9$). Adolescent subjects were reared in foraging

demand naïve conditions but with a VFD reared mother ($n = 4$) or with a Control reared mother ($n = 6$). Thus, four comparison groups were included in this study: (1) adult Control reared, (2) adult VFD reared, (3) foraging demand naïve adolescent Controls reared by Control reared females (Control offspring), and (4) foraging demand naïve adolescent Controls reared by VFD females (VFD offspring).

Five of the ten offspring were adolescent daughters of VFD ($n = 3$) or Control ($n = 2$) subjects included in the present study. The remaining five were adolescent daughters of adult VFD- or Control-reared adult females that were unavailable for study. No adult or adolescent subject had ever been part of any experimental protocol except that adults had been exposed to early VFD/control rearing.

The average age of Control-reared females (mean age = 9.75 years, range = 7–13 years) was comparable to VFD-reared females (mean age = 8.76 years, range = 7–10 years), who were all older than adolescent Control offspring (mean age 3.13 years, range = 3–3.5 years) and adolescent VFD offspring (mean age = 3.19 years, range = 3–3.5 years). No offspring shared a common mother, but one set of paternal half siblings was included in the VFD offspring and one set of paternal half siblings was included in the Control offspring group. All animal procedures were conducted in accordance with the SUNY Downstate Medical Center Institutional Animal Care and Use Committee. Further, all research adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates.

Rearing Conditions

VFD and control reared adults—Adult subjects had been reared from birth until four months of age under comparable conditions. They were housed with their mothers in small social groups comprised of 3–6 mother–infant dyads in small enclosures (2 m × 3 m × 2.5 m) in the SUNY Downstate Medical Center non-human primate facility. For 3 months between 4 and 8 months of age, the mothers of VFD subjects were exposed to a foraging device [a modified cart with wood chips to hide food, see Rosenblum and Paully, 1984 for full description]. VFD group members were alternately required to forage either little or extensively for food, in 2-week blocks over 12 weeks. This variability in foraging demand created unpredictable environmental conditions for experimental animals. Adult Control females were either able to readily retrieve food from this device (low foraging demand) or were never exposed to this device (foraging demand naïve). Adults exposed to control low foraging demand conditions in infancy did not differ significantly from adult foraging demand naïve subjects in our analysis.

Prior to 1 year of age, VFD and Control subjects had been housed with conspecifics exposed to the same experimental rearing condition. At 1 year of age, adult VFD- and Control reared females were then separated from mothers and natal groups and housed in peer groups of the same rearing conditions [Coplan et al., 1996]. Once they reached reproductive age, they were relocated to social groups that were mixed in terms of VFD-reared, Control reared, and foraging demand naïve members, so VFD- and Control-reared adults were exposed to conspecifics of all rearing histories after about 3 years of age.

VFD and control offspring—Adolescent VFD- and Control offspring were reared in the same caging and social conditions to adult subjects and to each other, but were never exposed to foraging demand procedures. VFD off-spring were reared by mothers who had experienced VFD conditions as 4- to 8-month-old infants. Control offspring were reared by mothers who had not experienced VFD stress in infancy. All VFD offspring were sired by VFD reared males, and all but one Control offspring had Control-reared fathers. One Control offspring had a VFD-reared father. The sires of these adolescents had been housed in social groups long enough to impregnate females and were then removed. Thus, females never lived in social groups with their fathers. From birth, adolescent VFD and Control offspring were housed in groups that were mixed in terms of rearing history, composed of VFD-reared, Control-reared, and foraging-demand naïve adult females and their offspring.

Behavioral Testing

Rationale—To elicit individual differences in stress response, we examined the behavior of our adult and adolescent subjects under four different stressful conditions conducted during four consecutive weeks. Removal from familiar social groups and relocation to relatively novel conditions is a common parameter for assessing individual stress reactivity to different stimuli [Ainsworth and Bell, 1970; Capitanio et al., 2005; Champoux et al., 2002; Kalin et al., 1991]. Individual responses in novel environments are thought to be more trait-like rather than learned, because subjects have little or no previous experience with specific aspects of the testing situation. The procedures described below were designed to measure subjects' individual differences in behavioral stress adaptation across time and conditions. We tested subjects' behavioral response during 2 weeks of daily relocations and 1 week of daily "human intruder" testing [modified from Kalin et al., 1991]. Subjects were observed for a variety of normal and anxiety-related behaviors in individual testing cages during each stress condition.

