

Research Report

Evidence for a Gene-Environment Interaction in Predicting Behavioral Inhibition in Middle Childhood

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ABSTRACT—*Gene-environment interactions are presumed to shape human behavior during early development. However, no human research has demonstrated that such interactions lead to stable individual differences in fear responses. We tested this possibility by focusing on a polymorphism in the promoter region of the gene for the serotonin transporter (5-HTT). This polymorphism has been linked to many indices of serotonin activity. Specifically, we tested the hypothesis that an interaction between children's 5-HTT status and maternal reports of social support predicts inhibited behavior with unfamiliar peers in middle childhood. Results were consistent with this hypothesis: Children with the combination of the short 5-HTT allele and low social support had increased risk for behavioral inhibition in middle childhood.*

Various mammalian species exhibit stable individual differences in fearful behavior that reflect gene-environment interactions. For example, studies in rodents show that environmental influences produce individual differences in fear responses through interactions with genes (Gross & Hen, 2004; Meaney, 2001). Recently, studies have implicated a functional polymorphism in the promoter region of the serotonin transporter (5-HTT) in these

interactions. The 5-HTT gene consists of two alleles, the short and the long, with the short allele conveying diminished 5-HTT transcription, lower transporter levels, and reduced serotonin uptake, with functional effects on neural circuits regulated by serotonin (Hariri et al., 2002). The short allele has been associated with predispositions to anxiety and negative emotionality in adult humans (Munafo et al., 2003). Studies relating presence of the short allele to the neural substrates of anxiety or fear include one that found the presence of the S allele to be associated with heightened amygdala activation to fear faces in adults (Hariri et al., 2002). A second study found that this allele is associated with greater coupling between amygdala and ventromedial prefrontal cortex compared with the long allele (Heinz et al., 2005).

Findings in children with the short allele are, however, mixed. We (Schmidt, Fox, Rubin, Hu, & Hamer, 2002) found no relation between the 5-HTT gene and *behavioral inhibition*, which refers to a fearful temperament or style of reacting when confronted with novelty. Arbellet et al. (2003) found that the long form of this gene was associated with questionnaire-based reports of shyness. Battaglia et al. (2005) found that heightened shyness in children (measured via questionnaire and observation) was associated with the homozygous short-short 5-HTT allele status.

Some children show continuity in behavioral inhibition across childhood, whereas others display discontinuity of this behavioral disposition (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001). This temperament is associated with enhanced amygdala activation in response to faces (Schwartz, Wright,

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Shin, Kagan, & Rauch, 2003), which suggests that a neural “fear circuit” plays a role in this phenotype. However, no study has investigated the possibility that variability in continuity of behavioral inhibition across childhood is a function of gene-environment interaction.

In a study of nonhuman primates, the interaction of maternal caregiving and a 5-HTT promoter polymorphism predicted fearfulness (Suomi, 2004). Preliminary studies in humans also implicate 5-HTT in gene-environment interactions. These studies suggest that a stressful environment may exert greater influences on emotional behavior and fear-circuit function in adults with the short as opposed to the long allele of the 5-HTT gene (Caspi et al., 2003). Caspi et al. found that adults who both had the short allele and reported high levels of stressful life events were more likely to exhibit major depressive disorder than individuals who had two long alleles. The only other study demonstrating a gene-environment interaction in children involving this polymorphism is one by Kaufman et al. (2004). These authors examined 101 children, 57 of whom had been removed from their parents because of a history of abuse or neglect. Kaufman et al. reported that the maltreated children with the short allele and no positive social supports had higher depression scores than children having all other combinations of social support and genotype (and their scores were twice those of nonmaltreated children with the same genotype).

Both of these studies (Caspi et al., 2003, and Kaufman et al., 2004) focused exclusively on measures of psychopathology, particularly major depressive disorder. To our knowledge, however, there are no data on whether gene-environment interactions predict variation in the normative trajectories of personality during childhood. The present study extends the research on 5-HTT gene-environment interactions by examining the developmental effects of such interactions on fear-related behaviors in young children. Although gene-environment interactions are presumed to shape human behavior during early development, no research has demonstrated that such interactions lead to stable individual differences in fear responses.

In order to investigate environmental influences on children identified as having the temperament of behavioral inhibition, we focused on social support, as experienced by the mother, as an index of environmental stress. Prior studies have suggested that a lack of social support is a marker of family risk via its influence on parenting (Osofsky & Thompson, 2000) and have established a firm link between maternal report of low social support and children’s stress (Adamakos, Ryan, Ullman, & Pascoe, 1986; Deater-Deckard, 1998). Parent-reported negative life events have been implicated in gene-environment interactions predicting maladaptive behavior in adolescents (Silberg, Rutter, Neale, & Eaves, 2001). As another measure of environmental stress, we collected mothers’ responses on the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). Elevated scores on this measure suggest an increased number of symptoms associated with depression.

