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Genetic influences on behavioral inhibition and anxiety in juvenile rhesus macaques

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Abstract

In humans and other animals, behavioral responses to threatening stimuli are an important component of temperament. Among children, extreme behavioral inhibition elicited by novel situations or strangers predicts the subsequent development of anxiety disorders and depression. Genetic differences among children are known to affect risk of developing behavioral inhibition and anxiety, but a more detailed understanding of genetic influences on susceptibility is needed. Nonhuman primates provide valuable models for studying the mechanisms underlying human behavior. Individual differences in threat-induced behavioral inhibition (freezing behavior) in young rhesus monkeys are stable over time and reflect individual levels of anxiety. This study used the well-established human intruder paradigm to elicit threat-induced freezing behavior and other behavioral responses in 285 young pedigreed rhesus monkeys. We examined the overall influence of quantitative genetic variation and tested the specific effect of the serotonin transporter promoter repeat polymorphism. Quantitative genetic analyses indicated that the residual heritability of freezing duration (behavioral inhibition) is $h^2 = 0.384$ ($P = 0.012$) and of 'orienting to the intruder' (vigilance) is $h^2 = 0.908$ ($P = 0.00001$). Duration of locomotion and hostility and frequency of cooing were not significantly heritable. The serotonin transporter polymorphism showed no significant effect on either freezing or orienting to the intruder. Our results suggest that this species could be used for detailed studies of genetic mechanisms influencing extreme behavioral inhibition, including the identification of specific genes that are involved in predisposing individuals to such behavior.

Keywords

5HTTLPR; anxiety; freezing; heritability; primate; serotonin transporter; SLC6A4; temperament; vigilance

Introduction

In humans, as in other animals, behavioral responses to stimuli that are perceived to be threatening are an important component of temperament. In children, extreme behavioral inhibition elicited by exposure to novelty or unfamiliar individuals predicts the development of anxiety disorders (Rosenbaum *et al.* 1993) and depression (Caspi *et al.* 1996). Several studies

also indicate that genetic differences among children affect their risk of developing extreme behavioral inhibition or excessive anxiety (Eley *et al.* 2003; Kovacs & Devlin 1998). However, a more detailed understanding of the genetic processes that affect such risk is needed, including identification of specific genes that influence susceptibility and investigation of interactions between genetic predisposition and developmental experience.

Nonhuman primates provide valuable animal models for studying mechanisms underlying human behavior and susceptibility to psychiatric disorders. Among young rhesus monkeys, individual differences in threat-induced freezing behavior are stable over time and reflect individual levels of anxiety (Kalin & Shelton 1998, 2003). Freezing, the complete cessation of vocalizations and gross motor activity, is an adaptive response to potential threats from predators. While freezing, monkeys are vigilant, visually scanning the environment to assess changes in the threat. Individual differences in threat-induced freezing are positively correlated with metabolic activity of the amygdala and bed nucleus of the stria terminalis (Kalin *et al.* 2005). This is significant because these structures contribute to neural circuits associated with the processing of emotion, fear and anxiety, and increased amygdala reactivity is reported among human adults with childhood histories of extreme behavioral inhibition (Schwartz *et al.* 2003). Extreme threat-induced freezing in rhesus monkeys can be considered the behavioral manifestation of an underlying anxious endophenotype consisting of behavioral, emotional and physiological features that are shared with the endophenotype inferred to exist in children who exhibit extreme behavioral inhibition (Kalin & Shelton 2003).

