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Moderate Level Fetal Alcohol Exposure and Serotonin Transporter Gene Promoter Polymorphism Affect Neonatal Temperament and LHPA Axis Regulation In Monkeys

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Abstract

Background—A length polymorphism in the serotonin (5-HT) transporter gene promoter region in humans and rhesus monkeys affects functional characteristics of the brain 5-HT system. Prenatal alcohol exposure (FA-exposure) can have an impact on brain and psychosocial development that could interact with genetic endowment. This study determined whether moderate FA-exposure interacts with polymorphism in the 5-HT transporter gene to increase the incidence or severity of fetal alcohol effects in rhesus monkeys.

Methods—The offspring of monkeys who did or did not consume moderate amounts of alcohol during pregnancy were assessed for temperament as neonates and adreno-corticotrophic hormone (ACTH) and cortisol (CORT) in response to mother-infant separation at 6 months of age. 5-HT promoter region genotypes (homozygous s/s or heterozygous s/l, versus homozygous l/l), were determined.

Results—FA-exposed carriers of the s allele exhibited increased neonatal irritability and increased ACTH and CORT compared to FA-exposed monkeys homozygous for the l allele and monkeys that were not FA-exposed regardless of genotype.

Conclusions—The s allele of the 5-HT transporter increases the probability of neonatal irritability and increased stress responsiveness in FA-exposed monkeys, and this gene-environment interaction may affect psychosocial development. It is probable that FA-exposure contributes to 5-HT transporter gene-environment interactions in humans.

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Keywords

serotonin transporter gene; infant negativity; fetal alcohol exposure; rhesus monkey; cortisol (CORT); adrenocorticotrophic hormone (ACTH); LHPA-axis

Introduction

Aberrations in central nervous system serotonin (5-HT) function are thought to play a role in human depression, obsessive compulsive disorder, eating disorders, impulsive aggression, alcoholism, and suicide (1,2,3,4,5). Synaptic levels of serotonin are regulated, in part, via the serotonin reuptake transporter (5-HTT). In humans, there is a length polymorphism in the serotonin transporter gene promoter (5-HTTLPR) referred to as the long (*l*) and short (*s*) alleles. The cellular effects of having the *s/s* or *l/s* combination of alleles of the 5-HTTLPR gene, by comparison to *l/l*, include reduced gene transcription (6). Although studies are variable (7) reduced synaptic transporter density and re-uptake has also been reported in some studies (8, 9,10).

The 5-HTTLPR *l/l* versus *l/s* or *s/s* genotypes influence the early patterning of the brain through effects on neurite growth, migration, and synaptogenesis in mice (4,11). Differential genotype also plays a role in 5-HT neurotransmission by affecting the uptake of 5-HT into the pre-synaptic neuron (8). This effect appears to vary in relation to the functional characteristics of other neurotransmitter or neurohormonal systems that can be affected by developmental experience (3,4,11). Among these are the brain catecholamine systems and the limbic-hypothalamic-pituitary-adrenal (LHPA) axis (12,13,14).

An orthologous, 21 bp functional length polymorphism in the rhesus monkey (*Macaca mulatta*) rh5-HTTLPR has been shown to reduce central nervous system (CNS) 5-hydroxyindoleacetic acid (5-HIAA) output into cerebrospinal fluid (CSF) (15). The objective of the present study was to determine whether the effects of fetal alcohol exposure (FA-exposure) on temperament and LHPA axis responsiveness to stressors vary in relation to rh5-HTTLPR genotype.

Carrying the 5-HTTLPR *s* allele may have a direct effect on many aspects of human brain function (16,17). Some studies indicate that individuals carrying the *s* allele are more likely to demonstrate anxiety-related personality traits than are individuals homozygous for the *l* allele (1,3) but do not show any relationship between allelic variation and anxiety-related personality traits or psychopathology (18,19,20,21,22,23,24). This may result, in part, from the fact that expression of the effects of the *s* allele can interact with exposure to environmental factors (15,25,26).

The 5-HTTLPR *s* allele interacts with exposure to environmental stress and adversity to increase risk for psychopathology and influence psychobiological function (26,27,28). Studies in rhesus monkeys have also identified gene by environment interactions in brain neurochemistry and behavior. Monkeys carrying the *s* allele by comparison to *l/l* monkeys differ in levels of CSF 5-HIAA (15), neonatal behavior (29), and LHPA responsiveness (25) and these effects depend on the post-natal rearing environment.

