DOCUMENT RESUME

ED 368 458 PS 022 101

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TITLE Genetic and Common Environmental Influences on Behavioral Inhibition in Infancy and Early Childhood.
PUB DATE Mar 93
NOTE 11p.; Paper presented at the Biennial Meeting of the Society for Research in Child Development (60th, New Orleans, LA, March 25-28, 1993).
PUB TYPE Reports - Research/Technical (143) -- Speeches/Conference Papers (150)

EDRS PRICE MF01/PC01 Plus Postage.
DESCRIPTORS *Affective Behavior; Age Differences; Family Influence; *Heredity; Individual Differences; *Infant Behavior; *Inhibition; Longitudinal Studies; Nature Nurture Controversy; Play; *Toddlers; *Twins

ABSTRACT

This study was designed to assess genetic influence on behavioral inhibition and its varying expression in 92 monozygotic and 86 dizygotic twin pairs. Infant behavior and mother-child interaction were observed and videotaped during structured play sessions at age 14, 20, 24, and 36 months. Analysis of the results suggests that most of the overlap in behavioral inhibition is due to overlap in genetic influences, especially at 24 months. In contrast, there is no evidence that shared twin environment contributed appreciably to overlap in the measures. Twins do not appear to be subject to a strong familial influence that affects their response to all situations where behavioral inhibition might be measured. (MDM)

GENETIC AND COMMON ENVIRONMENTAL INFLUENCES ON BEHAVIORAL INHIBITION IN INFANCY AND EARLY CHILDHOOD

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Poster presented at the 60th Anniversary Meeting of the Society for Research in Child Development, New Orleans, LA, March, 1993.

ABSTRACT

Temperamental styles are hypothesized to be organized patterns of behavior which appear early in life, persist into childhood, and have a partial biological basis. One style, the tendency to respond to the unfamiliar with wariness or avoidance, has come to be known as behavioral inhibition". This study reports a behavioral genetic analysis of overlap and situational specificity for five assessments of behavioral inhibition using two situations and four time points between 14 and 36 months of age. The implications of the findings for the hypothesized genetically driven broad-scale temperamental domain of behavioral inhibition are discussed.

SUMMARY

Temperamental styles are hypothesized to be organized patterns of behavior which appear early in life, persist into childhood, and have a partial biological basis. One style, the tendency to respond to the unfamiliar with wariness or avoidance, has come to be known as "behavioral inhibition". Previous developmental research has shown it to be fairly stable through age five: about one-half preserve the phenotype (Kagan, Reznick, & Snidman, 1988). Previous research on twins participating in the MacArthur Longitudinal Twin Study has suggested that behavioral inhibition may be genetically influenced in children as young as 14 months old, and that changes in behavioral inhibition observed in a laboratory setting at 14 months and again at 24 months may also be genetically influenced (Robinson, Kagan, Reznick, & Corley, 1992).

The goal of the current study is to assess the heritable nature of behavioral inhibition and its varying expression at four time points over the interval between 14 and 36 months as assessed in two laboratory situations. One structured play situation involves unfamiliar toys and an unfamiliar adult; the other is a peer social situation in which twins played together with members of an unfamiliar twin pair in an unfamiliar room. For each situation a composite score of behavioral inhibition is derived. The structured play situation composites were derived at 14, 20, and 24 months; the peer free play composites were derived at 24 and 36 months. For a sample of 292 twins, the correlation at 24 months between the structured play composite and the peer play composite was 0.32 ($p < .001$), indicating that at the phenotypic level, there is both significant overlap between the methods of assessing behavioral inhibition and appreciable situational specificity.

The longitudinal sample consisted of the first 100 monozygotic (MZ) and first 100 dizygotic (DZ) same-sex twin pairs participating in the MacArthur Longitudinal Twin Study through the age of 24 months. This sample consists of 106 female pairs and 94 male pairs. The parents of twins participating in this study are slightly older and somewhat better educated on the average than the mean values for parents of newborn children in Colorado, and are less likely to be African-American or Hispanic.

