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Developmental consequences of behavioral inhibition: A model in rhesus monkeys (*Macaca mulatta*)

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Abstract

In children, behavioral inhibition is characterized by a disposition to withdraw in the presence of strangers and novel situations. Later in life, behavioral inhibition can result in an increased risk for anxiety and depression and a decrease in social behavior. We selected rhesus monkeys that, during infancy, showed evidence of behavioral inhibition in response to separation, and contrasted them with non-inhibited peers. To understand the development of behavioral inhibition at juvenile age, we collected behavioral data in response to relocation; in response to a human intruder challenge; and in naturalistic outdoor field corrals. At four years of age (young adulthood), we again collected behavioral data in the outdoor field corrals to understand the adult social consequences of behavioral inhibition. We also included sex, dominance rank, and number of available kin in our analyses. Finally, to understand the consistency in behavior in behaviorally inhibited animals, we conducted exploratory analyses contrasting behaviorally inhibited animals that showed high vs. low durations of non-social behaviors as adults. At juvenile age, behaviorally inhibited animals continued to show behavioral differences in the novel testing room and during the human intruder challenge, generally showing evidence of greater anxiety and emotionality compared to non-inhibited controls. In their outdoor corrals, behaviorally inhibited juveniles spent more time alone and less time in proximity and grooming with mother and other adult females. In young adulthood, we found that behavioral inhibition was not related to time spent alone. We did find that duration of time alone in adulthood was related to time alone exhibited as juveniles; sex, dominance rank, or the number of kin were not influential in adult non-social duration, either as main effects or as moderators. Finally, exploratory analyses revealed that behaviorally inhibited females that were more sociable (less time spent alone) as adults had spent more time grooming as juveniles, suggesting that high quality social interaction at a young age might mitigate the social consequences of behavioral inhibition. Overall, we believe that the many similarities with the human data that we found suggests that this monkey model of naturally occurring behavioral inhibition can be valuable for understanding social development.

Introduction

Behavioral inhibition (BI) reflects a disposition to respond warily to strangers (Coll, Kagan, & Reznick, 1984; Kagan, Reznick, & Snidman, 1987; Schmidt et al., 1997) and novel situations (Marshall, Reeb, & Fox, 2009) and can manifest as social anxiety (Biederman et al., 2001; Degnan et al., 2014; Essex et al., 2010; Perez-Edgar & Fox, 2005). BI has been shown to demonstrate continuity from toddler age to early childhood; for example, toddlers displaying extreme inhibition show, in childhood, signs of anxiety and distress when challenging situations are encountered (Kagan, 1989) and reduced sociability in peer groups (Kagan et al., 1988; Kagan et al., 1984; Rubin et al., 1997). Although BI has shown continuity in some studies, other data have suggested that continuity of BI may be moderated by external factors such as parental caregiving (Belsky, Fish, & Isabella, 1991; Hane, Cheah, Rubin, & Fox, 2008; Nelson & Garduque, 1991; Rubin, Burgess, & Hastings, 2002; Williams et al., 2009), and non-parental caregiving (i.e. daycare and peers) environments (Almas et al., 2011; Fox et al., 2001; Gazelle & Ladd, 2003), and by internal factors including attention processes (Perez-Edgar et al., 2010; White et al., 2011), and sex of the individual (Caspi, Bem, & Elder, 1989; Henderson, Marshall, Fox, & Rubin, 2004; Kagan, Snidman, & Arcus, 1998; Stevenson-Hinde & Shouldice, 1995).

Rhesus monkeys provide an important model to study the persistence of BI, because of their similarity in temperament with humans (Gosling & John, 1999) and their faster rate of development. Several laboratories have attempted to establish models of BI in rhesus monkeys. In one set of studies, BI was assessed by conducting behavioral observations on animals of a wide range of ages (1–19 years old) and then BI was defined by those animals that were least likely to approach new stimuli and new challenging situations, most anxious, and most constrained in social interactions (Boyce et al., 1998). In another set of studies, BI was characterized by use of a common behavioral testing paradigm, a human intruder test; behaviorally inhibited animals displayed significantly higher durations of freezing behavior (complete cessation of vocalizations and motor activity; Davidson, Kalin, & Shelton, 1993; Fox et al., 2008; Kalin & Shelton, 1989, 1998; Rogers et al., 2008). While the previously mentioned studies aimed to establish monkey models of BI, the determination of BI was quantified in a cross-sectional manner with regard to age; use of this model to study continuity, however, would require following cohorts of animals through multiple time-points. A longitudinal approach would permit examination of the stability of BI over the lifespan, and would allow for the study of relevant life-history (i.e. sex, dominance rank, number of kin) and other factors that might moderate the effects of BI on later social development.

Previous work in our lab has characterized BI in infant rhesus monkeys to understand the association of BI and negative health outcomes (Capitanio et al., 2011; Chun et al., 2013). When infants were 3–4 months old, they were relocated and separated from their mother for a 25-hour period (Section 2.2.1). The criteria used to characterize BI included: a) low Emotionality during the behavioral observations conducted in the first hour following relocation consisting of decreased rate of vocalizations, threats, lipsmacks, and scratching; b) high Vigilance from temperament ratings conducted at the end of the 25-hour period, which was a composite of: vigilant, not depressed, not tense, and not timid; and c) a blunted

cortisol response 7 hours after relocation/separation (Capitanio et al., 2011; Chun et al., 2013). Low emotionality (Kalin & Shelton, 1989, 1998; Rogers et al., 2008) and high vigilance (Fox et al., 2005; Kagan et al., 1987) are characteristics that are similar to those used to describe behaviorally inhibited monkeys and children in previous studies. The relationship between BI and levels of cortisol is unclear in previous studies, however; BI has been linked to a blunted cortisol reactivity (Gunnar et al., 2009), high baseline levels (Kagan et al., 1987; Schmidt et al., 1997), or no reactivity changes (de Haan et al., 1998) in humans. Although there are mixed results for cortisol values in the literature, the current study utilized the same criteria described in our two previous studies (Capitanio et al., 2011; Chun et al., 2013), which, in combination, did show predictive validity for an important health-related outcome.

The current study aimed to understand the persistence of BI from infancy to adulthood in a rhesus monkey model. First we hypothesized that animals identified as behaviorally inhibited as infants would continue this pattern of behavior as juveniles by showing more anxious behaviors (i.e. scratching, yawning, claspings, vocalizations) in response to stressful situations (Cross & Harlow, 1965; Hinde & Rowell, 1962; Maestripieri, Schino, Aureli, & Troisi, 1992; Troisi et al., 1991). To test this hypothesis we quantified animals' responses to relocation and to an acute challenge by a human intruder. Our second hypothesis was that behaviorally inhibited infants would spend more time alone (i.e., have a higher duration of time spent "non-social") in the naturalistic environment as juveniles, as others have suggested (Caspi et al., 2003; Caspi & Silva, 1995; Essex et al., 2010; Perez-Edgar et al., 2010; Rubin et al., 2002). To test this hypothesis, we observed animals at one year of age in their current living cages (outdoor field corrals) and quantified their social behavior. Our third hypothesis was that behaviorally inhibited animals would continue to spend more time non-social into young adulthood; we expected to find a weaker relationship with BI at the adult age point, however, inasmuch as the intervening years might cause other monkey-relevant life history factors (sex, dominance rank, or number of kin) to moderate the relationship between early inhibition and later social behavior. Rhesus monkeys live in large multi-male/multi-female social groups characterized by female philopatry and male dispersal. In addition, females form dominance hierarchies according to their matrilineal kinship, thus sex, dominance rank, and number of kin seem likely candidates as moderators of social behavior in adulthood. To test this hypothesis, animals were observed in their outdoor corrals at a second time-point as young adults, and we tested whether BI was associated with time spent alone at that age. Because behavioral consistencies might exist between juvenile and adult periods even though the underlying psychological processes that influence the expression of these behaviors could differ at the two ages, we also conducted exploratory analyses to determine whether life-history factors (described above) or other behavior observed when the animals were juveniles, might moderate the relationship between BI and adult social behavior.