Researchers (Gelfand & Teti, 1990) have noted relations between mothers’ reports of depression and negative outcomes for their children. We included this measure to examine the degree to which maternal report of social support was mediated by depression.

We used a longitudinal framework and direct observation of behavior to examine the manner in which early gene-environment interactions shape development of behavioral inhibition. Early interactions between genes and the environment are particularly salient because of the plasticity of the child’s brain. This methodology allowed us to watch these interactions unfold instead of relying on parents’ reports of children’s early temperament and environment. We tested the hypothesis that behavioral inhibition in middle childhood is predicted by the combination of low social support reported by the mother and the short 5-HTT allele in the child. We also examined this model with mothers’ report of their own depressive symptoms as a proxy for family stress.

METHOD

Participants and Design

The data came from a cohort of 153 children who had received multiple assessments of temperament. In this study, we analyzed observational measures of temperament at ages 14 and 84 months, along with maternal reports of shyness at age 84 months. To index genetic and environmental influences on children’s fear-related behavior, we assessed mothers’ perceptions of social support, mothers’ depressive symptoms, and the children’s 5-HTT status. Seventy-three children had complete data and were used in the analyses. On the observational measures of behavioral inhibition at 14 and 84 months and parental report of shyness and social support, these children did not differ from the remaining children with incomplete data.

Procedure

Behavioral Inhibition at 14 Months

At age 14 months, infants were observed in a paradigm modeled after one utilized by Kagan to assess behavioral inhibition (Kagan, Reznick, & Snidman, 1987). Their behaviors toward and latencies to approach novel objects and an unfamiliar adult were assessed. First there was a brief period of free play. Next, an unfamiliar adult sat silently, then dumped blocks out of a dump truck, and then played with a robot that flashed and made clicking sounds as it walked. The unfamiliar adult prompted the child to play with the objects multiple times.

We created a single aggregate of behavioral inhibition using latencies to vocalize to the stranger, to the robot, and during free play; latencies to approach the stranger, to approach the robot, and to touch a toy during free play; and time spent in proximity to the mother during the appearance of the stranger, the appearance of the robot, and free play. The summed index

was standardized, and scores on the index ranged from -1.86 to 2.95 . Intercoder reliability was calculated on 15% of the sample using percentage agreement. Pearson's correlations between pairs of coders on individual measures ranged from $.85$ to 1.0 .

Behavioral Inhibition at 84 Months

At 84 months, the children were observed in play quartets consisting of unfamiliar same-sex peers. Each play group consisted of 1 previously inhibited child, 1 previously exuberant child, and 2 typical children. The children were categorized by their scores from observations when they played in quartets at 48 months of age. For both the 48- and 84-month observations, the children were left alone in the playroom for 15 min with age-appropriate toys. (See Fox et al., 2001, for a complete description.)

Rubin's (2001) Play Observation Scale was used to code behaviors in the play sessions. Behavioral inhibition was measured as the proportion of time that the child was reticent. Reticent behavior was calculated as the sum of the duration of onlooking behavior and unoccupied behavior. The ratio of time spent in reticent behavior was then submitted to a natural-log transformation. The standardized score of social reticence was used as the index of behavioral inhibition at 48 and 84 months. The 84-month reticence scores ranged from -2.95 to 2.79 . Approximately ninety 10-s coding intervals were coded for each child. Three independent observers double-coded 30% of the sample to achieve intercoder reliability. For the full variable matrix, Cohen's kappas ranged from $.81$ to $.94$.

Questionnaires

In the current study, we indexed the children's psychosocial stress by assessing social support in the family when the children were 48 months of age. Our measure was the Personal Resource Questionnaire (PRQ; Brandt & Weinert, 1981), which assesses five components of perceived social support: intimacy, social integration, self-esteem, nurturance, and assistance. Mothers made ratings on 25 Likert-scale statements that assessed perceived levels of support. Total scores ranged from 25 to 127, with higher scores representing higher degrees of perceived social support. After standardization, the data ranged from -1.86 to 2.29 . The Colorado Child Temperament Inventory (CCTI; Rowe & Plomin, 1977) was used to obtain maternal reports of the children's shyness at 84 months. These ratings were standardized and ranged from -1.58 to 2.21 . The BDI (Beck et al., 1961) was used to obtain maternal reports of symptoms linked to depression. Mothers were asked to rate themselves on 21 items, which measured characteristic attitudes and symptoms of depression. After standardization, the data ranged from -1.18 to 2.66 .