In humans, both the post-natal social environment, as well as prenatal exposure to alcohol, tobacco and other drugs are associated with adverse developmental outcomes. FA-exposure is associated with deficits in learning, memory, attention, impulsivity, school achievement, adaptive social behaviors, and depression (30,31). In infancy, FA-exposure is associated with increased blood cortisol (CORT) and crying in response to sampling procedures at 18 months

of age (32). Not everyone exposed to alcohol in utero shows impairments, however. One source of variation could be related to genetic factors. Since the 5-HTTLPR polymorphism is common, it is inevitable that carrying a *s* 5-HTTLPR genotype and FA-exposure will converge in a proportion of the population. In the United States, 60% of women of childbearing age consume alcohol (33) and many women are unaware of their pregnancy until 6 - 8 weeks gestation (34).

We previously reported that FA-exposed infant monkeys exhibited reduced attention, impaired muscle tone and coordination (35,36), and poor emotion regulation compared to controls (37). Adrenocorticotrophic hormone (ACTH) responses to stressful situations were increased, and behavioral adaptation to a 3 day maternal separation when the infants were 6 months old was compromised (38). In adolescence, FA-exposed monkeys took more trials to learn a Nonmatch-to-Sample task (39). FA-exposed monkeys, compared to controls, also exhibited altered striatal dopamine (DA) system function in adulthood (40).

In this study, we tested the hypothesis that 5-HTTLPR genotype alters the impact of FA-exposure on LHPA systems and temperament in rhesus monkeys. We conducted a genotype-based regrouping of previous data (36,38). We reassessed whether FA-exposed rhesus monkeys carrying the *s* allele (*s* group) showed different neonatal temperament (PNNAS) (41,42), as well as LHPA axis responses to stress in comparison to FA-exposed monkeys homozygous for the *l* allele (*l/l* group).

Materials and Methods

Adult Female Subjects

This study was conducted in accordance with the guidelines of the University of Wisconsin-Madison Animal Care and Use Committee. The monkeys in this study were part of an ongoing longitudinal study investigating the effects of moderate level FA-exposure alone or in conjunction with prenatal stress on behavior and brain function (36,38). At the onset of the experiments, healthy adult female rhesus monkeys that consistently and voluntarily consumed 0.6 g/kg of a 6% volume/volume (v/v) alcohol solution sweetened with NutraSweet (300 mg/100 ml) (Equal Sweetener, Merisant US, Inc., Chicago, IL) were identified. This is comparable in effect to an average size woman consuming two servings of alcohol beverages. Moderate alcohol consumption is defined as 4 to 14 alcohol beverage servings per week (43). Twenty-two adult female rhesus monkeys that would voluntarily consume alcohol were randomly assigned at breeding to one of four groups (control or one of three experimental groups) in a 2 × 2 factorial design with alcohol consumption and/or stress as the independent variables (36): 1) control (no alcohol, no stress), 2) alcohol alone, 3) stress alone, and 4) alcohol-stress. Fifteen sires were used in the study and no female-sire pairs produced more than one offspring in the experiment. Hence, in subsequent statistical analyses, full- or half-sibling genetic loadings across treatment groups were not an issue.

Maternal Alcohol Consumption and Stress

During pregnancy (depending on experimental condition) the monkeys were provided with either 0.6 g/kg body weight of alcohol in a 6% (v/v) aspartame sweetened alcohol solution or an equivolemic and equicaloric (8 g/100 ml water) sucrose solution. This yields a blood alcohol concentration (BAC) between 20–50 mg/dl 60 minutes later, measured prior to pregnancy (36). The solutions were presented at 1600 hr five days per week beginning 5 days prior to breeding, throughout gestation, and ending at parturition. The mothers in the stress treatment groups (stress alone, and alcohol-stress) were exposed to a noise stressor five times per week at 1530 hours on gestational days 90 to 145. The pregnant females were transported to a darkened room where three noise bursts (1300 Hz pitch at 115dB) were randomly administered

over a 10-minute period. This treatment increased plasma cortisol (CORT) levels in these monkeys from 25.2 ± 2.2 $\mu\text{g/dl}$ at baseline to 34.8 ± 2.4 $\mu\text{g/dl}$ -post stress treatment (mean $\pm$ SEM, $p < 0.05$) (44).

