What is an "Adverse" Environment? Interactions of Rearing Experiences and *MAOA* Genotype in Rhesus Monkeys

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Background: Studies have been inconsistent in demonstrating that early adversity and specific genotype can be joint risk factors for poor behavioral outcomes. Using a rhesus monkey model, we examined how social context and different forms of early adversity influence whether a specific genotype (polymorphism in the promoter region of *monoamine oxidase A* [*MAOA*]) affects display of aggressive, fearful, and anxious behaviors.

Methods: Rhesus monkey infants ($n = 473$) were exposed to brief social challenge at age 3–4 months. Infants were reared 1) with mothers and up to 150 other animals in large cages; 2) with mothers in smaller social groups; 3) with mother and access to, at most, one other mother–infant pair; and 4) without mother but with access to a same-age peer in a nursery.

Results: No effects of genotype were found for infants reared by mothers in large social cages, although several genotype by rearing environment interactions were evident. Animals reared in smaller social groups were more likely to display aggression, which was especially true of animals possessing the low-activity *MAOA* genotype. In addition, animals with low-activity genotypes that had experienced restricted mother rearing showed more anxious behavior (scratch, yawn).

Conclusions: Among mother-reared animals, broader contextual features, associated with the social environment and experience of the mother, can affect the extent to which genotype contributes to behavioral expression under conditions of challenge. Results also suggest that different forms of early adverse experience can affect the types of responses displayed by animals of different genotypes.

Key Words: Aggression, anxiety, fear, *MAOA*, rearing, rhesus

Several studies with humans have demonstrated interactions of genotype and early adversity on later behavior. Particular attention has been paid to a polymorphism in the promoter region of *monoamine oxidase A* (*MAOA*), an X-linked gene that codes for an enzyme involved in deamination of monoamine neurotransmitters. In an early report, Caspi *et al.* (1) found that individuals possessing the genotype associated with lower transcriptional activity of *MAOA* showed increased risk for antisocial behavior, but only if they had experienced an adverse early environment. Other studies have found similar interactions for behaviors reflecting conduct disorder (2), antisocial behavior and other mental health problems (3), antisocial alcoholism (4), and physical aggression (5). Not all studies have found gene–environment interactions, however; Huizinga *et al.* (6) described several differences between their work and earlier studies that could account for their differing results, including the age of, and criteria for, early "adversity." Providing qualified support for the interaction hypothesis, Widom and Brzustowicz (7) found the expected interaction only for white participants; no such interaction was evident for non-whites.

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Widom and Brzustowicz's work (7) suggests the importance of other contextual factors when looking for interactions of genes and experience. Their failure to find a protective effect of genotype among nonwhites who suffered abuse suggested that such an effect may be evident only in "the absence of other environmental stressors or adversities" (p. 688). The broader context could be influential at the point of the early adverse event (e.g., abuse may be only one aspect of an adverse environment for a young nonwhite) or at the point of the later outcome (e.g., the development of later violence and antisocial behavior could be attributed to multiple risk factors among nonwhites, such as socioeconomic status and racism).

A nonhuman primate model is an excellent means to study the role of broader contextual factors. The ability to raise nonhumans under different conditions can permit study of the multiple factors that might contribute to an adverse environment during development. Rhesus macaques also possess a variable nucleotide tandem repeat polymorphism in the promoter region of *MAOA*, and functional assessments indicate that the 5- and 6-repeat alleles confer high transcriptional activity, whereas the 7-repeat allele produces low activity (8). In the only published behavioral study with nonhuman primates, Newman *et al.* (8) contrasted 3- to 5-year-old male monkeys that had been reared either with their mothers in small social groups or in the nursery with same-aged peers, an environment generally considered adverse. Contrary to expectation, mother-reared monkeys with low-activity genotypes were more aggressive than were low-activity, nursery-raised animals or any animals with high-activity genotypes. Newman *et al.* (8) provided several possible explanations, including that nursery rearing is characterized by a total absence of experience with adults and provides little experience with models of aggression. Obviously, these components of the early environment stand in marked contrast to the type of adversity experienced by young, abused, children. Thus, al-

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Table 1. Number of Individuals for *MAOA* Genotypes by Sex

	Genotypes						
	5/5 or 5/-	6/6 or 6/-	7/7 or 7/-	6/5	7/5	7/6	Total
Females	43	74	100	58	65	73	413
Males	60	87	109				256
Total	103	161	209	58	65	73	669

though nursery rearing is often described as an "adverse" environment, the results of Newman *et al.* (8) suggest that *MAOA* genotype does not interact with the qualities of this particular environment to affect aggression-related outcomes.