Testing environment—The testing environment was standardized for all conditions. During testing, all adult and adolescent animals were housed in individual cages in a testing room (6 m × 3 m × 4 m) in the primate facility at SUNY Downstate Medical Center. Four quadruple cages were used to house animals during testing. Each of these quadruple cages included four individual cages (two on top and two on bottom), each measuring 1 m × 0.8 m × 1 m. These cages were connected by steel dividers that prevented visual and tactile contact between animals. Quadruple cages were on wheels, which were locked during testing. Quadruple cages were placed against opposite walls (two quads against each wall) in the testing room and faced a center aisle. Animals were housed in individual top cages only, so that animals were tested at a height of 1.2 m, putting subjects at eye level with the standing observer for all tests. This meant that up to eight animals were observable within one session (or cohort) as each of four quads had two top cages.

Animals were tested in the same cohort of seven or eight animals every day, and all animals were placed in individual housing described above before testing began. Each animal was tested in the same individual cage for all conditions during all testing days. All subjects had visual, auditory, and/or olfactory access to other conspecifics (always animals from other social groups) housed in adjacent cages during testing. For practical reasons, this multiple

individual housing practice is sometimes used when experimentally observing macaque individual behavior [Golub et al., 2008; Kinnally et al., 2008; Rosenblum et al., 2001]. We ensured that all subjects had the same social experience with their neighboring conspecifics in the testing room, in that each could see two or more other animals that were not members of their home social group. A maximum of two animals from the same social groups were tested in the same cohort, but animals from the same social groups were housed on the same side of the room, with one or two cages between them, to prevent visual contact.

Animals were not clustered by rearing group in the testing room. Animals were intermixed in their caging position with animals from all four rearing conditions.

Testing day procedures—Testing was conducted between 0900 and 1300 hr. On testing days, animals were removed from social housing and placed in transport boxes (0.3 m × 0.5 m × 0.3 m). Within one hour, animals were moved to individual temporary individual housing in the testing room described above. To prevent time of day or testing order effects, cohorts were tested in a different order every day. Order of testing within cohort was also rotated by testing cage number daily. These procedures were used to control for the potential confound introduced by novelty of the testing conditions, time of day of individual testing, and relative order of individual testing within cohort.

Immediately after the last animal was placed in the testing cage, all animal care staff vacated the testing room, and the observer entered the room and observation of the first subject for one of the test conditions (described in detail below) commenced. The same female observer conducted all observations, and stood at least 1 m from the testing cage at all times during observation, avoiding direct eye contact with subjects. Standard macaque anxiety-related and normal behavior was observed during trials [Golub et al., 2009; Kalin et al., 1991; see Table 1 for Ethogram descriptions]. The observer recorded all behavioral responses during all tests. Prior to observation, the observer had reached 85% or better agreement on all ethogram behaviors for 10 consecutive 1-min reliability trials with another observer. Inter-observer reliability was calculated as the number of behaviors the two observers agreed upon divided by the total number of observed behaviors (agree/[agree + disagree]). The observer was blind to subjects' rearing histories. After testing, subjects were returned to their social groups and enclosures within 1 hr, and the total amount of time an animal was removed from social groups did not exceed three hours per day. These procedures were repeated for each of the following conditions. Conditions were administered daily for each subject over four weeks. All tests were conducted on different days, but in fixed order so that all subjects were tested in the same condition on the same day.

Relocation: During the first 2 weeks of testing, animals were relocated to individual housing in the testing room on six different days (three consecutive days in weeks 1 and 2). For these trials, only a female observer was present during observation. This observer recorded behavioral frequencies for each animal during a 1-min trial during each relocation (see Table 1 for ethogram).