Method

2.1 Subjects and living arrangements

Fifty-two rhesus monkeys (*Macaca mulatta*; 26 males) born in any of 17 outdoor 0.2-hectare enclosures at the California National Primate Research Center (CNPRC) were observed in infancy and as juveniles. As four-year-olds (young adulthood), thirty animals (11 males) still residing in the outdoor enclosures were included in the study. Sample size in adulthood was reduced due to permanent relocation to indoor housing ($n = 15$), relocation to a different type of social housing ($n = 2$), and death ($n = 5$). Each enclosure contained up to 150 animals of all ages and both sexes approximately reflecting the composition of wild troops of rhesus monkeys. Animals were provided with water ad libitum and monkey chow (Lab Diet #5038) twice daily between 0700 h and 0800 h and between 1400 h and 1500 h. Supplemental fruits and vegetables were provided twice weekly.

During the 25-hour infant assessment (BioBehavioral Assessment Program; BBA, Section 2.2.1), infants were transported to an individual holding cage (61 cm $\times$ 69 cm $\times$ 81 cm, Lab Products Inc., Seaford, DE) that contained a cloth diaper, stuffed terry cloth duck, and a novel object. Infants were provided with water ad libitum, monkey chow, fruit-flavored juice, and fruit supplements.

For juvenile assessments (Sections 2.2.2, 2.2.3), animals were relocated from their outdoor field corrals and placed in an individual holding cage (same construction and dimensions as during the infant assessments) for a total of four days, and then returned to the outdoor field corral. Behavioral testing for Day 1 is presented here, while data collected during Days 2, 3, and 4 are presented elsewhere (Chun et al., 2013). Juveniles were provided with a Nylabone Dental Chew (Nylabone, Neptune, NJ), a mirror, water ad libitum, and monkey chow twice daily between 0700 h and 0900 h and between 1400 h and 1500 h.

2.2 Procedures

Data were collected from animals at three time points: when they were infants (3–4 months of age) during the CNPRC's BBA program (see Section 2.2.1 below); as juveniles (1–2 years of age) in response to relocation and individual housing (Section 2.2.2) and using the human intruder paradigm (Section 2.2.3) five hours after relocation, and in their outdoor field corrals (Section 2.2.4); and as young adults (4 years of age) in their outdoor field corrals using the same methods as during the juvenile outdoor corral assessments (Section 2.2.4). We also obtained measures of dominance rank (Section 2.2.5) and number of kin (Section 2.2.6) during infancy, juvenile age, and adulthood.

2.2.1 BBA Program – infancy—At a mean age of 110.1 days (range = 90–124 days), infants were separated from their mothers and relocated to an individual indoor testing cage at 0900 for a 25-hour period, during which time several assessments were made (see Golub, Hogrefe, Widaman, & Capitanio, 2009). After the animal was in the holding cage for at least 15 minutes, behavioral data were collected by a technician in the room for 5-minutes on each animal using a predetermined random order. Behavioral data from more than a thousand animals were subjected to exploratory and confirmatory factor analyses to identify

underlying latent traits explaining the data. Scales were constructed by summing z-scores for items that loaded on a given factor and final scales were z-scored again. One scale, labeled “Emotionality”, consisted of rate of vocalizing and whether animals displayed threats, lipsmacks, and scratching. At 1600 h, each animal was manually restrained and blood was sampled via femoral venipuncture to measure the animals’ cortisol concentrations. At the end of the 25-hour period, a technician rated the animals’ overall temperament using a scale of 1 to 7 on 16 trait adjectives. Factor scores were calculated by summing z-scores for all of the adjectives loading on a given factor then z-scoring each scale. One scale labeled “Vigilant” consisted of: vigilant, not depressed, not tense, and not timid.

Based on the data collected in the BBA program, twenty-six animals (13 males) were identified as behaviorally inhibited based upon showing a) low Emotionality during the behavioral observations conducted during the first hour following relocation, b) high Vigilance from the temperament ratings conducted at the end of the 25-hour period, and c) a blunted cortisol response 7 hours after relocation/separation (Capitanio et al., 2011; Chun et al., 2013). The remaining 26 non-BI subjects (13 males) were selected from the pool of BBA assessed animals that did not fit the previously mentioned criteria of BI. All 52 animals were tested as juveniles. In adulthood, our sample size decreased to 13 behaviorally inhibited (4 males) and 17 non-inhibited animals (7 males).

2.2.2 Behavioral observation in relocation cage – juveniles—To determine whether animals identified as behaviorally inhibited in infancy continued to show evidence of BI under stressful conditions, animals (mean = 1.8 years, range = 1.5 – 2.0) were relocated to individual testing cages as pairs – one behaviorally inhibited and one non-inhibited animal. After 15 minutes of habituation, we collected 5-minutes of behavioral data on videotape from each animal in a pre-determined random order. Observations were made using a comprehensive ethogram (Table S1) consisting of behavioral categories reflecting activity states (e.g. locomote, sleep, hang) and events such as negative emotional behaviors (e.g. threats, fear grimace), positive emotional behaviors (lipsmacks), anxiety-related behaviors (e.g. scratches and yawns), and vocalizations (e.g. coo, bark) (Cross & Harlow, 1965; Hinde & Rowell, 1962; Troisi et al., 1991). Data were summarized on The Observer 5.0 (Noldus Information Technology, Wageningen, Netherlands). Inter-observer reliability exceeded 85% for all behaviors.

2.2.3 Human Intruder Challenge – juveniles—The human intruder challenge assesses the responsiveness of the animal to a standardized and graded series of challenges, and has been described by Gottlieb and Capitanio (2013). Five hours after relocation from the field corral, a technician in protective clothing presented her profile at 1 m distance from the animal’s cage for 1-min (“Profile Near”), and then moved to 0.3 m away for 1-min (“Profile Far”). Next the technician returned to the 1 m position and maintained eye contact for 1-min (“Stare Far”). Finally, she continued to maintain eye contact as she relocated to the 0.3 m position for 1-min (“Stare Near”). We used an ethogram similar to the one described in Section 2.2.2 where behaviors were recorded for the animal during each of the four conditions; in addition, we quantified the amount of time animals spent in the front of the cage (see Table S1). All data were summarized in The Observer 5.0 (Noldus Information

Technology, Wageningen, Netherlands). Inter-observer reliability was greater than 85% for all behaviors.