Twin analyses of genetic influence compare the resemblance of MZ twin pairs, who are genetically identical, and DZ twin pairs, who on average, share half their genes. Three sources of influence on individual differences for the traits under consideration are estimated: additive genetic, shared or common environmental, and residual or non-shared environmental. Longitudinal, multivariate twin data on behavioral inhibition provide an opportunity to explore the genetic and shared environmental contributions to both the overlap and the specificity of multiple assessments of this behavioral dimension between 14 and 36 months of age. In this report, a triangular or Cholesky decomposition of the genetic and environmental influences on the five measures of behavioral inhibition at the four ages was used to examine the importance of common genetic and environmental influences acting on the multiple measures of behavioral inhibition. The procedure used is similar to methods discussed for decomposing MZ and DZ twin covariance matrices using structural equation programs like LISREL or EQS (Fulker, Baker, & Bock, 1983), but modified to maximize a log-likelihood function appropriate for twin family pedigrees in which some missing data are found.

INTRODUCTION

Temperamental styles are hypothesized to be organized patterns of behavior which appear early in life, persist into childhood, and have a partial biological basis. One style, the tendency to respond to the unfamiliar with wariness or avoidance, has come to be known as "behavioral inhibition". Previous developmental research has shown it to be fairly stable through age five: about one-half preserve the phenotype (Kagan, Reznick, & Snidman, 1988). Our previous research on twins participating in the MacArthur Longitudinal Twin Study has suggested that behavioral inhibition may be genetically influenced in children as young as 14 months old, and that changes in behavioral inhibition observed in a laboratory setting at 14 months and again at 24 months may also be genetically influenced (Robinson, Kagan, Reznick, & Corley, 1992).

The current study is an attempt to explore the heritable nature of behavioral inhibition at four time points over the interval between 14 and 36 months as assessed in two laboratory situations. To what extent do genetic and common environmental influences acting on twins contribute to the covariance among measures of behavioral inhibition?

SAMPLE

The sample consisted of 92 monozygotic (MZ) and 86 dizygotic (DZ) twin pairs who completed the 14 month assessment; 85 MZ and 77 DZ pairs who completed the 20 month assessment; 76 MZ and 81 DZ pairs who completed the 24 month structured play episodes and 77 MZ and 74 DZ pairs who completed the 24 month peer play situation; and 58 MZ and 46 DZ pairs who completed the 36 month peer play situation. These twins represent all twin pairs from the first 100 MZ and 100 DZ twin pairs with unambiguous zygosity who participated in the MacArthur Longitudinal Study through age 24 months. The sample at 36 months is a subset of these children. The twins were recruited from monthly reports of birth supplied by the Colorado Department of Health and were selected preferentially for high birth weights. Sixty-two percent of the twins had birth weights greater than 2500 grams.

Over one-half of the parents contacted agreed to participate. Over 90 percent were Caucasian, the remaining families were primarily Hispanic. Participating parents were slightly older (30 years) and somewhat better educated (14.5 years) than the average Colorado parent of a newborn (28 years old and 12.5 years of education).

Zygosity of twins was determined through aggregation of independent tester ratings on the similarity of eight physical attributes across age. The attributes were selected based on the diagnostic rules developed by Nichols and Bilbro (1966). When the features of the pair of twins were rated consistently as highly similar (scores of one or two on a 5-point scale), the classification of MZ was made. When two or more features were somewhat similar (a score of three) or if one feature was not at all similar (score of four or five), the classification was DZ.

PROCEDURES

Twin pairs came to the lab with their mother at 14, 20, 24, and 36 months for roughly 2 1/2 hours. Behavioral inhibition was assessed during the second half of the visit.