DNA Preparation and Genotyping

Children were analyzed in two groups, the long-allele group ($n = 18$) and the short-allele group ($n = 55$; see Table 1 for fre-

TABLE 1
Allele Frequencies

Allele	<i>n</i>
l/s	38
s/s	17
l/l	18

quencies), which were identified by data on 5-HTT status obtained at age 48 months. Genomic DNA was collected with buccal swabs. The swabs were air dried and then sent to the Laboratory of Biochemistry at the National Institutes of Health for extraction within 3 days. DNA was prepared by absorption to a bead matrix and heat elution. The 5-HTT gene promoter polymorphism (5-HTTLPR) was analyzed by polymerase chain reaction amplification and agarose gel electrophoresis as described by Lesch et al. (1996). (See Schmidt et al., 2002, for further details.)

RESULTS

Predicting Behavioral Inhibition at 84 Months

The hypothesis was supported, $t(61) = -2.167$, $p < .05$, as indicated by a significant genotype-by-social-support interaction in predicting observed behavioral inhibition at 84 months (see Table 2). Social support was differentially related to behavioral inhibition at 7 years depending on whether the child had the short or long 5-HTT allele. To test and interpret these

TABLE 2
Predicting Behavioral Inhibition and Shyness at 84 Months

Variable	β
Behavioral inhibition	
Step 1 ($df = 3, 62$; $R^2 = .03$; adjusted $R^2 = -.021$)	
Behavioral inhibition at 14 months	.061
5-HTT allele	.154
Social support at 48 months	-.045
Step 2 ($df = 4, 61$; $R^2 = .1$; adjusted $R^2 = .036$)	
Behavioral inhibition at 14 months	-.013
5-HTT allele	.196
Social support at 48 months	.309
5-HTT Allele $\times$ Social Support	-.456*
Shyness	
Step 1 ($df = 3, 68$; $R^2 = .063$; adjusted $R^2 = .022$)	
Behavioral inhibition at 14 months	.229
5-HTT allele	.154
Social support at 48 months	.020
Step 2 ($df = 4, 67$; $R^2 = .122$; adjusted $R^2 = .069$)	
Behavioral inhibition at 14 months	.168
5-HTT allele	.188
Social support at 48 months	.388
5-HTT Allele $\times$ Social Support	-.449*

* $p < .05$.

interactions, we followed Aiken and West's (1991) standard procedures for decomposing interactions. Controlling for behavioral inhibition at 14 months, 5-HTT genotype status exhibited increasingly strong associations with the 84-month behavioral-inhibition score, $t(61) = 2.50, p < .05$, at increasingly low levels of social support (see Fig. 1, top panel). Removing behavioral inhibition at 14 months from the regression

did not result in an appreciable difference in the gene-environment interaction.

In contrast to the significant interaction between allele status and social support in this model, the model including maternal BDI scores did not show a significant interaction between child's genotype and mother's BDI score, $t(61) = 1.17$, n.s. Maternal reports of social support and BDI scores were only modestly correlated ($r = -.26, p < .05$).