During the intervening week between relocation and Human Intruder testing, five daily observations were conducted in the same manner as relocation only conditions. In this

condition, however, a second male human stood next to the observer, 1 m from the testing cage, averting eye contact with the subject throughout the 1-min observation. This condition was included to acclimate animals to the second human before initiating the “human intruder” trials described below. Because behavioral frequencies were lowest during this condition, to reduce the number of variables for statistical analysis, the data from this condition is not included in our analysis.

Relocation with human intruder: During four modified “Human Intruder” [Kalin et al., 1991; Kinnally et al., 2010] trials in week 4, one male and one female observer stood at a 1 m distance from subjects. The female observer recorded behavior during a 1-min baseline trial, while both humans averted eye contact with the subject. Within 10 sec, the human intruder trial was initiated, during which the (non-observer) second male human initiated and maintained eye contact with the animal for the duration of the one-minute trial. The observer recorded all behavioral frequencies during this trial. One baseline and one human intruder trial was conducted per day over 4 days. We only consider the data from the eye contact condition in the present analysis.

Data Analysis

Behavioral analysis—We used factor analysis [Maruyama, 1998] to detect latent factors underlying behavioral expression in two stressful contexts. Our subject to item ratio was somewhat low (2.1:1) according to typically used factor analytic methods, but previous studies have demonstrated that when communalities (a measure of indicator reliability) are high (>0.6) among items, the model is relatively robust to error [MacCallum et al., 2001]. Our preliminary analysis demonstrated average communality of 0.759 among our items, and hence, we proceeded with analysis. Only seven behaviors were observed more than once per trial (i.e., observed in more than one animal in one trial), and these behaviors (summed across trials) were included in the factor analysis. These behaviors were: environmental explore (Relocation frequency range 0–16, mean 2.83; Human Intruder frequency range 0–8, mean = 1.70), hang (Relocation frequency range 0–8, mean = 0.80; Human Intruder frequency range 0–3, mean = 0.233), lipsmack (Relocation frequency range 0–51, mean 14.47; Human Intruder frequency range 1–44, mean = 26.37), locomotion (Relocation frequency range 0–49, mean = 10; Human Intruder frequency range 0–47, mean = 11.67), self-directed behavior (Relocation frequency range 0–7, mean 0.80; Human Intruder frequency range 0–2, mean = 0.33), tooth grind (Relocation frequency range 0–28, mean = 2.17; Human Intruder frequency range 0–19, mean = 2.97), and vocalize (Relocation frequency range 0–89, mean = 9.87; Human Intruder frequency range 0–54, mean = 14.60). We conducted an outlier analysis for each behavior within each condition and initially removed two data points that were >3.5 standard deviations from the population mean. Their removal did not change the factor structure significantly, so these data points were retained for the final analysis. We entered the frequencies of these behaviors across all trials of relocation and the frequencies of these behaviors across trials of human intruder testing into a principal components analysis using Promax rotation. Analysis yielded five factors that explained 75% of the variance in behavioral expression. Factor loadings greater than 0.4 were considered to contribute to the latent factor. Scores were generated using regression. The factors included the following behaviors: (1) hang during relocation (lipsmack during

relocation, locomotion during relocation, vocalize during relocation, tooth grind during relocation), (2) Lipsmack/Locomote (lipsmack during relocation, locomotion during relocation, lipsmack during human intruder, locomotion during human intruder, and tooth grind during human intruder), (3) Environmental Exploration (environmental exploration during relocation, environmental exploration during human intruder, locomotion during relocation, tooth grind during human intruder), (4) self-directed behavior (self-directed behavior during relocation, self directed behavior during human intruder), and (5) Hang and vocalize during human intruder.

Effects of rearing on behavioral stress response—The effects of rearing history and condition on factor scores was tested using repeated measures ANOVA, with five factors used as repeated measures and rearing history (four groups: VFD-reared, Control-reared, VFD offspring, and Control off-spring) as a between subjects factor. Post hoc testing was conducted using Fisher's Least Significant Difference comparisons of mean differences between groups. Two-tailed significance was set at $p < 0.05$.