Numerous studies demonstrate that individual variation in human temperament, including elements related to behavioral inhibition and anxiety, is significantly influenced by genetic differences (Kendler *et al.* 1995; Kovacs & Devlin 1998; Loehlin 1992). One gene that is associated with variation in behavior and underlying neurobiological traits and has been extensively studied in humans and nonhuman primates is the serotonin transporter locus, gene symbol SLC6A4 (Barr *et al.* 2004a,b; Brown & Hariri 2006; Hariri & Holmes 2006; Lesch *et al.* 1996, 1997). Allelic differences in the promoter of the serotonin transporter gene (also referred to as 5HTTLPR) predispose to increased neuroticism (Lesch *et al.* 1996; Schinka *et al.* 2004) and are associated with reduced volume and increased reactivity of the amygdala (Pezawas *et al.* 2005). Studies of similar variation in the SLC6A4 gene among rhesus monkeys also demonstrate effects on brain function and behavior (Barr *et al.* 2004a,b; Bennett *et al.* 2002).

In this study, we used the human intruder paradigm (Kalin & Shelton 1989) to elicit threat-induced freezing behavior in a pedigree of young rhesus monkeys. We examined overall quantitative genetic effects on behavioral responses as well as the specific effect of the serotonin transporter promoter repeat polymorphism.

Materials and methods

Subjects

Two hundred eighty-five young rhesus monkeys (169 males and 116 females) from the Harlow Primate Laboratory and the Wisconsin National Primate Research Center (Madison, WI) were tested. All study subjects were reared indoors by their mothers, with the majority (80%) of subjects raised in mixed-sex social groups of various sizes and age-sex compositions. The average size of social groups was approximately 5 animals, with only one fourth of groups consisting of 10 animals or more. The 20% of study subjects not raised in groups were reared as a mother-infant pair. The average age at testing was 19.3 ± 13.2 months. The genealogy of these study subjects was known, and therefore, pair-wise kinship among all study subjects could be calculated from the pedigree. All animal procedures were reviewed and approved by the University of Wisconsin (Madison) Institutional Animal Care and Use Committee and

adhered to all relevant United States Department of Agriculture (USDA) and Public Health Service (PHS) policies and guidelines.

Behavioral testing

Subjects were tested using a modified human intruder paradigm (Kalin & Shelton 1989; Kalin *et al.* 1991). Each study animal was placed in a cage ($79 \times 76 \times 71$ cm) by itself for the first 10 min. Following that period, a human entered the room and presented his or her profile to the monkey for another 10 min, while standing motionless 2.5 m from the cage and avoiding any eye contact (no eye contact, NEC) with the animal. Behavior and vocalizations were recorded on videotape. Extensively trained raters performed the behavioral ratings using previously validated methods. Using these videotapes, we scored the duration and/or frequency of five behaviors: (1) freezing (defined as a period of at least 3 seconds of tense body posture, no vocalizations and no movement other than slow movements of the head), (2) orient to the intruder (defined as the length of time the subject stares intently at the human intruder), (3) locomotion (any voluntary movement within the cage, including walking, jumping and climbing), (4) cooing (a high-pitched vocalization made by rounding and pursing the lips and characterized by an increase, then decrease in frequency and intensity) and (5) hostility to the intruder (any hostile behavior directed toward the intruder, such as barking, head bobbing or ear flapping).

We also calculated the pair-wise linear correlations between all pairs of behavioral measures using standard statistical methods. We do note that while these correlation coefficients are accurate, the *P*-values associated with these coefficients are only approximate because the study subjects are not independent but genealogically related. The statistical methods used to generate the *P*-values for correlation coefficients assume independence among subjects, and therefore, the *P*-values should only be considered approximate.

Quantitative genetic analysis

Univariate quantitative genetic analyses were conducted using the five measured behaviors observed for each monkey. These analyses employed maximum likelihood variance decomposition methods, implemented in the computer package SOLAR (Almasy & Blangero 1998). In order to determine whether any of the measured phenotypes are significantly influenced by genetic differences among individuals, i.e. are heritable, we took advantage of the known pedigree relationships (kinship) among study subjects. While various approaches can be employed, maximum likelihood variance components analysis using data from extended pedigrees is an effective approach that allows simultaneous estimation of the effects of genetic variance as well as any number of other potential factors such as age, sex or other environmental variables (Lange & Boehnke 1983). One strength is that this approach uses all kinship information, including full sibships, half sibships, parent–offspring and more distant relationships. In essence, variance components heritability analyses test the hypothesis that information about kinship relationships among pairs of individuals is useful in predicting relative phenotypic similarity or difference among these pairs. Individuals with non-zero kinship will share some fraction of their genes in common by virtue of inheriting those genes from a common ancestor. Hence, if genetic differences among individuals influence variation in a given trait, then on average across the population, the closer the kinship relationship between any two individuals, the smaller will be their observed pair-wise phenotypic difference.