Here we report data from rhesus monkey infants reared under four standardized protocols at the California National Primate Research Center (CNPRC). Three protocols involved rearing infants with mothers, although there were differences in numbers of social companions and amount of physical space available to infants. The fourth protocol involved nursery rearing. We had two goals. First, we were interested in whether the effects of *MAOA* genotype were influenced by broader, contextual features of the animals' experience. This was accomplished by contrasting the three sets of mother-reared animals. Second, we were interested in the role that genotype might play in different adverse early environments. For this goal, we contrasted nursery-reared animals with monkeys from the mother-reared group that was most restrictive. Restrictive mother-reared infants display more agonistic behavior in later infancy and beyond (9), and nursery-rearing produces monkeys that display greater emotionality (10). We predicted that genotypic influences on behavioral expression would differ for animals reared in an environment that predisposes to high emotional reactivity compared with those reared in an environment that predisposes to aggression.

Methods and Materials

Subjects and Living Arrangements

MAOA genotype was determined for 669 infant rhesus monkeys, but only males (which are hemizygous) and homozygous females were included in the behavioral analyses, because we do not know, for heterozygous females, which alleles are silenced as a result of X inactivation. Mean age for the final sample (n = 473) was 107.9 days (range: 89–130 days) at time of behavioral testing, which corresponds roughly to age 12–16 months for human infants (11).

Field-cage-raised (FR; n = 375) monkeys were born to mothers living in 1 of 17 half-acre outdoor enclosures, constructed of pipe and chain link and measuring 40 × 64 × 9 m. Each cage contained multiple perches, climbing structures, smaller shelters for protection against rain and wind, and up to 150 animals of all age and sex classes. Monkeys raised in corncribs (CR; n = 26) lived in small outdoor structures (approximately 4 m in diameter) in social groups with their mothers, an adult male, and typically two to five other adult females and their offspring. Infants that were mother reared (MR; n = 22) resided with their mothers in standard-sized (.58 × .66 .81 m), indoor housing cages. In most cases, two mother–infant pairs were allowed to interact with each other by opening a divider between adjacent cages for approximately 8 hours per day. Nursery-reared (NR; n = 50) infants were derived from the field cages by separating them from their mothers on the day of birth and placing them in incubators (each containing a stuffed animal toy and towels to cling to) for 30 days, after which they were moved into individual units of a quad cage (.46 × .61 × .69 m) constructed of stainless steel. After 1 or 2 days of habituation, barriers between adjacent units were removed so that infants could interact in pairs. In one birth year, infants were formed into pairs for 6 hours per day, whereas in the subsequent 2 years, barriers were removed permanently, resulting in continuous, dyadic, peer rearing. Mean age of delivery of the mothers for the FR, CR, and NR animals ranged from 7.5 to 8.3 years and were not significantly different; mothers of MR monkeys, however, were significantly older (11.9 years).

Behavioral Assessment

Five hours after infants were separated from their rearing environment and companion(s) (see Supplement 1), a Human Intruder Test was conducted to assess responsiveness of the infants under standardized and graded conditions of challenge (12). This test is an abbreviated version of a test that has been described by others (13,14), although important differences in procedures (age of animals, duration of exposure [and distance] to the intruder, size of cage, and amount of time since separation from mother) and responses exist. Four consecutive trials, each lasting 1 min, were conducted in the test cage as follows: an unfamiliar human "intruder" sat approximately 1 m in front of the cage, presenting the left profile (profile-far); after 1 min, the intruder, maintaining the profile orientation, moved to within .3 m in front of the cage [profile-near]; after 1 min, the intruder returned to the 1-m location and attempted to maintain direct eye contact with the infant [stare-far] for 1 min, and finally, the human moved again to within .3 m of the test cage and attempted to maintain eye contact [stare-near] for 1 min. Behavioral responses were videotaped for later transcription. Because of minimal differences in the two profile conditions, we examined data only from the two stare conditions involving far (mild-challenge) and near (high-challenge) distances. Behaviors of interest reflected fear (grimace), aggression (threat), and anxiety (scratch, yawn) (15–17). The ethogram and definitions can be found in Supplement 1. Correlational analyses (n = 473) revealed that, within each challenge condition, rates of behavior were generally independent: in the mild-challenge condition, Pearson correlation coefficients among the four variables ranged from –.02 to .09 and were nonsignificant, and in the high-challenge condition, scratch and yawn were correlated (r = .20, $p < .001$), as were threat and grimace (r = –.10, $p < .05$). Across challenge conditions, correlations were higher and significant (all ps $< .001$), suggesting reasonable reliability of the individual differences: threat (r = .37); grimace (r = .55), scratch (r = .43), and yawn (r = .41).

Genotyping and Sequencing

See Supplement 1.

Statistical Analysis

The principal objective of the analysis was to determine the association between *MAOA* genotype and behavioral responses of rhesus monkeys in the context of early rearing conditions. Evaluation of the significance of genotype-by-environment inter-

Table 2. Number of Individuals for *MAOA* Genotype Activity by Rearing Condition

	Rearing Condition				
	CR	FR	NR	MR	Total
Low activity	15	156	27	11	209
High activity	11	219	23	11	264
Total	26	375	50	22	473

CR, corncrib reared; FR, field-cage-reared; MR, indoor mother reared; NR, nursery reared.