The monkeys were fed a standard ration for pregnant and nursing mothers of Purina Monkey Chow supplemented 3 times weekly with fresh fruit at 0800 hr daily. There was no solid food (chow) left by 1600 hr when the alcohol or sucrose solutions were introduced. Tap water was available ad libitum. All females were housed under identical conditions, undisturbed except for necessary routine animal husbandry, with the exception of the stress procedure for those females in stress-alone and alcohol-stress conditions. Lighting and temperature housing conditions were controlled with 16 hrs light (0600 on): 8 hrs dark, and temperature $21^\circ \pm 0.5^\circ$ C.

Research Subjects - Offspring

The subjects were 41 offspring, 23 females and 18 males, derived from the mothers in the four treatment groups described above: control (10 males and 3 females), prenatal stress-only (6 males and 2 females), alcohol-only (3 males and 7 females), and prenatal stress-alcohol (6 males and 4 females). All infant monkeys were housed with their mothers during the first 6 months except for weekly separations for testing during the first month. At 6 months, they were separated from their mothers for weaning and then were reared until adolescence in mixed-sex peer groups consisting of 5 – 6 monkeys from the same prenatal conditions (see 36,38 for details).

Neonatal temperament measure

Neonatal temperament was assessed during the first month of life using the Primate Neonatal Neurobehavioral Assessment (PNNAS), a 20 minute battery of developmental tests (41,42), now used in other nonhuman primate laboratories (29). The PNNAS yielded four composite scores: Orientation, Activity, Motor Maturity, and State Control that parallel composite scores of the Brazelton Neonatal Behavioral Assessment Scale for humans (45,46).

Temperament ratings were based on behaviors noted during the administration of all items during the test. The “Irritability” measure indicated whether the infant showed a negative, avoidant, and fussy response by comparison to a positive calm and alert response during the overall neonatal assessment (35,41,44). Four experimenters, blind to subject treatment condition, conducted the neonatal assessments. Inter-rater reliability on each scale was $r > 0.90$; test-retest reliability of the test battery has been reported previously and ranges from $r = 0.34$ – 0.54 for the Irritability measure (41).

Social Separation procedure

The three days following the separation of monkeys from their mothers at six months of age for weaning was used to stress reactivity. Separation Day 1 began at 0900 hr on when monkeys were separated from the mother and moved to another room where they were housed individually in wire-mesh cages.

Biological sampling and assays

Blood samples for ACTH and CORT were collected 1 week prior to separation at 1100 hr (baseline), on Separation Day 1 at 1100 hr (2 hours post-separation) and on Separation Day 2 at 1100 (26 hours post-separation) whereas blood samples for genotyping were collected when the animals were approximately 6 – 9 years old. DNA was isolated from whole blood using standard extraction methods.

Statistical analysis

Irritability scores were first analyzed in analyses of variance using a 2 (genotype) $\times$ 2 (prenatal stress) $\times$ 2 (prenatal alcohol) ANOVA design. Between subjects grouping factors for ANOVA were rh5-HTTLPR – “l/l” (l/l homozygous) versus “s” (l/s heterozygous and s/s homozygous) and “FA-exposed” for prenatal alcohol exposed subjects versus “NotFA-exposed” for those not exposed to prenatal alcohol. ACTH and CORT data were analyzed using a mixed design ANOVA in a 2 (genotype) $\times$ 2 (prenatal stress) $\times$ 2 (prenatal alcohol) $\times$ 3 (time pre-post-separation) design with repeated measures on the last factor. ACTH was analyzed as log base 10. For presentation, the linear logs were then transformed to match the raw means and standard deviations. To assess differences in the magnitude of LHPA axis response to separation, change in scores from baseline in ACTH and CORT concentration at 2 hr and 26 hr post separation were calculated and averaged and are referred to as “ACTHresponse” and “CORTresponse” measures. These data were analyzed using mixed design ANOVA. Alpha rejection levels were adjusted for departures from sphericity by the Huynh-Feldt correction.