2.2.4 Outdoor field corral behavioral data collection – juveniles and adults—

Eight 10-minute focal samples were collected on each subject over a 2-week period. Subjects were observed from August 2009 through October 2009 as juveniles (mean = 1.4 years, range = 1.3 – 1.6) and again from August 2012 to September 2012 as young adults (mean = 4.3 years, range = 4.2 – 4.5). We conducted observations between 0800 h and 1200 h, 4 days per week, and animals were observed in a pre-determined randomized order. A voice recorder (Olympus DSS player version 6.3.0, Center Valley, PA) was used to record frequencies and durations of proximity (within arm's reach of another animal), non-aggressive physical contact, play (shoving, grabbing, chasing, wrestling, and/or mouthing behavior accompanied by a play face), and grooming (picking and examining the fur of another monkey with fingers or mouth). We identified immediate family members (mother and siblings), age/sex classes of other interactants (i.e. adult male and female, juvenile male and female, yearling male and female, and infant), and which animal initiated and terminated each interaction. Duration of time spent in non-social activity (i.e., alone) was calculated by subtracting all social durations from total observation duration. Data were summarized on The Observer 5.0 (Noldus Information Technology, Wageningen, Netherlands). Sums of behaviors across observations were used for data analyses. Inter-observer reliability, determined prior to data collection, exceeded 85% for all behaviors.

2.2.5 Dominance Rank – infancy, juvenile age, and adulthood—At the field corrals, CNPRC behavioral management staff collected dominance rank data at least 3 times per month. Scan sampling was employed to record dyadic displacement interactions (where one animal approaches another animal causing him to move) between individuals. All data were uploaded and queried for total displacements by individual each month. All animals were arranged in a rank order, and for analyses, dominance rank was normalized for each subject by dividing subject rank by the number of same-sex individuals present in the field corral. Normalized ranks that were less than 33% were categorized as high ranking, between 33% and 66% were mid-ranking, and above 66% were low ranking. Dominance rank was assessed during the infant and juvenile time-points as maternal dominance rank, inasmuch as animals at these ages have not yet achieved their own rank.

2.2.6 Number of Kin – infancy, juvenile age, and adulthood—The number of kin present in each subject's outdoor cage was compiled by counting the number of animals in the subject's matriline, which includes a female, all of her offspring, and any subsequent offspring of her female offspring. The number of kin was identified for each subject during the infant (mean = 15.60, range = 2 – 41), juvenile (mean = 17.06, range = 2 – 48), and adult (mean = 10.23, range = 1 – 24) time-points.

2.3 Data Analysis

Our first hypothesis was that animals characterized as behaviorally inhibited during infancy would show, as juveniles, more anxious behaviors (i.e. scratching, yawning, claspings, and vocalizations) in response to relocation and during the human intruder challenge (Cross &

Harlow, 1965; Hinde & Rowell, 1962; Maestripieri et al., 1992; Troisi et al., 1991). We tested hypotheses about responses to relocation using temperament classification as the between-subjects variable and behavior as the outcome variable. Multivariate ANOVA was used to better understand how behaviorally inhibited animals differed from non-inhibited animals on durations and frequency of positional states. “Sleep” and “Rock” were omitted from the analyses because fewer than 3 subjects displayed either behavior. Behavioral measures that had a high number of zeros were converted into a dichotomous variable of whether the animal displayed the behavior or not, and these variables were examined using chi-square. To test hypotheses about responses during the human intruder test, we used chi-squares (when very few subjects displayed behaviors) and Mann-Whitney tests using temperament as the between-subjects variable and behavior as the outcome measure. We focused on only 2 conditions: “Profile Far” was used as a baseline condition and the “Stare Near” was used to reflect behavior under conditions of maximum challenge.

Our second hypothesis was that behaviorally inhibited animals would spend more time alone as juveniles in the outdoor corrals. A one-way ANOVA was used to test this hypothesis with BI as the between-subjects variable and non-social duration as the outcome measure. To better understand with whom behaviorally inhibited animals were interacting, we used multivariate ANOVAs with BI as the between-subjects variable and separate analyses for field cage behaviors specific to each age/sex classes as the outcome measure. For example, the amount of time grooming, in contact, and in proximity with mother was tested with one multivariate ANOVA, while the same behaviors with adult females was tested in a separate multivariate ANOVA, etc. Subjects ($n=2$) whose mothers were not present in the cage during field cage observations were taken out of analyses for mother-directed behaviors. Owing to heteroscedasticity, all field corral analyses were performed on transformed variables, while figures show non-transformed values.

Prior to testing our third hypothesis that BI would be related to adult behavior, we examined whether the 22 animals that were lost to follow-up at the adult time point differed in meaningful ways from the 30 animals that we studied as adults. Field cage, relocation cage behaviors, and human intruder behaviors from the juvenile time point were outcome variables and adult status (present for our observations vs. lost to follow-up) was tested using multivariate ANOVAs for separate age/sex class behaviors, or chi-squares as appropriate. To understand how behaviorally inhibited and non-inhibited animals differed in adult social behavior, we first used a one-way ANOVA with BI as the predictor variable and duration of field corral non-social behavior as the outcome variable. Next, we used separate multivariate ANOVAs with BI as the between-subjects variable and field cage behaviors related to specific age/sex class as the outcome measure. Finally, we used multiple regression to examine which factors (sex, dominance rank, number of kin, BI during infancy, juvenile non-social duration, juvenile grooming, and juvenile grooming with mom) were contributing to adult non-social behavior. The outcome measure had two outliers (values greater than two standard deviation above the mean) and one animal was missing maternal dominance rank during the juvenile time-point, thus our sample size for multiple regression analyses was reduced to $n = 27$. We investigated moderators of BI by testing interaction terms of covariates with BI. Models using centered and un-centered independent variables were run and both yielded the same results, thus, statistics from centered variables are

reported. We performed additional exploratory analyses, as described below. All statistical analyses were performed using SPSS 18.0 (IBM Corporation, Armonk, NY).

Results

3.1 Hypothesis 1: BI in infancy predicts anxious behaviors at juvenile age

Means and standard deviations of juvenile variables are presented in Table S2. Animals identified as behaviorally inhibited (BI) in infancy showed evidence of greater emotionality and anxiety in response to relocation: a higher proportion of BI animals displayed scratching (*Chi-Square* (1) = 4.33, $p = .037$), drinking (*Chi-Square* (1) = 6.26, $p = .012$), and vocalizations (barks: *Chi-Square* (1) = 6.93, $p = .008$; coos: *Chi-Square* (1) = 9.774, $p = .002$) compared to non-BI animals. BI animals also showed positional differences compared to non-BI (Pillai's Trace = .303, $F(7,44) = 2.87$, $p = .015$). BI animals displayed higher frequencies of position changes ($F(1,50) = 9.05$, $p = .004$), spent more time standing ($F(1,50) = 4.32$, $p = .043$), and hanging from the side of the cage ($F(1,50) = 5.20$, $p = .027$) compared to non-BI animals. Groups did not differ in durations of locomotion ($F(1,50) = 3.34$, $p = .074$), sitting ($F(1,50) = .30$, $p = .298$), lying ($F(1,50) = 1.97$, $p = .166$), or crouching ($F(1,50) = .010$, $p = .922$). Analyses for other behaviors were non-significant ($p > .153$).

During the human intruder challenge, our analyses revealed significant effects for coo vocalizations and clasps. During the low-challenge "Profile Far" condition, only 2 animals displayed coo vocalizations, both were behaviorally inhibited (*Chi-Square* (1) = 2.08, $p = .149$). A Mann-Whitney test indicated that coo vocalizations during the high-challenge "Stare Near" condition was greater for BI than for non-BI animals ($U = 247.00$, $p = .012$; Figure 1). During the "Profile Far" condition, there were no differences between groups for clasps ($U = 317.50$, $p = .599$), while during the "Stare Near" condition BI animals displayed more clasps than non-BI animals ($U = 258.00$, $p = .033$). No other group differences were found ($p > .113$).