RESULTS

We observed a main effect of rearing on behavioral factor scores ($F(3, 26) = 3.01$; $p = 0.048$; partial $\eta^2 = 0.258$; see Fig. 1). Adolescent VFD Offspring exhibited significantly lower factor scores across all behavior types (multiple behaviors during relocation, Lipsmack/locomotion during both conditions, environmental exploration during both conditions, self directed behavior during both conditions, and hang/vocalization during human intruder trials) compared with Control offspring (Fisher's LSD, $p = 0.011$). Adolescent VFD offspring did not differ significantly from Adult Control (Fisher's LSD, $p = 0.096$) or Adult VFD subjects (Fisher's LSD, $p = 0.333$). Control offspring also did not differ from adult Controls (Fisher's LSD, $p = 0.180$), but displayed significantly higher factor scores than VFD adults (Fisher's LSD, $p = 0.027$). Adult Control and VFD did not differ from each other in overall behavioral reactivity scores (Fisher's LSD, $p = 0.313$). No interaction between rearing groups across factor scores were observed. Age (in days) was not correlated with any factor score (all $r < 0.144$; all $p > 0.449$).

DISCUSSION

Previous studies report that the experience of early VFD stress is associated with reduced behavioral responsiveness to social and non-social novel conditions in infancy, an effect that persists into at least adolescence in bonnet macaques [Andrews and Rosenblum, 1994; Rosenblum and Pauly, 1984; Rosenblum et al., 2001]. This behavioral inhibition may reflect an energy saving strategy [Champoux et al., 1993], or an anxious psychological response to stress [Bell and Ainsworth, 1972; Denenberg, 1964; Kagan et al., 1987; Rosenblum et al., 2001; Stevens et al., 2009; Suomi, 1983]. We found that behavioral expression during stressful circumstances did not differ significantly between adult VFD reared and Control reared females. One reason for the difference between studies may be that while previous studies comparing VFD and control behavior observed behavioral responses to a fearful stimulus while animals remained in their home cages [e.g., Rosenblum et al., 2001], we relocated animals to a new environment. If the effects of VFD stress are

only observed in social circumstances, we may not have been able to detect these differences with our testing paradigm. However, it is also possible that the difference between our results and those from earlier studies may be due to our adult subjects' experiences. Throughout their lives in the primate facility, our adult subjects were likely accustomed to conditions similar to those in our study, including relocation and exposure to humans. Over time, this familiarity may have overcome the effects of early VFD stress and equalized somewhat adult stress response. It is also possible that behavioral reactivity became developmentally attenuated with advancing age, as has been demonstrated in rhesus macaques [Corcoran et al., 2012; Zhang et al., 2012].

Intriguingly, though our sample size was small, adolescent Control offspring displayed significantly higher behavioral expression than VFD adults or adolescents. These differences spanned the range of behaviors we observed, including composites of behaviors (hanging, lip-smacking, locomotion, vocalization, and tooth grinding) exhibited during relocation, hanging, and vocalizing during human intruder testing, environmental exploration during both conditions, self-directed behavior during both conditions, and lipsmacking/locomotion during both conditions. Though one set of paternal half siblings was included in the Control Offspring and VFD offspring group, relatedness did not appear to explain our results. In both cases, these half-siblings were dissimilar in their behavioral response to stress: in the VFD offspring, one was the highest of the group in reactivity scores within all tests while its half sibling was the lowest. This general downregulation of behavior in response to multiple types of stress is consistent with previous reports of behavioral inhibition in VFD reared rodents, bonnet macaques, and squirrel monkeys [Andrews and Rosenblum, 1993, 1994; Champoux et al., 1993; Coutellier et al., 2009; Rosenblum et al., 2001], except that we observed behavioral downregulation in non-exposed daughters of exposed females. These findings must be replicated in a larger sample before firm conclusions can be drawn. Nonetheless, we suggest that the behavioral effects of experimental early VFD stress may abate in adulthood in females, but may be evident under stressful conditions in the next generation.