The variance components approach we used (Almasy & Blangero 1998) is an extension of the strategy developed by Amos (1994). We test for significant heritability by comparing two models, one that assumes no genetic effect (i.e. no predictive information contained in the kinship relations among animals) and the alternative that assumes an influence of shared

genetics (kinship). The covariance matrix among all pairs of individuals in a pedigree is given by

$$\mathbf{\Omega} = 2\mathbf{\Phi}\sigma_g^2 + \mathbf{I}\sigma_e^2 \quad (1)$$

where σ_g^2 is the genetic variance due to residual additive genetic factors (heritability), $\mathbf{\Phi}$ is the kinship matrix representing the pair-wise kinship coefficients among all animals, σ_e^2 is the variance due to individual-specific environmental effects and $\mathbf{I}$ is an identity matrix. Significance of heritability was tested by comparing the likelihood of the model in which the additive genetic effect (σ_g^2) is constrained to 0 with that of a model in which σ_g^2 is estimated. Twice the difference between the two $\log_e$ likelihoods of these models yields a test statistic, which is asymptotically distributed as a 1/2:1/2 mixture of a χ_1^2 variable and a point mass at 0 (Self & Liang 1987).

A series of covariates (age, age², sex, age $\times$ sex interaction and age² $\times$ sex interaction) were also incorporated into the statistical model. Variance components analysis affords the opportunity to simultaneously test, using likelihood methods, for the effects of any number of parameters (Almasy & Blangero 1998), and we tested this series of covariates in SOLAR as part of the test for genetic effects. The phenotypes we analyzed are not normally distributed, and therefore, we either transformed the raw data to achieve a normal distribution or performed the variance components analyses using the *t*-distribution utility within SOLAR, which makes the analyses robust to this circumstance. Prior to variance components genetic analysis, the data set was evaluated by counting the number of phenotypic values that were 3 SD or more from the population mean. This evaluation of data distribution is important because extreme outliers can have a substantial impact on the estimated heritability of a trait. There were no datapoints for duration of freezing or frequency of cooing that were more than 3 SD from the mean. Seven values for duration of locomotion, 2 values for 'orient to intruder' and 10 values for 'hostility to intruder' were outside that threshold and thus were blanked prior to quantitative genetic analysis.

Genotyping the serotonin transporter promoter polymorphism

We amplified the polymorphic region of the rhesus monkey serotonin transporter (SLC6A4) promoter (Lesch *et al.* 1997) using the following polymerase chain reaction (PCR) primers (forward – 5'cagcacctaaccctaatgtccctg3' and reverse – 5'gattctggtgccactagacgccag3') and the PCR conditions described by Rogers *et al.* (2006b). Due to lack of DNA or tissue for some subjects, only 173 individuals with behavioral data also had genotype data for the serotonin transporter polymorphism.

Testing the effect of serotonin transporter genotype on behavior

SOLAR was used to examine the quantitative effect of the assayed genetic variation in the SLC6A4 promoter repeat polymorphism. The same pedigree data used for heritability studies were employed in these analyses. To test for the influence of SLC6A4 genotype on phenotypic variance, we classified all study animals as *l/l*, *l/s* or *s/s* genotype and added this as one more potential contributing factor to phenotypic covariance among all pairs of animals. SOLAR was used to calculate the statistical significance of SLC6A4 genotype as an additional factor beyond the general effect of kinship. These analyses compared the likelihood of the model that assumed SLC6A4 genotype has no effect to a series of models in which the effect of the SLC6A4 genotype was allowed to vary. We tested one model in which the three possible genotypes are allowed to have independent effects and a second model in which *l/s* and *s/s* genotypes were grouped and tested against *l/l* genotypes. For all parameterizations of the model, the maximum

likelihood values of the genotype effects were estimated as independent variance components separate from additive genetic variance accounted for by kinship (i.e. heritability).