3.2 Hypothesis 2: Behaviorally inhibited infants would spend more time non-social as juveniles

In the field corrals, juveniles identified as behaviorally inhibited spent more time alone ($F(1,50) = 6.29$, $p = .015$; Figure 2a) compared to non-inhibited monkeys. To further understand this result, we examined in more detail the pattern of interactions that the juveniles had with various age/sex groups. Behaviorally inhibited animals differed from non-inhibited animals in behaviors with mother (Pillai's Trace = .251, $F(3,46) = 5.14$, $p = .004$) and other adult females (Pillai's Trace = .247, $F(3,48) = 5.25$, $p = .003$). Behaviorally inhibited animals spent less time in proximity and grooming with their mother (proximity: $F(1,48) = 7.09$, $p = .011$; grooming: $F(1,48) = 6.35$, $p = .015$; Figure 2b), but there were no differences in time in contact ($F(1,48) = .014$, $p = .907$). Behaviorally inhibited animals also spent less time in proximity ($F(1,50) = 10.18$, $p = .002$) and grooming ($F(1,50) = 6.26$, $p = .016$) with adult females compared to non-inhibited animals, but there were no differences in contact. No group differences in behavior were found for other age/sex classes of interactants ($p > .313$).

3.3 Hypothesis 3: Continuity of behavioral inhibition into adulthood and the role of moderators

The comparison of the data from the juvenile assessments between those animals available to test as adults versus those that were unavailable for testing revealed that behaviorally inhibited animals differed on relocation cage behaviors (Pillai's Trace = .294, $F(7,44) = 2.62$, $p = .024$). Animals that continued to be tested as adults had spent more time hanging ($F(1,50) = 9.83$, $p = .003$) as juveniles. All other behaviors were non significant ($p > .087$).

To understand the continuity of BI into adulthood, we tested to see if BI was related to any of the adult outdoor field corral behaviors, but none of the adult field cage behaviors were associated with BI ($p > .206$). Next, we examined bivariate relationships between adult non-social duration and various predictors (Table 1). (Bivariate relations of these variables during juvenile age can be found in Table S3). We found that animals that spent more time non-social as juveniles also spent more time non-social as adults ($r = .418$, $p = .022$).

We used multiple regression to better understand which factors were predicting adult non-social behavior ($n = 27$, Table 2). Sex, maternal and adult dominance rank, and number of kin as juveniles and adults were covariate predictors in Step 1; in Step 2 we added BI, and in Step 3 we added either non-social duration from the juvenile assessments, total juvenile grooming, or juvenile grooming with mother. In Step 4, we added moderators of BI including the interaction between BI with sex, BI with maternal and adult dominance rank, BI with juvenile and adult kin, and either BI with juvenile non-social duration, total juvenile grooming, or grooming with mother. Maternal rank and number of kin during infancy were highly correlated with juvenile maternal rank and number of kin during juvenile time-point (Table S3). Models and steps using total juvenile grooming, or juvenile grooming with mother as predictors were overall non-significant ($p > .061$), thus only the model including juvenile non-social duration is discussed next and shown in Table 2. All predictors had VIF values (a measure of multi-collinearity) of less than 6.58. For the prediction of adult non-social behavior, only Step 3 significantly added to the predictive power of the model, as indicated by the adjusted R^2 at the bottom Table 2. In the final model, Step 4 was non-significant and all variables were not significantly associated with adult non-social duration ($p > .144$).

Our analyses thus far indicated that adult non-social duration was not associated with BI, but rather that juvenile non-social duration was the best predictor; life-history variables important to this species, such as number of kin and rank, were not influential. An investigation of the correlation between juvenile and adult non-social behavior duration split between groups showed that non-inhibited animals had a positive correlation ($r = .796$, $p < .001$), while behaviorally inhibited animals did not show the same relationship ($r = -.096$, $p = .765$). To better understand the altered relationship between BI and juvenile vs. adult nonsocial behavior, we conducted exploratory analyses. Using the full sample of $n=30$ adults, we calculated mean values for juvenile non-social duration and for adult non-social duration, and classified individuals into the resulting four quadrants. Figure 3 displays a scatterplot of the data, and shows that nearly all of the behaviorally inhibited animals fell above the mean for juvenile non-social duration, a result consistent with Hypothesis 2, above. Thus focusing on only behaviorally inhibited animals, we next contrasted BI animals

that were above the mean in adult non-social duration with those that were below the mean in adult non-social duration (the upper right vs. lower right quadrants, respectively, in Figure 3). Because there was only one male in each group, we restricted our exploratory analyses to females (high group: 5 females; low group: 4 females; Fig 3: shading indicates the behaviorally inhibited animals; the two non-shaded BI animals in these quadrants were the males), and re-framed our question as, “Among BI animals that were above the mean in non-social duration as juveniles, what factors distinguish between those that remain above the mean in non-social duration as adults (high group), versus those that spend less time non-social as adults (low group)?” We found that these groups did not differ in juvenile non-social behavior, as expected, but they did differ in total grooming (initiated and received) as juveniles (high group mean (se) = 71.66 (43.33) sec; low group mean (se) = 254.47 (70.39) sec; $F(1,8) = 5.96$, $p = .045$). Moreover, members of the low group spent more time initiating groom as juveniles (high group mean (se) = 27.46 (14.50) sec; low group mean (se) = 208.37 (51.87) sec; $F(1,8) = 17.62$, $p = .004$). As adults, animals in the low group had lower durations of non-social behavior ($F(1,8) = 13.59$, $p = .008$) as expected, but also continued to have higher durations of total grooming (initiated and received; $F(1,8) = 11.29$, $p = .012$), and had higher durations of proximity with adult females ($F(1,8) = 8.07$, $p = .025$) and with infants ($F(1,8) = 22.36$, $p = .002$). These two groups did not differ in dominance rank, number of kin, or any other field cage behaviors collected during the juvenile and adult age periods.

Discussion

Using a longitudinal design, we found that BI characterized in infancy showed consistency into juvenile age but BI did not show continuity into adulthood, at least with respect to social behavior. As juveniles, behaviorally inhibited animals exhibited increased anxious behaviors in response to relocation and during the human intruder test, and in their naturalistic environment behaviorally inhibited juveniles showed decreased social functioning. As adults, we found that juvenile non-social duration was significantly related to adult non-social behavior; however, animals characterized as behaviorally inhibited during infancy did not show any behavioral differences from non-inhibited animals in the naturalistic environment as adults. Furthermore, our multiple regression analysis showed that our expected covariates (number of kin, rank, and sex) also did not predict adult non-social behavior, either by themselves or as moderators of BI. Our exploratory analyses, however, did suggest that behaviorally inhibited females that showed the least non-social behavior (i.e., more social interaction) as adults were more likely to have had higher quality social interaction (i.e., grooming) as juveniles.