SITUATION 1: (14, 20, 24 Months). One twin accompanied mother and examiner to a small room (9 x 12 feet); s/he was placed on the floor containing a set of toys. A cabinet three feet high was situated to the right of the doorway and contained an unfamiliar object. At each age the object was: 14 months - a 2' stuffed blue toy monster; 20 months - a 2' robot made from tin cans; 24 months - a 14" remote-control, gray plastic robot. Mother sat on a sofa, allowing her child to accompany her or leave her; mother was given two questionnaires to complete and was instructed to pay minimal attention to her child unless the child requested her attention. The examiner then left the room. Two cameras recorded the episode. The inhibition index was composed of data from three episodes at 14 and 20 months: 1) a freeplay episode during the first 12 minutes in which the child could explore the room and toys; 2) a stranger episode which involved an unfamiliar female who entered the room holding a novel toy (a truck) and engaged in a standard approach sequence with the child; 3) the presentation of the object, approximately 20 minutes after entering the room, involving a standard approach sequence. The procedures differed somewhat at 24 months because the stranger episode was not presented. Instead, the examiner returned to the room after 12 minutes and engaged the child in reading a book as the remote-control robot was introduced into the room.

SITUATION 2: (24, 36 Months). At 24 months, two sets of same-sex twins (four boys or four girls) were brought together in a laboratory playroom containing age-appropriate toys, including a play kitchen, a shopping cart, and a large plastic tunnel. The children were allowed to play without restraint for 25 minutes. The mothers were told to interact with the children as little as possible, but to engage with each other in conversation. At 36 months the procedure was modified so that only one member of each twin pair was in the playroom at the same time.

VARIABLES

Videotapes of the structured play sessions at 14 and 20 months were coded for four latencies: 1) to leave mother upon entering the playroom; 2) to approach the toys; 3) to approach the stranger, and 4) to approach the unfamiliar object. In addition, the total proportion of time the child spent proximal to mother was coded during the 1) free play, 2) stranger, and 3) unfamiliar object phases. At 24 months, three latencies were coded: (1) to leave mother upon entering the playroom; (2) to approach the toys; and (3) to approach the unfamiliar object. The total proportion of the time the child spent proximal to mother was coded during the freeplay and unfamiliar object phases. An aggregate index (standard score) of the inhibited/ uninhibited dimension was computed by averaging the standard scores for the variables at each age.

During the peer play situation, behaviors of the children were coded live by trained coders who each rated the behaviors of one child. Approximately 10% of the sessions had to be coded from tape because of a shortage of coders at the time the children were tested. The index of behavioral inhibition in the peer play situation was created from four measures among ten coded: 1) proximity to mother; 2) inverse of the number of times child approached an unfamiliar child; 3) time spent staring at an unfamiliar child; and 4) latency to touch a toy for the first time. Each measure was standardized, then averaged to create the index at 24 and again at 36 months. Details of the peer play situation and aggregate construction can be found in DiLalla, Kagan, & Reznick (in press).

ANALYSES & RESULTS

Twin analyses of genetic influence compare the resemblance of MZ twin pairs, who are genetically identical, and DZ twin pairs, who on average, share half their genes. Three sources of influence on individual differences for the traits under consideration are estimated: additive genetic, shared or common environmental, and residual or non-shared environmental. In this report, a triangular or Cholesky decomposition of the genetic and environmental influences on the five measures of behavioral inhibition at the four ages was used to examine the importance of common genetic and environmental influences acting on the multiple measures of behavioral inhibition. The procedure used is similar to methods discussed for decomposing MZ and DZ twin covariance matrices using structural equation programs like LISREL or EQS (Fulker, Baker, & Bock, 1983), but modified to maximize a log-likelihood function appropriate for twin family pedigrees in which some missing data are found. The factorization of genetic, common environmental, and specific environmental influences acting on the behavioral inhibition measures yields developmental insights into processes of continuity and change (Cherny, 1993; Cherny, et al., 1993).

The phenotypic association between measures for our sample is given in TABLE 1. Comparable measures at adjacent time points are typically correlated at around 0.3; the correlations for non-adjacent time points, or for different situations at adjacent time points are lower. Thus the phenotypic picture is one of both situational and time-point specificity.