In subsequent analyses, prenatal stress was not included as a factor because there were no substantial interactions of genotype with prenatal stress. Also, ANOVAs were performed with sex as a factor. There were no substantial interactions of genotype with sex. Addition or deletion of the prenatal stress factor and/or sex of the monkeys did not affect the significance of the analyses presented herein. Three subjects were excluded because of incomplete data for repeated measures of CORT or ACTH, leaving N = 38 subjects.

After collapsing prenatal stress and sex factors into the overall design, the NotFA-exposed group consisted of 9 males and 10 females (N = 19). The FA-exposed group consisted of 8 males and 11 females (N = 19). The l/l group consisted of 10 males and 11 females (N = 21), and the “s” group (s/l and s/s) consisted of 7 males and 10 females (N = 17). Analyses of FA-exposure $\times$ genotype interactions were based on the following group divisions : N(FA-exposed, l/l allele) = 10; N(FA-exposed, s allele) = 9; N(NotFA-exposed, l/l allele) = 11; and, N(NotFA-exposed, s allele) = 8. Subsequent breakdown of subject numbers by statistical analysis is provided in the Results.

Results

Temperament

Figure 1 shows that FA-exposure increased neonatal Irritability in monkeys carrying the s rh5-HTTLPR allele by comparison to l/l FA-exposed monkeys. Irritability of the NotFA-exposed monkeys did not differ as a function of genotype, whereas Irritability of FA-exposed monkeys did (FA-exposure $\times$ Genotype interaction, $F(1,34) = 5.79$; $p = 0.022$). FA-exposed monkeys carrying the s allele exhibited the highest levels of Irritability. Hence, the effect of genotype on Irritability depended on whether the monkeys were FA-exposed.

LHPA Axis –ACTHresponse and CORTresponse

Figure 2a shows that by comparison to baseline, ACTH levels increased 2hr post-separation and declined to baseline or below by 26 hr post-separation (Main effect of Time Post-Separation, $F(2,68) = 32.20$, $p < 0.0001$). ACTH was higher in FA-exposed compared to NotFA-exposed monkeys. This is due to the increased ACTH levels at 2 hr post-separation in monkeys bearing the s allele (FA-exposure $\times$ Time Post-Separation interaction, $F(2,68) = 4.01$, $p = 0.023$). ACTH levels were also higher in monkeys carrying the s allele by comparison to l/l monkeys. This was due to peak ACTH levels at 2 hr post-separation in monkeys carrying the s allele (FA-exposed, s allele versus all other groups at 2 hr post-separation, $F(1,36) = 4.90$, $p = 0.03$). Hence, the combination of FA-exposure and carrying the s 5-HTTLPR allele increases LHPA responsiveness to mother-infant separation.

Figure 2b shows that CORT levels increased 2 hr post-separation and then declined somewhat by 26 hr post-separation (Main effect of Time Post-Separation, $F(2,68) = 90.24$, $p < 0.0001$). CORT was higher overall in monkeys carrying a *s* allele by comparison to *l/l* monkeys (Main effect of genotype, $F(1,34) = 5.36$, $p = 0.027$). This was attributable to higher CORT at 26 hr post-separation in monkeys carrying a *s* allele (CORT x Genotype interaction $F(2,68) = 4.59$, $p = 0.013$). Thus, there was a difference between monkeys carrying a *s* allele by comparison to *l/l* monkeys at 26 hrs (F-test, $F(1,34) = 6.844$, $p = 0.0132$). Also, at 2 hr post-separation the FA-exposed group carrying a *s* allele had increased CORT by comparison to all other groups (F-test, $F(1,36) = 5.629$, $p = 0.0235$).

Figure 3 shows the mean values for ACTHresponse (mean change from baseline at 2 hr and 26 hr post-separation) for FA-exposed and NotFA-exposed “*l/l*” or “*s*” monkeys. ACTHresponse in FA-exposed monkeys was increased by comparison to NotFA-exposed monkeys (Main effect of FA-exposure $F(1,34) = 6.60$, $p = 0.015$). FA-exposed monkeys with the *s* allele had the largest positive ACTHresponse to separation. The effect of separation on ACTHresponse in NotFA-exposed monkeys with the *s* allele was in the opposite direction. By comparison, there was a modest positive ACTHresponse in monkeys with the *l/l* 5-HTTLPR allele after separation whether or not they were FA-exposed (FA-exposure X Genotype interaction, $F(1,34) = 8.32$, $p = 0.007$). The key point in Figure 3 is that ACTH output in response to separation averaged out to be below baseline in NotFA-exposed carriers of the *s* allele.