4.1 Hypothesis 1: BI in infancy predicts anxious behavior at juvenile age

We found that BI characterized in infancy was associated with increased frequencies of anxious behaviors during the juvenile period (Cross & Harlow, 1965; Hinde & Rowell, 1962; Maestripieri et al., 1992; Troisi et al., 1991) in both the relocation cage and during the human intruder test, results that are consistent with both human and monkey studies of BI (the manifestation of BI into anxiety-related behaviors; Boyce et al., 1998; Fox et al., 2005; Rapee, 2002). We note that the fact that the characterization of BI included low behavioral

output (i.e., emotionality) in infancy, but was associated with greater behavioral output (more anxious behavior) at the juvenile age is an example of heterotypic continuity (Caspi & Roberts, 2001), in which behaviors early in development may not predict similar behaviors at later ages, but may still be associated with behaviors that are conceptually consistent with the earlier behavior. Heterotypic continuity characterizes developmental change in BI in humans: BI is characterized in infants and young children by recording their latencies to approach various novel stimuli, whereas in older children, it can be measured through levels of reluctance to interact with unfamiliar adults or peers, or reactivity to unfamiliar people (Fox et al., 2001). The change in assessment from reactivity to novel objects to reactivity to unfamiliar people reflects the type of stimulus situation that is expected to elicit BI in older children. Responses to novel objects may reflect BI in infants and younger children because of the lack of control over their actions (Gunnar-vonGnechten, 1978). Older children are less likely to display BI to novel objects, but are more likely to exhibit BI in novel social situations. The same may be applied to rhesus monkey development. For example, Kalin and Shelton (1998) found that during the human intruder paradigm, coo vocalization frequency decreased from 4 month to 8 month olds, but this was not due to repeated testing. Because coo vocalizations are thought to be indicative of the infant's dependence on mother, the decrease in coo vocalizations may be due to the maturation of the infant, particularly to its increased independence from the mother. In the current study, one component of our characterization of BI in infancy is decreased emotionality in response to separation and relocation, which then manifests into increased display of anxious behaviors compared to non-inhibited animals when a second separation and relocation occurs at juvenile age. The novelty of the initial separation from the social group and mother is stressful; however, the reaction to this situation may change over time as it does in humans, and the differences in response between behaviorally inhibited and non-inhibited animals at the two time points is consistent with differences in the perceived situation by these two sets of animals.

The current monkey model of BI has similarities and differences from Kalin's model. Kalin's group characterized BI in animals displaying high durations of freezing behavior (complete cessation of vocalizations and motor activity) during the human intruder test and characterized BI at different ages between 3-month of age into juvenility (Davidson et al., 1993; Fox et al., 2008; Kalin & Shelton, 1989, 1998; Rogers et al., 2008). In the current study, one component of infant BI was characterized similarly to Kalin as decreased emotionality, and then the continuity of BI was characterized as display of anxiety-related behaviors during the relocation observations and human intruder test and decreased social behavior during juvenile age. Kalin has studied the consistency of BI during the first year of life using the human intruder paradigm with multiple assessments at 3, 8, and 12 months of age (Kalin & Shelton, 1998) and other studies included older juveniles assessed at one time-point (Davidson et al., 1993; Fox et al., 2008; Rogers et al., 2008). All of the previously mentioned studies characterized BI as involving increased durations of freezing behavior at all ages (Davidson et al., 1993; Fox et al., 2008; Kalin & Shelton, 1989, 1998; Rogers et al., 2008), while the current study found that animals characterized as BI during infancy showed increased frequencies of anxious behaviors as juveniles (that is, higher, not equally low, behavioral output as juveniles). In addition, the current study includes measures of social behavior as an additional tool to characterize BI, while Kalin's studies do not. It's possible

that some of the differences between our study and Kalin's studies of BI in monkeys may be due to differences in methodology for the human intruder tests: the test conditions in Kalin's human intruder paradigm are much longer (9–30 min depending on the study vs. 1 min in the current study); sometimes did not include a "Stare" condition (Fox et al., 2008; Rogers et al., 2008); involved different distances between the intruder and the subject (2.5 m vs. 1 and 0.3 m in the current study); and were conducted at different times following separation (immediately following separating vs. 5 hours post-separation in current study). These methodological differences may contribute to the differences in anxiety-related behaviors quantified in our BI group at juvenile age.

4.2 Hypothesis 2: BI infants would spend more time non-social as juveniles

We confirmed our hypothesis that behaviorally inhibited animals would spend more time alone when in their naturalistic environment, a result consistent with data from human studies showing that children characterized as behaviorally inhibited were more likely to avoid interactions with peers (Coll et al., 1984; Rubin et al., 2002). We then further examined the pattern of interactions that the juveniles had with various age/sex groups. During early development, rhesus monkey infants spend much of their time with their mother, while as juveniles, they spend less time with mother and interact more with other members in the group including related adult females, same-aged animals, and siblings (Hinde & Spencer-Booth, 1967). We found that, as juveniles, behaviorally inhibited animals had decreased durations of interaction with both their mother and adult females, which deviates from the usual pattern of social development of rhesus monkeys. A limitation of our study was that our field cage observations did not involve recording the identities of all interactants, except for mother and siblings; consequently, it is possible that behaviorally inhibited animals spent more time with matrilineal kin than did non-inhibited animals. Follow-up studies that record individual identities of all interactants would help further characterize differences between BI and non-inhibited animals at this age.

4.3 Hypothesis 3: Continuity of behavioral inhibition into adulthood and the role of moderators

We hypothesized that BI would show continuity in social behavior from juvenile age to young adulthood. We did find continuity in social behavior, in that time spent alone as a juvenile was related to time spent alone as an adult; however, BI characterized in infancy was not related to time spent alone in adulthood. To understand what factors were influencing adult non-social behavior we used multiple regression to study the effects of sex, dominance rank, and number of kin, as well as temperament. We were interested in investigating these monkey-relevant life-history factors as moderators of BI because the human literature has shown that the stability of BI can be influenced by different factors of the child's environment including peer group interactions and the presence of non-parental care (Almas et al., 2011; Fox et al., 2001; Gazelle & Ladd, 2003). Our final regression model revealed no further main effects or moderators of BI predicting adult non-social behavior, however. Our finding that sex was not a moderator of BI is in contrast to the human literature (Caspi et al., 1989; Henderson et al., 2004; Kagan et al., 1998; Stevenson-Hinde & Shouldice, 1995). It may be that the moderating effects of sex on BI may be

evident in other outcomes including mating behavior, or number of offspring later in life. Dominance rank and number of kin were also non-significant moderators of BI.

Although BI has shown continuity during childhood in human samples, there is evidence that subsets of behaviorally inhibited individuals become non-inhibited later in life (Kagan, 1989). We further examined our data to understand whether there were differences between animals that showed continuity in juvenile and adult non-social behavior versus those that did not. We found that there was an overall positive correlation between juvenile and adult non-social behavior for non-inhibited animals, while behaviorally inhibited animals did not show this same correlation (Fig 3). This difference in correlation between groups for juvenile and adult non-social behavior may be related to differential susceptibility of these two groups to the environment as described by Belsky, Hsieh, and Crnic (1998) and reviewed in Ellis et al. (2011). The Differential Susceptibility Theory (DST) and Biological Sensitivity to Context Theory (BSCT) both converge on the idea that certain individuals may be more susceptible to both negative and positive aspects of the environment. The behaviorally inhibited group is relatively non-social as juveniles and some animals continue this same pattern, while others become more sociable as adults suggesting that something about the environment (i.e. maternal care, social group dynamics, etc.) may be influencing this change. We found that the non-inhibited group showed more variation in juvenile non-social behavior and had a positive correlation between juvenile and adult non-social duration, an indication that these animals may be less susceptible to environmental factors.