We do not yet know how offspring acquired different stress coping strategies based on their mother's early experiences. We hypothesize that the effects of early VFD stress were transmitted from mother to daughter. Adolescents' behavioral stress responses were not likely learned from other adult social group members because adolescent VFD- and Control offspring resided in mixed social groups that consisted of VFD-, Control-, and/or foraging naïve-reared adults. It is possible that VFD offspring learned coping strategies by observing their mothers' responses to challenging conditions during development. Maternal behavior may also have played a role, although one of the limitations of our study is that we cannot ascertain whether maternal behavior quality or maternal parity explain our findings. Moreover, although we evaluated the adult females, it was at an age after they had raised the offspring evaluated in this study, and so we do not know the degree to which VFD mothers differed in behavioral response to stress at the time they rearing their infants. If maternal behavior played a role, we might predict that the insecure attachment that VFD females experienced with their own mothers [Andrews and Rosenblum, 1994] may have influenced VFD maternal behavior, a cycle that has been described in humans [van IJzendoorn et al., 1995], and to a certain extent, in rodents [Francis et al., 1999]. Insecure attachment can

inhibit exploration, because the mother was not a secure base for the infant to develop exploratory tendencies [Ainsworth, 1985]. In this way, insecure attachment to VFD reared mothers may have caused VFD offspring to inhibit their responses in our testing conditions. Another possibility is that neurobehavioral changes following VFD exposure are reproduced in the next generation through physiological or molecular alterations in the gestational environment the mother provides for her offspring [e.g., HPA regulation: Yehuda et al., 2005]. Epigenetic inheritance may also play a role. A recent study has implicated germ line epimutation in the intergenerational effects of early life stress between fathers and sons in mice [Franklin et al., 2010]. Since all but one biological father of our adolescent subjects were reared in the same conditions as their mothers, we cannot preclude the possibility that maternal and/or paternal germline epimutation played a role in offspring development. A key step in this research will be to identify the behavioral and molecular mechanisms of the transgenerational effects of early VFD stress in macaques.

An important limitation of this study was the small sample size of adolescent subjects. Only four female offspring of VFD reared females and six female offspring of Control reared females were available for study. These findings must therefore be replicated in a larger cohort of VFD and Control offspring. Additionally, because we focused on females, we cannot confirm that male offspring would exhibit the same transgenerational sensitivity. Many studies have demonstrated intergenerational transmission of traits in same-sex, but not necessarily opposite sex, offspring [Dunn et al., 2011; Fairbanks, 1989; Francis et al., 1999; Franklin et al., 2010; Maestripieri et al., 2005]. Future studies should determine whether the effects of maternal early life stress are evident in male offspring.

The present study suggests that a concentrated exposure to VFD stress early in life affected behavioral development in descendants of VFD-exposed individuals. This observation requires replication, and if confirmed, has important evolutionary implications. While the persistence of the effects of early stress across generations may initially seem maladaptive, in variable or consistently stressful ecological conditions, transgenerational inheritance of stress coping strategies may accelerate stress adaptation in the next generation.

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Fig. 1.

Adolescent VFD offspring exhibit lower behavioral expression during stress than adolescent control offspring. Factors are summarized here with the primary behaviors included in factor scores, but see the Data Analysis Section for a comprehensive list of behaviors included in each factor. Behavior abbreviations: EE, Environmental Exploration; HA, Hang; LS, Lipsmack; LO, Locomotion; SD, Self-directed Behavior; TG, Tooth Grind; VO, Vocalization. Means are presented standard error of the mean.

TABLE 1**Behavioral Ethogram and Definitions**

Cage shake	Uses hands or feet to shake cage, usually by grasping bars
Crouch	Ventrum close to floor; head at/below the level of the shoulders
Environmental Explore	Uses hands or mouth to manipulate the environment
Fear Grimace	Exaggerated display of teeth
Hang	All limbs off the base of the cage, suspended by at least one limb
Lip smack	Rapid pursing of lips, may be accompanied by smacking sound
Locomote	Animal moves on all limbs. Bouts should be scored every 3 sec
Scratch	Uses hands or feet to move back and forth on fur or skin
Self-directed Behavior	Appendage sucking, self-picking, grooming, chewing
Threat	Any two: Head bob, open mouth threat, ear flatten, cage shake
Tooth grind	Gnashing teeth (usually molars) together
Vocalize	Screeching, calling, barking
Yawn	Common usage