Figure 4 shows that monkeys carrying the *s* 5-HTTLPR allele exhibited increased CORTresponse change from baseline to separation by comparison to *l/l* monkeys whether or not they were FA-exposed (Main effect of genotype, $F(1,34) = 8.78$, $p = 0.005$).

Overall, the results presented in Figures 2, 3, and 4 suggest that FA-exposure interacts with the *s* 5-HTTLPR allele to influence ACTH release in response to stress, and/or LHPA axis sensitivity to CORT feedback. Carrying the *s* 5-HTTLPR variant ultimately leads to increased CORTresponse, regardless of ACTH output. The regulation of CORT in relation to ACTH output seems to be substantially affected by FA-exposure, but only in subjects with one or more *s* alleles. Whether ACTH levels in a single sample correspond to circulating CORT levels differs depending on whether the monkey was FA-exposed or not. NotFA-exposed monkeys carrying the *l/l* allele exhibit an increase in ACTHresponse to separation (Figure 3) and an increase in CORTresponse (Figure 4). NotFA-exposed monkeys carrying the *s* allele, however, exhibit a blunted ACTHresponse (Figure 3), corresponding to a decline in mean ACTH. Nevertheless, there is an increase in CORTresponse (Figure 4). This response pattern is different than that observed in monkeys carrying the *l/l* allele.

Correlations Among Variables

Table 1 shows the correlations among Irritability, ACTH and CORT mean values, and ACTH- and CORTresponse measures for FA-exposed and NotFA-exposed groups. In the FA-exposed group (Top Panel) all correlations were significant ($p < 0.05$) with the exceptions of ACTHresponse vs. CORT mean, and ACTH mean vs. CORTresponse.

The bottom panel of Table 1 shows that there were no significant correlations between Irritability and any measures of LHPA axis response in NotFA-exposed monkeys. Indeed, the only significant correlation in this group was that between CORTresponse and CORT mean. Carrying the *s* versus *l/l* 5-HTTLPR allele affects LHPA axis activity measured by CORT.

Figure 5 shows the scatter plot of the Irritability and ACTHresponse correlation in the FA-exposed group. Monkeys carrying a *s* allele are distinguished from *l/l* monkeys by different symbols (filled versus open circles respectively). The upper end of the distribution for

Irritability and for ACTH response is comprised mostly of monkeys carrying the *s* allele. If the data from monkeys carrying the *s* allele are removed, the correlation between Irritability and ACTH response is practically zero ($N = 10$, $r = 0.080$). Conversely, if the data from the *l/l* monkeys are removed, then the numerical value of the correlation between Irritability and ACTH response ($N = 9$, $r = 0.568$) is comparable to the overall correlation ($N = 19$, $r = 0.588$). Therefore, significant correlations between Irritability and LHPA measures in FA-exposed monkeys seem attributable largely to effects of FA-exposure on monkeys carrying a *s* allele. From another perspective, for this group, if one did not have prior knowledge of their FA-exposure, the overall correlation between increased Irritability and increased ACTH response would certainly be attributed to an effect of genotype alone.

Discussion

The results indicate that there was a substantial interaction between FA exposure and genotype on outcome measures of neonatal Irritability and measures of LHPA axis responsiveness to an acute stressor. This finding has important implications for understanding how FA-exposure may affect psychobiological development, and in the interpretation of results of studies in monkeys and humans where the probability of FA-exposure ought to be considered. Monkeys exposed to prenatal alcohol and also carrying the *s* 5-HTTLPR allele differed from monkeys in all other groups in three respects: 1) they were more irritable as neonates, 2) their LHPA axis was more responsive to stress at 6 months of age and appeared to be regulating differently than other monkeys, and 3) neonatal Irritability was correlated with measures of their LHPA axis responsiveness to stress at 6 months of age.