TABLE 2 shows the MZ and DZ twin intraclass correlations for the five measures, as well as the estimates for individual additive genetic variance (h^2) and shared or common environmental variance (c^2) derived from the Cholesky decomposition procedure. For each measure, the MZ twin correlations are higher than the DZ twin correlations, consistent with our earlier research that suggested a heritable component to each of the three inhibition composites derived from the structured play situation. However, the estimate of the common environmental variance is negligible for all except the 24 month variables.

TABLE 3 shows that the MZ cross-twin, cross-measure correlations are also typically higher than the comparable DZ correlations, suggesting that there is a genetic component to the covariance between measures of behavioral inhibition. This hypothesis will be explicitly tested below.

FIGURE 1 shows the factor loadings for the Cholesky factorization of genetic and common environmental influences. Three factors are shown for the additive genetic influences acting on the five behavioral inhibition measures; two factors are shown for the common environmental influences. A complete Cholesky decomposition would estimate loadings on five factors (one for each measure) for each of the three sources of variance in the measure (genetic, common environment, non-shared environment). Loadings are forced to zero for the remaining factors, however, in order to keep the derived covariance matrices positive semi-definite (Fulker, Baker, & Bock, 1983). The genetic factorization suggests that appreciable new genetic variance is seen at both 20 and 24 months, with the third factor contributing to a relatively high degree of genetic covariance between the inhibition room and peer play room at 24 months. The common environmental factorization suggests a minimal contribution to the covariance among measures, with the two factors largely defined as contributors to the variance of the two 24 month measures.

TABLE 4 shows the derived estimates of the genetic and non-shared environmental correlations for the five measures. The common environmental correlation matrix is not shown, because there is so little evidence for any common environmental influence except at 24 months (The estimate for the common environmental correlation between 24 month measures is -0.04). These results suggest a nearly complete overlap between the genetic influences acting on the two 24 month measures, and in general, that the overlap between phenotypic measures may largely be due to genetic influences rather than due to non-shared environmental influences which truly appear to be non-shared not only with regard to the individual, but also to the particular measure being considered.

We explicitly test some hypotheses about the relative importance of these factors to the patterns of covariance in the MZ and DZ twin matrices in TABLE 5 using likelihood-ratio tests. Our first test is whether we can eliminate any non-shared environmental contribution to the covariance among measures. Then we test whether the common environmental contribution can be limited to two non-overlapping contributions to the 24 month measures. Finally, we test two genetic hypotheses: that of complete non-overlap between genetic contributions and its complement: that all genetic influences are the result of a single factor. Both of these simplified genetic model tests fail; we conclude that there is evidence for both genetic continuity and genetic change among our five measures.

FUTURE DIRECTIONS

1. Heart rate and its relationship to behavioral inhibition: Our preliminary analyses of heart rate at 14 months have indicated that uninhibited boys show significantly greater variability in their heart beat rhythms than inhibited boys. We wish to examine whether heart rate can be used to distinguish between subjects who will persist in their inhibited or uninhibited behavior.

2. Gender and its potential interactions with behavioral inhibition: The sample of twins from 200 families discussed in this poster represents half of the sample of twins who have been tested as part of the MacArthur Longitudinal Twin Study at 14, 20, and 24 months. We will have more power to explore issues involving the interaction of inhibitio and gender as coding is completed. Our preliminary analyses on our sample of 400 twins (Robinson, Kagan, Reznick, & Corley, 1992) suggested the possibility that at least at 20 months, genetic and environmental effects may differ by gender.

3. The persistence of inhibited and uninhibited behavior: We have found that there is an association between the status of a twin and co-twin status when categorizing individuals as stably inhibited, stably uninhibited, and changers of various kinds over the interval from 14 to 24 months. Our increased sample size will allow us to explore whether that association is stronger for MZ twins than for DZ twins.