Results

We found substantial individual variation among test subjects for all five behavioral measures. The means and standard deviations for these phenotypes are presented in Table 1. Threat-induced freezing and ‘orienting to the intruder’ were the most prominent behaviors expressed in response to the NEC condition. There is a *priori* reason to expect duration of freezing and duration of orienting to the intruder to be correlated because both are believed to reflect the underlying endophenotype of elevated anxiety and behavioral inhibition. We found a statistically significant, although modest, positive correlation between these two behaviors ($r = 0.318$, $P < 0.001$) (Fig. 1). By definition, neither locomotion nor cooing can occur when animals are freezing, and therefore, it is not surprising that these two behaviors are significantly negatively correlated with freezing duration (freezing vs. locomotion: $r = -0.561$, $P < 0.001$; freezing vs. cooing: $r = -0.385$, $P < 0.001$). Locomotion is also weakly negatively correlated with orienting to the intruder ($r = -0.174$, $P < 0.02$). Cooing is positively correlated with locomotion ($r = 0.311$, $P < 0.001$), and hostility to the intruder ($r = 0.286$, $P < 0.001$) and also weakly positively related to orient to intruder ($r = 0.109$, $P < 0.05$). Finally, freezing is negatively correlated, although only weakly, with hostility to the intruder ($r = -0.181$, $P < 0.02$).

Quantitative genetic analysis indicated that duration of freezing and duration of orienting to the intruder are significantly influenced by genetic differences among individuals but that the durations of locomotion and hostility to intruder and frequency of cooing are not. The heritability of freezing duration during the NEC condition is $h^2 = 0.384 \pm 0.20$ ($P = 0.012$). Covariate effects were estimated simultaneously with genetic effects. Sex and age $\times$ sex interaction were the only covariates that showed any detectable effect on this phenotype. Males and females showed a marginally significant difference in freezing duration ($P = 0.052$), with the mean for males (186.6 ± 11.1 seconds, $\pm$ SD) higher than that for females (157.4 ± 13.0 seconds). The age $\times$ sex interaction was also marginally significant ($P = 0.059$). However, these two covariates combined account for only 1.5% of the total variance in freezing duration. Our analysis of freezing duration indicates that, after removing the effects of all the tested covariates, additive genetic variation accounts for an estimated 38.4% of the residual phenotypic variance in this behavioral measure.

The estimated residual heritability of orienting to the intruder is greater than that of freezing, with $h^2 = 0.908 \pm 0.22$ ($P = 0.00001$). Age, age² and sex were all statistically significant covariates, with P -values less than 0.05. The age² $\times$ sex interaction was marginally significant ($P = 0.078$). Orienting behavior is negatively correlated with age ($r = -0.625$, $P < 0.001$), while females show significantly less orienting behavior than males. Together, all the covariates account for 44.9% of the total variation in duration of orienting to the intruder, and additive genetic variation accounts for 90.8% of the remaining or residual phenotypic variance. Consequently, we can calculate the proportion of total phenotypic variance in orienting that is attributable to genetic variation by multiplying the residual heritability by the variance not explained by covariates, and we obtain 50.0%.

We found no statistically significant evidence for an effect of genetic variation on duration of locomotion ($P > 0.10$) or hostility expressed toward the intruder ($P > 0.24$) or on the frequency of cooing ($P > 0.43$). However, age and sex are both significant covariates for cooing, while age is significant for hostility to the intruder. No covariate was significantly related to duration of locomotion.