The data invite the hypothesis that the timing of LHPA axis responsiveness to stressors, CORT feedback, and inhibition of ACTH release may differ between the *l/l* and *s* allele groups. If this is a basic effect of *l/l* versus *s* 5-HTTLPR genotype on LHPA axis dynamics, then it is also true that prenatal alcohol substantially affects its expression.

The finding that the *s* 5-HTTLPR allele is a risk factor for increased neonatal Irritability in rhesus monkeys and that this risk is increased if the fetus is exposed to alcohol is consistent with studies of human infants. Infants that were *s/s* homozygous have higher scores on Negative Emotionality and Distress to Limitations at 2 months of age compared to infants with the *l/s* or *l/l* genotypes (47).

The positive correlation between neonatal Irritability and LHPA responses to mother-infant separation at 6 months of age in FA-exposed monkeys suggests that the neuroendocrine and behavioral effects of FA-exposure may form a stable or trait-like quality of the individual. Previous studies in humans and rodents have shown that FA-exposure has enduring effects on LHPA axis measures and responses to stressors (38). FA-exposed human infants exhibit higher post stress CORT levels and increased crying when compared to controls in response to a blood draw at 13 months (32). Also, “anger-prone” 15 month-old infants exhibit augmented CORT response when confronted with a stranger or a frightening robot-toy (48). In rodents, FA-exposure leads to either LHPA axis hyper-responsiveness to stressors or delayed or deficient return to pre-stress level (49,50,51,52,53,54,55,56). In this study, the effects of FA-exposure on increased infant irritability and LHPA axis responsiveness were most prominent in rhesus monkeys carrying the *s* allele.

Individual differences in LHPA axis responsiveness coupled with individual differences in 5HTT production, may indicate mechanisms by which CORT regulates 5HTT production. In an in situ fibroblast model, for instance, glucocorticoid receptor (GR) activation leads to increased 5-HTT transcription, then to increased 5HT uptake, yet *s/s* cells are generally less sensitive to GR activation than *l/l* cells (8). At high GR agonist concentrations, however, the

s/s genotype approaches *l/l* genotype production of 5-HTT (8). Increased irritability and LHPA responsiveness leading to substantially increased CORT responses could be related to altered function in the 5HT system. There may be a relative insensitivity of the 5HT system to CORT feedback in individuals carrying the *s* allele.

Maternal responses to neonatal irritability can affect the psychobiological development of the child. More irritable babies present challenges above and beyond the already heavy demands of infant care (57). Indeed, some intervention studies associate infant negativity with exceptional risk for developmental psychopathology (58,59,60). Highly negative infants are more responsive to maternal care practices and environmental change than easygoing infants (61). Human infants scoring higher on negative emotionality are also disproportionately positively responsive to intervention (61). Thus, irritability in infancy does not appear to directly predispose individuals to behavior problems or negative trait-like characteristics. Instead, irritability may correspond to inordinate responsiveness to maternal care-giving style. Difficulties encountered in coping with irritable behavior in the infant, however, may most often lead to an increase in negative and controlling, or withdrawal ignoring type responses by the caregiver (59,60). Considering the probable interaction with FA-exposure, the irritable infant may be born to young parents with problems in coping with stressors per se and problems with alcohol dependence and abuse. Hence, FA-exposed and irritable infants may most often be reared by parents less able to cope with this aspect of infant behavior than others. Variation in the quantity and quality of parental care can have substantial effects on infant psychobiological development and the expression of trait-like behavior characteristics (62, 63,64,65,66).

A limitation of the study is that only rhesus monkey mothers that voluntarily consumed a moderate dose of alcohol were included in the study. It is possible that these mothers differed genetically from mothers that do not voluntarily consume alcohol, thereby reducing the generalization of the findings.