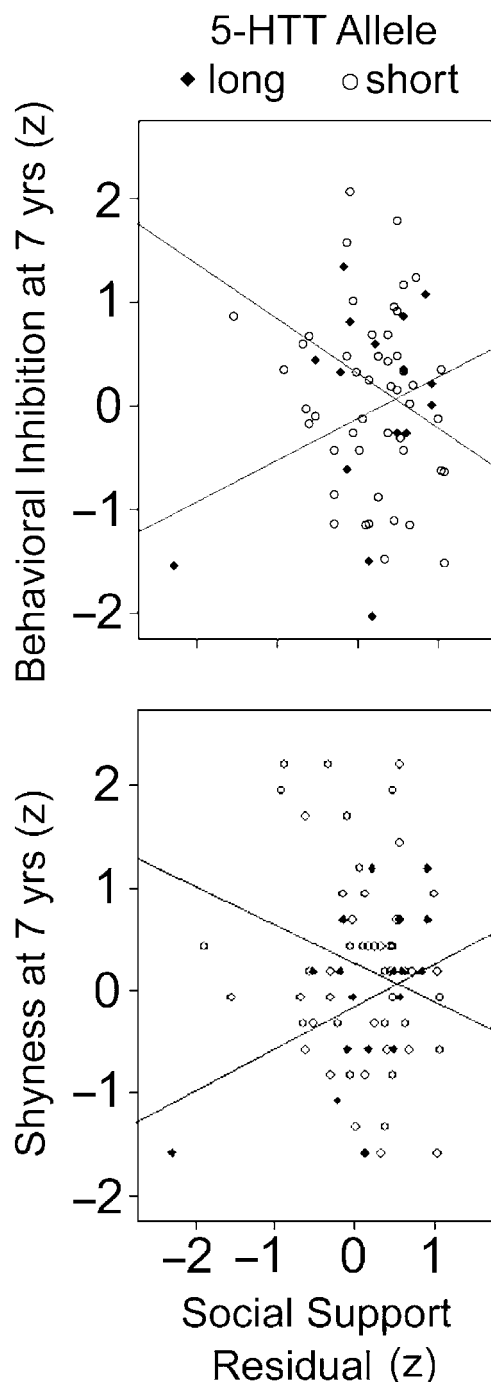


Fig. 1. Observed behavioral inhibition (top) and shyness (bottom) at age 7 as a function of level of social support and 5-HTT allele (long or short). The graphs show regression lines, as well as data from individual children.

Predicting Shyness at 84 Months

The model predicting mothers' reports of shyness at 84 months showed a significant genotype-by-social-support interaction, $t(67) = -2.112, p < .05$ (see Table 2), paralleling the results for observed behavioral inhibition. Social support was related to shyness at 7 years differently for children who had the short 5-HTT allele and those who had the long allele. Controlling for behavioral inhibition at 14 months, 5-HTT genotype status exhibited increasingly strong association with the 84-month shyness score, $t(67) = 2.49, p < .05$, at increasingly low levels of social support (see Fig. 1, bottom panel). Thus, we replicated the finding that for children in families with low levels of social support, having the short allele of the 5-HTT gene increased risk for behavioral inhibition (in this case, measured by maternal report), whereas for children in families with high levels of social support, the short allele did not convey increased risk for behavioral inhibition. Removing behavioral inhibition at 14 months from the regression did not result in an appreciable difference in the gene-environment interaction.

Again, the model including maternal scores on the BDI as a predictor of shyness did not show a significant interaction between child's genotype and mother's score on the BDI, $t(67) = -0.22$, n.s. Maternal report of shyness and observed behavioral inhibition at 7 years were correlated ($r = .36, p < .01$).

DISCUSSION

This study provides initial evidence for a gene-environment interaction (interaction between maternal report of social support and child's 5-HTT status) in predicting behavioral inhibition in middle childhood. These data are consistent with recent evidence in humans showing that 5-HTT status interacts with stress in the environment to confer risk for psychopathology (Caspi et al., 2003; Kaufman et al., 2004). The current data extend these prior findings in important respects. This study demonstrates a prospective association for a continuous measure of laboratory-observed temperament in young children, as opposed to an association for measures of psychopathology assessed via interview in later life. The data are longitudinal and do not rely on retrospective report. Moreover, parallel associations emerged with two distinct measures of child behavior, one derived from maternal report and the other derived from direct observation by examiners. The lack of a significant

gene-environment interaction involving maternal report of depressive symptoms on the BDI and the modest correlation between BDI and social-support scores suggest that maternal report of low social support was not solely a function of maternal dysphoric affect.

Despite the potential importance of these findings, we urge caution in interpreting them before they have been replicated, given prior false positives in psychiatric genetics. Current thinking suggests that samples considerably larger than the one in this study are required to provide adequate statistical power in association studies examining complex behavioral phenotypes. This thinking is based on the fact that prior association studies have generally found effect sizes that are moderate, as opposed to large (Risch & Merikangas, 1996). We, however, found a large effect in this study. Despite this, the current findings are in need of replication.

Current views on power emphasize flaws in prior studies of complex behavioral phenotypes, including the low reliability of the dependent measures, the incomplete understanding of brain-behavior associations for the typically employed phenotype, and the failure to consider gene-environment interactions (Merikangas & Risch, 2003). The current study overcame each of these major limitations. Thus, the magnitude of the effect detected may in fact be larger than the magnitude of the effects in prior association studies, making typical calculations for power and required sample sizes overly conservative. Moreover, the current study tested a relatively narrow, a priori hypothesis, based on two prior studies of gene-environment interactions (Caspi et al., 2003; Kaufman et al., 2004) that both detected statistically significant, relatively large effects (and Kaufman's study used a similarly small sample, $n = 101$). Finally, we detected the hypothesized interaction with two discrete measures, both of which assess aspects of social reticence. Moreover, these two measures were only modestly correlated, further tempering the possibility of a false positive.

In sum, this is the first study to describe a gene-environment interaction for the temperamental trait of behavioral inhibition in children. The data illustrate that the expression of this trait is a product of the child's underlying biology and environment. They demonstrate one of the important ways in which normative variations in social behavior unfold over the course of development.

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