In this population, the frequencies of the three SLC6A4 promoter repeat genotypes (also called 5HTTLPR genotypes) are $l/l = 0.606$, $l/s = 0.321$ and $s/s = 0.073$. This polymorphism showed no significant relationship to either duration of freezing behavior or duration of orienting to the intruder. We tested the effect of these serotonin transporter genotypes in several ways, including using each of the three genotypes as separate predictors, and combining l/s with s/s genotypes to compare the mean effect of l/l vs. l/s plus s/s . In all tests of freezing duration, the serotonin transporter repeat unit genotype effect never achieved a P -value of less than $P = 0.30$. Similarly, all models testing for SLC6A4 genotypic effects on orienting to the intruder exhibited P -values greater than 0.14. It is not appropriate to test for genotype effects on the other three behaviors because they did not show evidence for any overall genetic effect in the quantitative genetic (heritability) analyses.

Discussion

We found significant heritability for behavioral inhibition, i.e. the duration of freezing in response to the NEC challenge, in this population of macaques. We also found a significant genetic effect on a related behavior, vigilance or orienting to the intruder. Threat-induced freezing in rhesus monkeys and the analogous behavioral inhibition in humans are adaptive responses reflecting underlying anxiety, and in certain situations are protective (Kalin & Shelton 1989, 1998). Increased vigilance associated with freezing is adaptive because it facilitates ongoing evaluation of potential risk. However, extreme levels of behavioral inhibition and hypervigilance reflect excessive anxiety. In human children, this is associated with increased risk for developing anxiety and depressive disorders as well as comorbid substance abuse (Caspi *et al.* 1996; Kagan *et al.* 1988; Kalin & Shelton 2003; Rosenbaum *et al.* 1993).

Using young rhesus monkeys, we previously described behavioral and physiological components of the anxious endophenotype revealed by the human intruder challenge. In addition to excessive freezing and vigilance behavior, this endophenotype includes increased pituitary–adrenal activity (Kalin *et al.* 1998a,b), increased cerebrospinal fluid concentrations of the anxiogenic neuropeptide corticotrophin-releasing factor (Kalin *et al.* 2000) and asymmetric right frontal brain electrical activity (Kalin *et al.* 1998b). Increased cortisol and asymmetric right frontal brain activity also occur in extremely inhibited children (Kagan *et al.* 1988; Schmidt *et al.* 1999). Furthermore, individual differences in monkey freezing behavior are positively correlated with differences in metabolic activity of the amygdala and bed nucleus of the stria terminalis as assessed with high-resolution positron emission tomography imaging (Kalin *et al.* 2005). These brain structures are components of the neural circuitry associated with the processing of emotion, fear and anxiety. Human adults with a childhood history of extreme behavioral inhibition also exhibit increased amygdala reactivity (Schwartz *et al.* 2003).

The present study demonstrates that behavioral expression of this anxious endophenotype is significantly influenced by genetic differences among animals. We also note that much of the variation in behavioral response to this test is not attributable to genetic variation but to age, sex and unidentified environmental factors. The variance components approach applied to animals from multigeneration pedigrees efficiently exploits kinship information to test the hypothesis that individual behaviors are influenced by genetic differences among animals and simultaneously quantifies the relative significance of genetic and environmental factors.

Williamson *et al.* (2003) also used variance components methods to examine the heritability of anxiety and fearfulness in a different pedigree of young rhesus monkeys. They found that the latency to leave the mother and explore a novel play room, latency to inspect a novel food item and duration of exploratory behavior while separated from their mother were all

significantly heritable. While Williamson *et al.* (2003) did not directly assess freezing behavior, they did measure ‘movement’ during a modified human intruder test. Neither the amount of movement nor vocalizations during the NEC test were significantly heritable (Williamson *et al.* 2003), which is concordant with the results in our study.