Variation in the 5-HTTLPR polymorphism, whether in ongoing behavior or “risk” for anxiety disorders or depression, appears to be trait-like, but the relationship is found in some studies and not others (2,4,5,20,26,67). Fetal alcohol exposure may have effects on fetal development that are only apparent in individuals with a specific allele. In monkeys there are detrimental effects of carrying the *s* allele on behavior and LHPA axis regulation (25) that are compounded, based on our results, by prenatal exposure to alcohol. Moreover, FA-exposure affects neonatal temperament, but it depends upon genotype. If individuals carrying the *s* allele are excluded, then the effects of FA-exposure on irritability and LHPA axis function are not statistically significant. If one studied these measures in a human population in which a substantial but unknown proportion of the participants were FA-exposed, genotype may surface as a significant factor, but the fact that the genotype effect depended on FA-exposure would not be obvious. In humans the probability that there is a substantial effect of carrying the “*s*” allele combined with FA-exposure needs to be considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Interaction between Alcohol and 5-HTTLPR genotype on Irritability. Subjects per group: N (Alcohol, Long) = 10; N (Alcohol, Short) = 9; N (NoAlcohol, Long) = 11; N (NoAlcohol, Short) = 8. Irritability was significantly increased in the Alcohol monkeys carrying the short allele ($F(1,34) = 5.79$; $p = 0.022$) by comparison to all other groups.

Figure 2.

ACTH (a. Top Panel) and CORT (b. Bottom Panel) levels before and after mother-infant separation in Alcohol and NoAlcohol monkeys carrying the *s* allele (Short), or *l/l* (Long) genotype. Time Post Separation represents baseline samples (BASE), and samples collected at 2 hr and 26 hr after separation. Subjects per group: N (Alcohol, Long) = 10; N (Alcohol, Short) = 9; N (NoAlcohol, Long) = 11; N (NoAlcohol, Short) = 8.

Figure 2a. ACTH levels were highest in the Alcohol, Short group 2 hr post-separation by comparison to all other groups ($F(1,36) = 4.90$; $p = 0.03$).

Figure 2b. CORT was higher overall in monkeys carrying the short versus long allele at 26 hr post-separation ($F(1,36) = 5.60$; $p = 0.02$).

Figure 3.

Interaction between Alcohol and 5-HTTLPR genotype on ACTH response (mean difference from baseline at 2 and 26hr after mother infant separation). Subjects per group: N (Alcohol, Long) = 10; N (Alcohol, Short) = 9; N (NoAlcohol, Long) = 11; N (NoAlcohol, Short) = 8. The Alcohol, Short monkeys had the largest positive ACTH response to separation ($F(1,34) = 8.32$; $p = 0.007$).

Figure 4.

Effect of Alcohol and 5-HTTLPR genotype on CORT response (mean difference from baseline at 2 and 26hr after mother infant separation). Subjects per group: N (Alcohol, Long) = 10; N (Alcohol, Short) = 9; N (NoAlcohol, Long) = 11; N (NoAlcohol, Short) = 8. Monkeys with the short allele exhibited increased CORT response to separation by comparison to monkeys with the long allele ($F(1,34) = 8.78$; $p = 0.005$).

Figure 5.

Correlation between Irritability and ACTHresponse to mother-infant separation in Alcohol monkeys carrying the “s” allele, or *l/l* ($N = 19$, $r = 0.588$, $p < 0.01$). The correlation between Irritability and ACTH for *l/l* monkeys (Long) was $r = 0.080$, $N = 10$, $p = 0.83$. The correlation between Irritability and ACTHresponse for monkeys carrying the *s* allele (Short) was $r = 0.568$, $N = 9$, $p = 0.11$.

Table 1

Correlations between Neonatal Irritability and 6 Month measures of LHPA axis response to Mother-Infant separation. Top panel: Correlations in FA-exposed monkeys. Bottom Panel: Correlations in NotFA-exposed monkeys.

FA-exposed Monkeys (N=19)				
	Irritability	ACTHresponse	CORTresponse	Mean ACTH
Irritability	1			
ACTHresponse	0.588 ^{***}	1		
CORTresponse	0.523 ^{**}	0.512 ^{**}	1	
Mean ACTH	0.445 [*]	0.470 [*]	0.313	1
Mean CORT	0.465 [*]	0.283	0.781 ^{***}	0.550 ^{**}
NotFA-exposed Monkeys (N=19)				
	Irritability	ACTHresponse	CORTresponse	Mean ACTH
Irritability	1			
ACTHresponse	-0.322	1		
CORTresponse	-0.133	0.013	1	
Mean ACTH	0.052	0.062	0.389	1
Mean CORT	-0.131	0.021	0.858 ^{***}	0.353

* Probability =0.05

** 0.025

*** <0.01