To further understand why some behaviorally inhibited animals continued to be non-social, while others did not, and whether there were environmental factors influencing this change, we focused our exploratory analyses on the behaviorally inhibited group. We found that behaviorally inhibited females that became more sociable as adults (i.e., less time spent alone) were more likely to have displayed grooming as juveniles compared to adults that did show continuity in social behavior. However, we did not find differences between the two groups in maternal or adult dominance rank, or the number of kin. In rhesus monkeys, the mother plays a large role in the social development of the infant and it has been suggested that the degree to which macaque mothers encourage independence of their infants is influenced by the presence or absence of matrilineal kin (Berman, 1992; Hinde & Spencer-Booth, 1967; Silk, 1991). It may be that these behaviorally inhibited juveniles were encouraged by matrilineal kin and/or their mother to participate in grooming networks and this social environment facilitated increased sociability into adulthood. This is consistent with some human literature suggesting children became less inhibited when mothers were more intrusive, or would encourage the child to overcome anxieties (Park, Belsky, Putnam, & Crnic, 1997). Future studies may focus on identifying matrilineal kin interactions and direct measures of maternal caregiving as possible moderators of BI. Finally, we note that these exploratory results were only found in behaviorally inhibited females. Rhesus monkeys are characterized by female philopatry and male dispersal and there is evidence that maternal investment varies according to offspring sex (Bercovitch, Widdig, & Nürnberg, 2000). Consequently, mothers and other matrilineal kin may be less invested in integrating juvenile male offspring into grooming networks. Unfortunately, we were unable to investigate behaviorally inhibited males further because of the small number ($n = 4$) that were followed into adulthood.

4.4 Summary

BI characterized during infancy can be predictive of juvenile behavior in rhesus monkeys and we can better understand the continuity of BI into adulthood through the development of a longitudinal monkey model. In the current study, we selected animals as infants and showed that BI predicted social and nonsocial behavior at the juvenile age. We did not find the expected differences between behaviorally inhibited and non-inhibited animals in adult social behavior, but among behaviorally inhibited females, we identified some animals that appeared to engage in more social interaction as adults. It may be that the mother and/or matrilineal kin are influencing the stability of BI into adulthood, at least for females, a result similar to what some have reported in humans. Many results for our monkey model of BI are consistent with the human literature, and suggest this may be a valuable model for understanding how the early social environment can moderate the effects of temperament on later behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Almas, Alisa N., Degnan, Kathryn Amey, Fox, Nathan A., Phillips, Deborah A., Henderson, Heather A., Moas, Olga L., et al. The Relations between Infant Negative Reactivity, Non-maternal Childcare, and Children's Interactions with Familiar and Unfamiliar Peers. *Social Development*. 2011; 20(4):718–740. DOI: 10.1111/j.1467-9507.2011.00605.x [PubMed: 22563147]
- Belsky, Jay, Fish, Margaret, Isabella, Russell A. Continuity and discontinuity in infant negative and positive emotionality: Family antecedents and attachment consequences. *Developmental psychology*. 1991; 27(3):421–431.
- Belsky, Jay, Hsieh, Kuang-Hua, Crnic, Keith. Mothering, fathering, and infant negativity as antecedents of boys' externalizing problems and inhibition at age 3 years: Differential susceptibility to rearing experience? *Development and Psychopathology*. 1998; 10(02):301–319. [PubMed: 9635226]
- Bercovitch, Fred B., Widdig, Anja, Nürnberg, Peter. Maternal Investment in Rhesus Macaques (*Macaca mulatta*): Reproductive Costs and Consequences of Raising Sons. *Behavioral Ecology and Sociobiology*. 2000; 48(1):1–11. DOI: 10.2307/4601772
- Berman, Carol M. Immature siblings and mother-infant relationships among free-ranging rhesus monkeys on Cayo Santiago. *Animal Behaviour*. 1992; 44(Part 2):247–258. [http://dx.doi.org/10.1016/0003-3472\(92\)90031-4](http://dx.doi.org/10.1016/0003-3472(92)90031-4).
- Biederman J, Hirshfeld-Becker DR, Rosenbaum JF, Herot C, Friedman D, Snidman N, et al. Further evidence of association between behavioral inhibition and social anxiety in children. *American Journal of Psychiatry*. 2001; 158(10):1673–1679. DOI: 10.1176/Appi.Ajp.158.10.1673 [PubMed: 11579001]
- Boyce WT, O'Neill-Wagner P, Price CS, Haines M, Suomi SJ. Crowding stress and violent injuries among behaviorally inhibited rhesus macaques. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association*. 1998; 17(3):285–289.
- Capitanio JP, Miller LA, Schelegle ES, Mendoza SP, Mason WA, Hyde DM. Behavioral inhibition is associated with airway hyperresponsiveness but not atopy in a monkey model of asthma. *Psychosomatic Medicine*. 2011; 73(4):288–294. PSY.0b013e3182155c83 [pii]. DOI: 10.1097/PSY.0b013e3182155c83 [PubMed: 21536834]
- Caspi A, Bem DJ, Elder GH Jr. Continuities and consequences of interactional styles across the life course. *Journal of Personality*. 1989; 57(2):375–406. [PubMed: 2769561]

- Caspi A, Harrington H, Milne B, Amell JW, Theodore RF, Moffitt TE. Children's behavioral styles at age 3 are linked to their adult personality traits at age 26. *Journal of Personality*. 2003; 71(4):495–513. [PubMed: 12901429]
- Caspi A, Roberts Brent W. Personality Development across the Life Course: The Argument for Change and Continuity. *Psychological Inquiry*. 2001; 12(2):49–66. DOI: 10.2307/1449487
- Caspi A, Silva PA. Temperamental Qualities at Age-3 Predict Personality-Traits in Young Adulthood - Longitudinal Evidence from a Birth Cohort. *Child development*. 1995; 66(2):486–498. DOI: 10.1111/J.1467-8624.1995.Tb00885.X [PubMed: 7750379]
- Chun K, Miller LA, Schelegle ES, Hyde DM, Capitanio JP. Behavioral inhibition in rhesus monkeys (*Macaca mulatta*) is related to the airways response, but not immune measures, commonly associated with asthma. *PLoS ONE*. 2013; 8(8):e71575.doi: 10.1371/journal.pone.0071575 [PubMed: 23951195]
- Coll CG, Kagan J, Reznick JS. Behavioral-Inhibition in Young-Children. *Child development*. 1984; 55(3):1005–1019. DOI: 10.1111/J.1467-8624.1984.Tb03838.X
- Cross HA, Harlow HF. Prolonged and Progressive Effects of Partial Isolation on the Behavior of Macaque Monkeys. *Journal of Experimental Research in Personality*. 1965; 1(1):39–49.
- Davidson RJ, Kalin NH, Shelton SE. Lateralized response to diazepam predicts temperamental style in rhesus monkeys. *Behav Neurosci*. 1993; 107(6):1106–1110. [PubMed: 8136065]
- de Haan, Michelle, Gunnar, Megan R., Tout, Kathryn, Hart, Jordan, Stansbury, Kathy. Familiar and novel contexts yield different associations between cortisol and behavior among 2-year-old children. *Developmental Psychobiology*. 1998; 33(1):93–101. DOI: 10.1002/(SICI)1098-2302(199807)33:1<93::AID-DEV8>3.0.CO;2-N [PubMed: 9664174]
- Degnan KA, Almas AN, Henderson HA, Hane AA, Walker OL, Fox NA. Longitudinal trajectories of social reticence with unfamiliar peers across early childhood. *Dev Psychol*. 2014; 50(10):2311–2323. DOI: 10.1037/a0037751 [PubMed: 25181648]
- Ellis, Bruce J., Boyce, W Thomas, Belsky, Jay, Bakermans-Kranenburg, Marian J., van Ijzendoorn, Marinus H. Differential susceptibility to the environment: An evolutionary–neurodevelopmental theory. *Development and Psychopathology*. 2011; 23(01):7–28. DOI: 10.1017/S0954579410000611 [PubMed: 21262036]
- Essex MJ, Klein MH, Slattery MJ, Goldsmith HH, Kalin NH. Early risk factors and developmental pathways to chronic high inhibition and social anxiety disorder in adolescence. *Am J Psychiatry*. 2010; 167(1):40–46. DOI: 10.1176/appi.ajp.2009.07010051 [PubMed: 19917594]
- Fox AS, Shelton SE, Oakes TR, Davidson RJ, Kalin NH. Trait-like brain activity during adolescence predicts anxious temperament in primates. *PLoS ONE*. 2008; 3(7):e2570.doi: 10.1371/journal.pone.0002570 [PubMed: 18596957]
- Fox NA, Henderson HA, Marshall PJ, Nichols KE, Ghera MM. Behavioral inhibition: Linking biology and behavior within a developmental framework. *Annual Review of Psychology*. 2005; 56:235–262. DOI: 10.1146/Annurev.Psych.55.090902.141532
- Fox NA, Henderson HA, Rubin KH, Calkins SD, Schmidt LA. Continuity and discontinuity of behavioral inhibition and exuberance: psychophysiological and behavioral influences across the first four years of life. *Child Dev*. 2001; 72(1):1–21. [PubMed: 11280472]
- Gazelle H, Ladd GW. Anxious solitude and peer exclusion: a diathesis-stress model of internalizing trajectories in childhood. *Child development*. 2003; 74(1):257–278. [PubMed: 12625449]
- Golub MS, Hogrefe CE, Widaman KF, Capitanio JP. Iron Deficiency Anemia and Affective Response in Rhesus Monkey Infants. *Developmental Psychobiology*. 2009; 51(1):47–59. DOI: 10.1002/Dev.20345 [PubMed: 18814183]
- Gosling SD, John OP. Personality dimensions in nonhuman animals: A cross-species review. *Current Directions in Psychological Science*. 1999; 8(3):69–75. DOI: 10.1111/1467-8721.00017
- Gottlieb DH, Capitanio JP. Latent variables affecting behavioral response to the human intruder test in infant rhesus macaques (*Macaca mulatta*). *American Journal of Primatology*. 2013; 75(4):314–323. DOI: 10.1002/ajp.22107 [PubMed: 23229557]
- Gunnar MR, Wewerka S, Frenn K, Long JD, Griggs C. Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: normative changes and associations