DISCUSSION

Our results suggest that most of the overlap we see between measures of behavioral inhibition at four time points and in two situations is due to overlap in the genetic influences contributing to the variance of these measures. Particularly striking was the nearly complete overlap for the two measures collected at 24 months. But the picture is not one solely of continuity and overlap. We also know that new influences come into play at both 20 and 24 months.

In contrast, there is no evidence that shared twin environments contribute appreciably to overlap in the measures. Twins do not appear to be subject to a strong familial influence that affects their response to all situations where behavioral inhibition might be measured. At 24 months, where we found evidence of a common environmental effect, the influences that act on twins regardless of their zygosity appear to be largely situationally specific.

Those influences not common to twins, the non-shared influences, appear also to be both situational- and time-specific. We know that these measures have quite respectable internal consistencies and good rater reliabilities (Robinson, Kagan, Reznick, & Corley, 1992; DiLalla, Kagan, & Reznick, in press), so we are reluctant to conclude that the non-shared environmental influences are isomorphic with measurement error. However, there is certainly no strong evidence of systematic non-familial influences causing individual twins to act consistently inhibited or uninhibited.

TABLE 1

Phenotypic Intercorrelations of Five Inhibition Measures

	INH 14	INH 20	INH 24	PEER 24	PEER 36
INH ROOM 14	1.00				
INH ROOM 20	0.31**	1.00			
INH ROOM 24	0.18**	0.26**	1.00		
PEER PLAY 24	0.01	0.17**	0.31**	1.00	
PEER PLAY 36	0.09	0.17*	0.14	0.33**	1.00

* $p < .05$

** $p < .01$

Number of subjects for each correlation range from 170 to 300.

TABLE 2

Twin Intraclass Correlations and Derived Estimates

	MZ r	DZ r	h^2	c^2
INH ROOM 14	0.56**	0.24*	0.53	0.03
INH ROOM 20	0.44**	0.17	0.42	0.02
INH ROOM 24	0.56**	0.34**	0.31	0.22
PEER PLAY 24	0.86**	0.47**	0.44	0.38
PEER PLAY 36	0.24	-0.03	0.21	0.00

* $p < .05$

** $p < .01$

TABLE 3

MZ and DZ cross-twin, cross-measure intraclass correlations

(MZ correlations below diagonal, DZ above)

	INH 14	INH 20	INH 24	PEER 24	PEER 36
INH ROOM 14		0.17	0.01	-0.03	0.10
INH ROOM 20	0.34**		0.05	-0.03	-0.07
INH ROOM 24	0.20	0.32**		0.05	0.02
PEER PLAY 24	0.04	0.17**	0.44**		-0.05
PEER PLAY 36	0.05	0.26	0.24	0.24	

* $p < .05$

** $p < .01$

TABLE 4
Estimated genetic and non-shared environmental correlations
(Genetic correlations below diagonal, environmental above)