Fairbanks *et al.* (2004) demonstrated the heritability of approach behaviors expressed by adolescent and adult vervet monkeys (*Chlorocebus aethiops*) exposed to an unfamiliar conspecific. Although exposure to unfamiliar conspecifics may not be testing the same elements of temperament as response to a human intruder, this result is also consistent with our finding of significant heritability of responses to potentially dangerous stimuli. Overall, the results of Williamson *et al.* (2003) and Fairbanks *et al.* (2004) are consistent with our conclusion that individual variation in specific elements of primate behavior related to anxiety and fear is influenced by genetic differences among animals. Furthermore, these genetic effects are attributable to additive genetic variance, without the involvement of genotype $\times$ environment interaction effects.

Human studies have reported and replicated associations of neuroticism, major depression and other anxiety-related traits with the *s* allele of the serotonin transporter locus (Caspi *et al.* 2003; Kendler *et al.* 2005; Lesch *et al.* 1996; Schinka *et al.* 2004; Sen *et al.* 2004). Furthermore, previous studies in rhesus monkeys have detected an effect of a similar polymorphism in the same gene on adrenocorticotrophic hormone levels (Barr *et al.* 2004b) and alcohol preference (Barr *et al.* 2004a). However, we found no relationship between serotonin transporter promoter repeat genotype and either duration of freezing or duration of orienting to the intruder. Most of the published effects of the rhesus serotonin transporter genotype depend on an interaction between serotonin transporter genotype and the rearing environment experienced by the animal (Barr *et al.* 2004a,b; Bennett *et al.* 2002), suggesting that like some human studies, the rhesus serotonin transporter promoter polymorphism may exhibit its strongest influence in combination with adverse environmental experience. However, one study has reported an effect on the age at which male macaques disperse from their natal social groups under normal mother-rearing conditions (Trefilov *et al.* 2000).

We note that not all studies of the human serotonin transporter polymorphism are concordant (Lasky-Su *et al.* 2005; Munafo *et al.* 2005; Surtees *et al.* 2006; Willis-Owen *et al.* 2005). Most relevant to our findings are studies examining the relationship between the SLC6A4 short (*s*) allele and childhood behavioral inhibition or shyness. In a large sample of preschool children, no relationship was found between observed shyness and the *s* allele (Schmidt *et al.* 2002), whereas among second graders, the *s* allele was unexpectedly associated with decreased levels of shyness (Arbelle *et al.* 2003). In contrast, Battaglia *et al.* (2005) concluded that the *s* allele was associated with increased shyness among third and fourth graders. Finally, Fox *et al.* (2005) reported a gene $\times$ environment interaction such that 7-year olds with the *s* allele and low levels of social support showed increased inhibition when exposed to unfamiliar peers.

Bethea *et al.* (2004) reported a genetic association study investigating the effect of the serotonin transporter promoter polymorphism on anxiety-related behaviors in young rhesus monkeys. In the same population studied by Williamson *et al.* (2003), Bethea and colleagues analyzed a series of behavioral tests, including the modified human intruder challenge. They report that SLC6A4 promoter repeat genotypes (also called 5HTTLPR genotypes) are associated with one behavior during the human intruder test: the number of threat displays given by monkeys while the human stared directly at them. No association was found with any behaviors expressed during the NEC test, although SLC6A4 genotypes were reported to be associated with activity during a Free Play test and fear grimaces displayed to a threatening mechanical toy.

It is not clear how to interpret these genetic associations reported by Bethea *et al.* (2004). The same behavioral methods were used to study the same population of monkeys by Williamson *et al.* (2003), and the latter study did not find significant heritability for any of the behaviors reported by Bethea *et al.* to be associated with serotonin transporter genotypes. Bethea *et al.* did not provide an explanation for why they might find a genetic association with 5HTTLPR genotypes, while Williamson *et al.* could not detect any genetic effect across the entire genome. Bethea *et al.* also reported that they did not make use of the pedigree relationships among their study subjects in their analysis, although they acknowledge that some of the animals used were likely to be genealogically related. The analysis of heritability using variance components methods as we report here depends on using kinship relationships among individuals to estimate quantitative genetic effects, but the genetic association approach used by Bethea *et al.* (2004) depends on the assumption that animals are genealogically unrelated. Bethea *et al.* acknowledged that this is a concern regarding their analyses and suggested that it is possible that individuals exhibiting high levels of anxiety in their study may be influenced by other genetic polymorphisms but not by SLC6A4 (5HTTLPR) specifically.