with puberty. *Development and psychopathology*. 2009; 21(1):69–85. DOI: 10.1017/S0954579409000054 [PubMed: 19144223]

Gunnar-vonGnechten, Megan R. Changing a frightening toy into a pleasant toy by allowing the infant to control its actions. *Developmental Psychology*. 1978; 14(2):157–162. <http://dx.doi.org/10.1037/0012-1649.14.2.157>.

Hane, Amie Ashley, Cheah, Charissa, Rubin, Kenneth H., Fox, Nathan A. The Role of Maternal Behavior in the Relation between Shyness and Social Reticeence in Early Childhood and Social Withdrawal in Middle Childhood. *Social Development*. 2008; 17(4):795–811. DOI: 10.1111/j.1467-9507.2008.00481.x

Henderson HA, Marshall PJ, Fox NA, Rubin KH. Psychophysiological and behavioral evidence for varying forms and functions of nonsocial behavior in preschoolers. *Child development*. 2004; 75(1):251–263. [PubMed: 15015688]

Hinde RA, Rowell TE. COMMUNICATION BY POSTURES AND FACIAL EXPRESSIONS IN THE RHESUS MONKEY (MACACA MULATTA). *Proceedings of the Zoological Society of London*. 1962; 138(1):1–21. DOI: 10.1111/j.1469-7998.1962.tb05684.x

Hinde RA, Spencer-Booth Y. The behaviour of socially living rhesus monkeys in their first two and a half years. *Animal Behaviour*. 1967; 15(1):169–196. [http://dx.doi.org/10.1016/S0003-3472\(67\)80029-0](http://dx.doi.org/10.1016/S0003-3472(67)80029-0). [PubMed: 4961894]

Kagan J. Temperamental contributions to social behavior. *American Psychologist*. 1989; 44(4):668–674. <http://dx.doi.org/10.1037/0003-066X.44.4.668>.

Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. *Child development*. 1987; 58(6):1459–1473. [PubMed: 3691195]

Kagan J, Reznick JS, Snidman N, Gibbons J, Johnson MO. Childhood derivatives of inhibition and lack of inhibition to the unfamiliar. *Child development*. 1988; 59(6):1580–1589. [PubMed: 3208569]

Kagan J, Reznick J Steven, Clarke Charlotte, Snidman Nancy, Garcia-Coll Cynthia. Behavioral Inhibition to the Unfamiliar. *Child development*. 1984; 55(6):2212–2225. DOI: 10.2307/1129793

Kagan J, Snidman N, Arcus D. Childhood derivatives of high and low reactivity in infancy. *Child development*. 1998; 69(6):1483–1493. [PubMed: 9914634]

Kalin NH, Shelton SE. Defensive Behaviors in Infant Rhesus-Monkeys - Environmental Cues and Neurochemical Regulation. *Science*. 1989; 243(4899):1718–1721. DOI: 10.1126/Science.2564702 [PubMed: 2564702]

Kalin NH, Shelton SE. Ontogeny and stability of separation and threat-induced defensive behaviors in rhesus monkeys during the first year of life. *American Journal of Primatology*. 1998; 44(2):125–135. DOI: 10.1002/(Sici)1098-2345(1998)44:2<125::Aid-Ajp3>3.0.Co;2-Y [PubMed: 9503124]

Maestripieri D, Schino G, Aureli F, Troisi A. A Modest Proposal - Displacement Activities as an Indicator of Emotions in Primates. *Animal Behaviour*. 1992; 44(5):967–979. DOI: 10.1016/S0003-3472(05)80592-5

Marshall PJ, Reeb BC, Fox NA. Electrophysiological responses to auditory novelty in temperamentally different 9-month-old infants. *Dev Sci*. 2009; 12(4):568–582. DOI: 10.1111/j.1467-7687.2008.00808.x [PubMed: 19635084]

Nelson, Florence, Garduque, Laurie. The experience and perception of continuity between home and day care from the perspectives of child, mother, and caregiver. *Early Child Development and Care*. 1991; 68(1):98–111. DOI: 10.1080/0300443910680109

Park SY, Belsky J, Putnam S, Crnic K. Infant emotionality, parenting, and 3-year inhibition: exploring stability and lawful discontinuity in a male sample. *Developmental psychology*. 1997; 33(2):218–227. [PubMed: 9147831]

Perez-Edgar K, Bar-Haim Y, McDermott JM, Chronis-Tuscano A, Pine DS, Fox NA. Attention biases to threat and behavioral inhibition in early childhood shape adolescent social withdrawal. *Emotion*. 2010; 10(3):349–357. DOI: 10.1037/a0018486 [PubMed: 20515224]

Perez-Edgar K, Fox NA. Temperament and anxiety disorders. *Child Adolesc Psychiatr Clin N Am*. 2005; 14(4):681–706. viii. DOI: 10.1016/j.chc.2005.05.008 [PubMed: 16171698]

Rapee RM. The development and modification of temperamental risk for anxiety disorders: prevention of a lifetime of anxiety? *Biological psychiatry*. 2002; 52(10):947–957. [PubMed: 12437936]