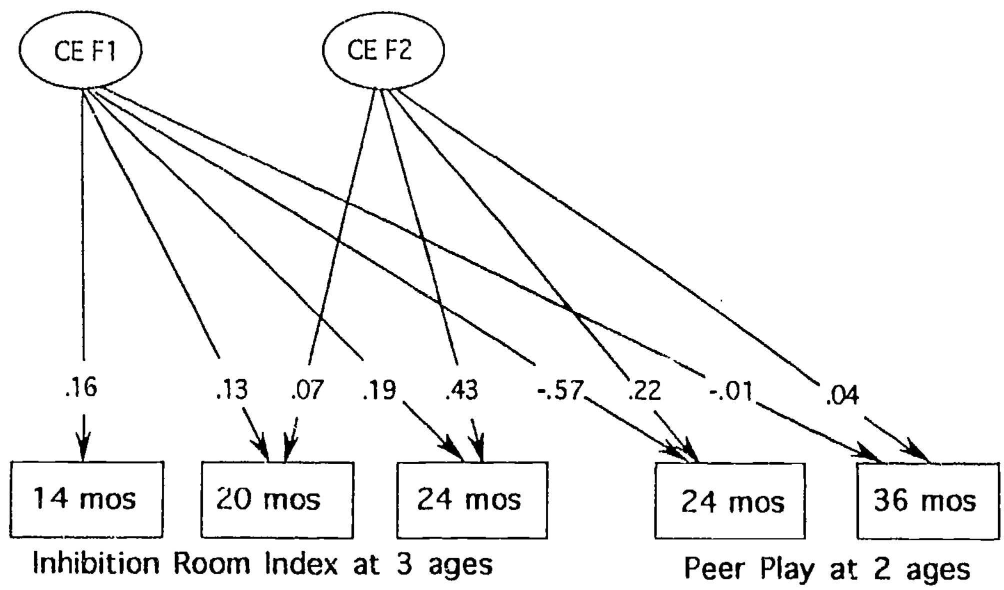
	INH 14	INH 20	INH 24	PEER 24	PEER 36
INH ROOM 14		-0.09	0.07	-0.18	0.05
INH ROOM 20	0.78		0.04	0.08	-0.08
INH ROOM 24	0.30	0.54		-0.15	-0.11
PEER PLAY 24	0.30	0.37	0.96		0.23
PEER PLAY 36	0.27	0.78	0.77	0.56	

TABLE 5
Tests of specific hypotheses about sources of covariance

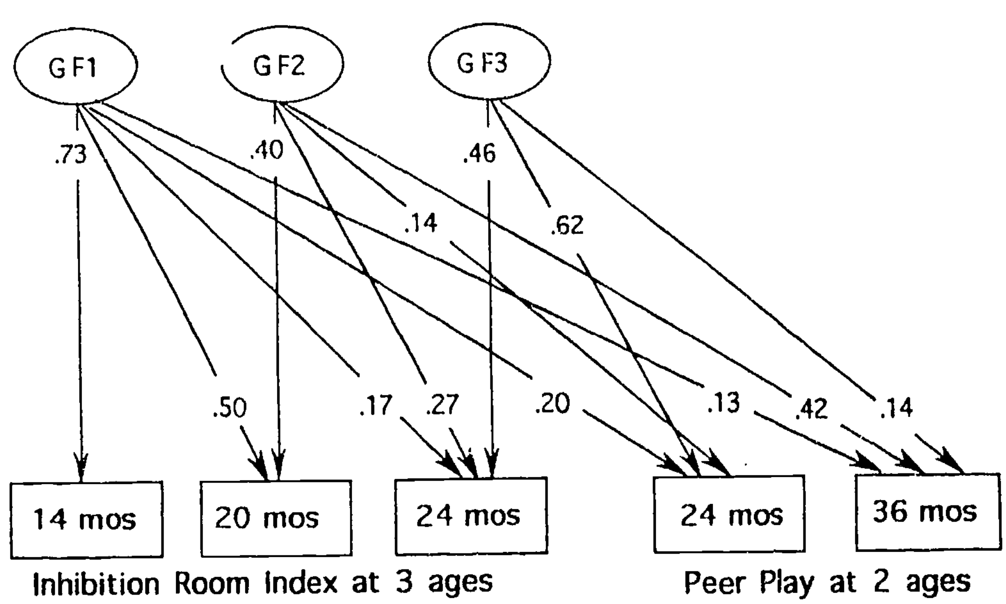
	Chi-Sq	D.F.	Prob.
TEST 1: No non-shared component to phenotypic covariance	11.46	10	> .25
TEST 2: No common environmental component to phenotypic covariance; 24 month only	1.10	12	> .95
TEST 3: No genetic contribution to phenotypic covariance	100.18	10	< .01
TEST 4: No new genetic influences emerging for later measures	51.62	10	< .01

Figure 1

Common Environment Parameters across three ages



Genetic Parameters across three ages



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