In general, the available data from humans and rhesus monkeys suggest that the serotonin transporter polymorphism does influence specific behavioral and neurobiological phenotypes, but its effects are not detected on all anxiety-related traits. It may be that among children and young rhesus monkeys, the major effects of serotonin transporter repeat unit polymorphism depend on interaction with adverse environmental experience. Like the situation for SLC6A4 and human psychopathology, it is not yet clear how much effect the serotonin transporter polymorphism has among monkeys raised in essentially normal circumstances. Because all our study subjects were raised with their mothers, as opposed to the peer-rearing condition employed in other studies (Barr *et al.* 2004a,b), we did not investigate genotype $\times$ environment interaction. We note, however, that several studies of human temperament and nonhuman primate behavior (Fairbanks *et al.* 2004; Williamson *et al.* 2003; this study) document additive genetic effects on anxiety-related traits that do not depend on abnormal developmental or environmental circumstances to be manifest.

In summary, we examined individual variation in behavioral responses to a standardized behavioral challenge, the human intruder test (Kalin & Shelton 1989/2003) among a large number of young rhesus monkeys from a multigeneration pedigree. Knowledge of the kinship relationships among animals allowed us to estimate the proportion of phenotypic variation attributable to additive genetic variation and to simultaneously estimate the influence of age and sex. We found significant heritability for two out of five behaviors. Both freezing and orienting to intruder are part of an anxious endophenotype we have demonstrated to be physiologically similar to extreme behavioral inhibition in children (Kalin & Shelton 2003). In addition to testing for overall genetic heritability, we tested for, but could not detect, specific effects of the serotonin transporter promoter polymorphism.

Longitudinal studies of children have documented a positive relationship between early childhood inhibition and both adolescent and adult anxiety disorders and depression (Caspi *et al.* 1996; Kovacs & Devlin 1998; Rosenbaum *et al.* 1993). Our genetic results for rhesus monkeys provide a foundation for in-depth examination of the genetic mechanisms underlying this predisposition. Primate models can be used to investigate the quantitative genetics of behavioral or neurobiological processes (Cheverud *et al.* 1990; Higley *et al.* 1993; Newman *et al.* 2005; Rogers *et al.* 2004) and to map quantitative trait loci that influence complex phenotypes (Havill *et al.* 2005; Kammerer *et al.* 2001; Martin *et al.* 2001; Rainwater *et al.* 2003). With the recent sequencing of the rhesus genome (Gibbs & the Rhesus Macaque Genome Sequencing and Analysis Consortium 2007) and publication of a whole-genome linkage map (Rogers *et al.* 2006a), whole-genome linkage analysis can now be used to locate and identify specific genes underlying the heritability of anxiety-related traits in this species.

Such studies in nonhuman primates may identify novel genetic pathways that influence the development of temperament, including predisposition to anxiety and depressive disorders in humans.

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Figure 1. Correlation between ‘freezing’ and ‘orient to the intruder’

This figure shows the observed phenotypic correlation between duration of freezing and duration of ‘orienting to the intruder’ among the study animals ($n = 285$).

Table 1

Results of behavioral analyses during the NEC phase of human intruder test

Behavior [*]	Mean	SDRange
Freezing	174.7	143.30.0–527.7
Orient to intruder	149.3	98.70.0–512.9
Locomotion	49.7	64.90.0–370.3
Cooing	22.9	53.10.0–304.0
Hostility to intruder	2.5	10.80.0–104.2

* All results are reported as observed durations in seconds during a 10-min test period, except 'cooing', which is reported as frequency during the same 10-min test.