- Rogers J, Shelton SE, Shelledy W, Garcia R, Kalin NH. Genetic influences on behavioral inhibition and anxiety in juvenile rhesus macaques. *Genes Brain and Behavior*. 2008; 7(4):463–469. DOI: 10.1111/J.1601-183x.2007.00381.X
- Rubin KH, Burgess KB, Hastings PD. Stability and social-behavioral consequences of toddlers' inhibited temperament and parenting behaviors. *Child Dev*. 2002; 73(2):483–495. [PubMed: 11949904]
- Rubin KH, Hastings PD, Stewart SL, Henderson HA, Chen X. The consistency and concomitants of inhibition: some of the children, all of the time. *Child development*. 1997; 68(3):467–483. [PubMed: 9249961]
- Schmidt LA, Fox NA, Rubin KH, Sternberg EM, Gold PW, Smith CC, et al. Behavioral and neuroendocrine responses in shy children. *Developmental Psychobiology*. 1997; 30(2):127–140. DOI: 10.1002/(Sici)1098-2302(199703)30:2<127::Aid-Dev4>3.0.Co;2-S [PubMed: 9068967]
- Silk, JoanB. Mother-infant relationships in bonnet macaques: Sources of variation in proximity. *International Journal of Primatology*. 1991; 12(1):21–38. DOI: 10.1007/BF02547556
- Stevenson-Hinde J, Shouldice A. 4.5 to 7 years: fearful behaviour, fears and worries. *J Child Psychol Psychiatry*. 1995; 36(6):1027–1038. [PubMed: 7593396]
- Troisi A, Schino G, D'Antoni M, Pandolfi N, Aureli F, D'Amato FR. Scratching as a behavioral index of anxiety in macaque mothers. *Behavioral and neural biology*. 1991; 56(3):307–313. [PubMed: 1759948]
- White LK, McDermott JM, Degnan KA, Henderson HA, Fox NA. Behavioral inhibition and anxiety: the moderating roles of inhibitory control and attention shifting. *J Abnorm Child Psychol*. 2011; 39(5):735–747. DOI: 10.1007/s10802-011-9490-x [PubMed: 21301953]
- Williams LR, Degnan KA, Perez-Edgar KE, Henderson HA, Rubin KH, Pine DS, et al. Impact of behavioral inhibition and parenting style on internalizing and externalizing problems from early childhood through adolescence. *J Abnorm Child Psychol*. 2009; 37(8):1063–1075. DOI: 10.1007/s10802-009-9331-3 [PubMed: 19521761]

Research Highlights

- Although behavioral inhibition can result in an increased risk for anxiety and depression and decreased social behavior later in life, some studies have found that the stability of behavioral inhibition can be moderated by different factors.
- In a rhesus monkey model of behavioral inhibition, behaviorally inhibited juveniles showed increased anxious behaviors in response to relocation and decreased social behaviors in naturalistic conditions.
- As adults, however, behaviorally inhibited animals showed discontinuity in social behavior, which may be related to the quality of interactions early in life.
- A non-human primate model of behavioral inhibition may be valuable in understanding how the early social environment may contribute to the continuity of behavioral inhibition.

Figure 1. Comparison of human intruder coo vocalizations between behaviorally inhibited and non-inhibited animals

Raw values shown in figure. Note: * $p < .05$.

Figure 2. Comparison of juvenile field cage behaviors between behaviorally inhibited and non-inhibited animals

(A) Durations of juvenile non-social duration. (B) Durations of proximity and grooming with mom as juvenile. Raw values shown in figure. Note: * $p < .05$.

Figure 3. Juvenile non-social duration by adult non-social duration

Reference line on x-axis represents mean of juvenile non-social duration, while reference line on y-axis represents mean of adult non-social duration. Shaded triangles represent female behaviorally inhibited animals used for exploratory analyses.

Table 1

Zero-order correlation coefficients of adult predictor and outcome measures.

Variable	1	2	3	4	5	6	7	8	9	10
1. Adult Non-Social Duration (n=30)	-									
2. Sex (n=30)	0.361 [*]	-								
3. Infant Maternal Rank (n=28)	0.353 ⁺	0.019	-							
4. Juvenile Maternal Rank (n=29)	0.365 ⁺	0.193	0.774 [*]	-						
5. Adult Dominance Rank (n=30)	0.404 [*]	0.075	0.250	0.373 [*]	-					
6. Infant Kin (n=30)	0.083	-0.022	-0.051	-0.366 ⁺	-0.216	-				
7. Juvenile Kin (n=30)	-0.089	-0.102	-0.235	-0.496 [*]	-0.410 [*]	0.825 [*]	-			
8. Adult Kin (n=30)	-0.082	-0.090	-0.333 ⁺	-0.597 [*]	-0.298	0.774 [*]	0.810 [*]	-		
9. BI (n=30)	-0.046	-0.107	0.098	0.168	0.185	-0.081	-0.169	-0.165	-	
10. Juvenile Non-Social Duration (n=30)	0.418 [*]	-0.096	0.136	0.103	0.278	-0.132	-0.274	-0.091	0.466 [*]	-
M	3289.610	0.367 ^a	2.000 ^b	2.000 ^b	2.000 ^b	12.433	13.230	10.230	0.430 ^d	3199.456
SD	631.093	-	-	-	-	9.016	9.313	7.463	-	494.117

Note:

^{*} p < .05;⁺ p < .10;^a Variables are dichotomous thus value represents proportion;^b Variables are ordinal thus value represents median.

Table 2

Unstandardized coefficients, standard errors in parentheses, adjusted R² and significance levels for various models of sex, maternal rank, dominance rank, juvenile kin, adult kin, BI, juvenile non-social duration, and interaction of BI and moderators (sex, dominance rank, and juvenile non-social duration)

Sequential multiple regressions testing the relationships between covariates of sex, maternal and adult dominance rank, and juvenile and adult kin (Step 1), BI (Step 2), juvenile non-social duration (Step 3), and the interaction of BI and moderators (Step 4) predicting adult non-social behavior. All analyses used centered variables.

Independent Variable	1	2	3	4
Constant	3371.41 [*] (83.18)	3371.41 [*] (85.16)	3371.42 [*] (74.97)	3451.99 [*] (97.71)
Sex	213.54 (176.78)	215.57 (181.30)	315.40 ⁺ (164.15)	243.60 (208.58)
Maternal Dominance Rank	185.55 (119.10)	183.76 (122.29)	205.87 ⁺ (108.00)	228.09 (134.72)
Adult Dominance Rank	65.89 (152.19)	58.28 (160.89)	89.44 (142.16)	199.86 (173.67)
Juvenile Kin	-19.72 (17.25)	-20.13 (17.79)	-0.64 (17.36)	1.86 (21.26)
Adult Kin	49.65 [*] (23.31)	50.31 [*] (24.11)	30.92 (22.49)	31.16 (27.16)
Behavioral Inhibition		34.59 (182.64)	-200.60 (184.36)	-142.08 (199.08)
Juvenile Non-social Duration			0.56 [*] (0.22)	0.40 (0.26)
Interaction of BI and Sex				-86.82 (436.30)
Interaction of BI and Maternal Rank				-35.83 (282.38)
Interaction of BI and Adult Rank				162.80 (366.50)
Interaction of BI and Juvenile Kin				21.04 (42.59)
Interaction of BI and Adult Kin				-16.54 (52.79)
Interaction of BI and non-social				-0.91 (0.55)
Adjusted R ²	.108	.065	.275 [*]	.221
N	27	27	27	27

Note:

^{*} p < .05;

⁺ p < .10.