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Evidence for interplay between genes and maternal stress *in utero*: monoamine oxidase A polymorphism moderates effects of life events during pregnancy on infant negative emotionality at 5 weeks

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The low activity variant of the monoamine oxidase A (*MAOA*) functional promoter polymorphism, *MAOA-LPR*, in interaction with adverse environments (G × E) is associated with child and adult antisocial behaviour disorders. *MAOA* is expressed during foetal development so *in utero* G × E may influence early neurodevelopment. We tested the hypothesis that *MAOA* G × E during pregnancy predicts infant negative emotionality soon after birth. In an epidemiological longitudinal study starting in pregnancy, using a two stage stratified design, we ascertained *MAOA-LPR* status (low vs. high activity variants) from the saliva of 209 infants (104 boys and 105 girls), and examined predictions to observed infant negative emotionality at 5 weeks post-partum from life events during pregnancy. In analyses weighted to provide estimates for the general population, and including possible confounders for life events, there was an *MAOA* status by life events interaction ($P = 0.017$). There was also an interaction between *MAOA* status and neighbourhood deprivation ($P = 0.028$). Both interactions arose from a greater effect of increasing life events on negative emotionality in the *MAOA-LPR* low activity, compared with *MAOA-LPR* high activity infants. The study provides the first evidence of moderation by *MAOA-LPR* of the effect of the social environment in pregnancy on negative emotionality in infancy, an early risk for the development of child and adult antisocial behaviour disorders.

Keywords: Infancy, life events, *MAOA* polymorphism, negative emotionality, pregnancy

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A genotype by environment interaction (G × E) occurs when a person's genotype moderates the effect of environmental experience on physical or mental health outcomes or when environmental experience moderates a genetic effect (Moffitt *et al*. 2005). One of the most widely studied G × E interactions involves polymorphisms of the monoamine oxidase (*MAOA*) gene which encodes the structure of the enzyme monoamine oxidase A. Monoamine oxidase A has a central role in the catabolism, and hence regulation, of key neurotransmitters, notably 5 hydroxytryptamine (5HT) and norepinephrine (NE). Possession of 3.5 and 4 copies of a 30 bp variable number tandem repeat in the upstream promoter region of the gene (the uVNTR), the *MAOA-LPR* high activity variant, is associated with higher *in vitro* expression compared with 3 or 5 repeat, low activity, variants (Sabol *et al*. 1998). The *MAOA-LPR* low activity variant in interaction with childhood maltreatment has been found to be associated with child behavioural and adult antisocial outcomes in many (Beach *et al*. 2010; Caspi *et al*. 2002; Enoch *et al*. 2010; Fergusson *et al*. 2011; Fergusson *et al*. 2012; Kim-Cohen *et al*. 2006) although not all (Huizinga *et al*. 2006; Prichard *et al*. 2008) studies. The plausibility of a causal role for the *MAOA-LPR* low activity variant is supported by findings that *MAOA* knockout mice display marked aggression and have elevated 5HT and NE levels, and in humans a rare functional knockout is associated with severe aggression (Buckholtz & Meyer-Lindenberg 2008). Furthermore *MAOA* is expressed in the brain during foetal development, and in animal studies prenatal inhibition of *MAOA* results in elevated aggression similar to that seen in *MAOA* knockout (Baler *et al*. 2008; Mejia *et al*. 2002). Modification of an *in utero* effect of *MAOA* genotype status by social stressors has not previously been reported.

Many studies of *MAOA* G × E only report on males because the gene is on the X chromosome, and there are uncertainties regarding the activity status of female heterozygotes (Caspi *et al*. 2002; Ferguson *et al*. 2011; Kim-Cohen *et al*. 2006). Some studies that have included females have found sex differences (Aslund *et al*. 2011), while others have not (Nikulina *et al*. 2012). The possibility of sex differences therefore needs to be examined in studies of *MAOA* G × E.

Repeated antisocial behaviours in adults are almost always preceded by conduct problems of aggression and oppositionality in early childhood (Baillargeon *et al*. 2007;

Odgers *et al*. 2008), so the study of processes preceding the appearance of childhood symptoms needs to start in infancy. High negative emotionality, a core component of infant temperament, confers early vulnerability to childhood conduct problems (Caspi & Silva 1995, Smeekens *et al*. 2007). In animal studies prenatal stress is associated with behavioural markers of increased emotional reactivity in the offspring (Weinstock 2008), and in humans, maternal cortisol levels during pregnancy predict infant emotional reactivity over the first days of life (Davis *et al*. 2011). We therefore examined whether *MAOA-LPR* genotype modifies the effects of social stressors during pregnancy on negative emotionality at 5 weeks of age.

Materials and methods

Design

The participants were members of the Wirral Child Health and Development Study, a UK prospective epidemiological longitudinal study of prenatal and infancy origins of conduct disorders (http://www.liv.ac.uk/psychology-health-and-society/research/first-steps/about/). This uses a two stage stratified design in which a larger general population sample of first-time mothers was recruited in pregnancy (extensive sample) and from which a sub-sample was drawn for more detailed assessment (intensive sample). All families in the extensive sample follow a brief assessment protocol while those in the intensive subsample receive an additional battery of labour intensive measurement, such as that used in this study to assess infant negative emotionality. The design allows general population estimates of means and associations to be derived for all extensive or intensive sample measures.

Approval for the procedures was obtained from the Cheshire North and West Research Ethics Committee (UK). The extensive sample was identified from consecutive first time mothers who booked for antenatal care at 12 weeks gestation between 12/02/2007 and 29/10/2008. The booking clinic was administered by the Wirral University Teaching Hospital which is the sole provider of universal prenatal care on the Wirral Peninsula, a geographical area bounded on three sides by water. Socioeconomic conditions on the Wirral range between the deprived inner city and affluent suburbs, but with few from ethnic minorities. The study was introduced to the women by clinic midwives, who asked for their agreement to be approached by study research midwives when they attended for ultrasound scanning at 20 weeks gestation. After complete description of the study to the women, written informed consent was obtained by the study midwives who then administered questionnaires and an interview in the clinic.

Sample

The extensive sample of 1233 mothers with surviving babies had a mean age at recruitment of 26.8 years (SD 5.8, range 18–51). Measures for this study were obtained from the extensive sample (from which the intensive sample was drawn) at 20 weeks gestation and from the intensive sample at mean 32.1 (SD 2.0) weeks of pregnancy ('32 weeks gestation') and when the infants were mean 5 weeks 2 days (SD 9 days). Of the 316 mothers recruited to the intensive subsample during pregnancy, 282 were assessed using the Neonatal Behavioural Assessment (Brazelton & Nugent 1995) from which ratings of infant negative emotionality were derived. Adequate DNA for *MAOA* analyses was obtained from 209 of these infants, 104 boys, 105 girls and they comprise the sample for this report.

Socioeconomic status was determined using the revised English Index of Multiple Deprivation (IMD; Noble *et al*. 2004) based on data collected from the UK Census in 2001. According to this system, postcode areas in England are ranked from most deprived (i.e. IMD of 1) to least deprived (i.e. IMD of 32 482) based on neighbourhood deprivation in seven domains: income, employment, health, education and training, barriers to housing and services, living environment and crime. All mothers were given IMD ranks according to the postcode of the area where they lived and assigned to a quintile based on the UK distribution of deprivation; 41.8% of the extensive sample had socioeconomic profiles found in the most deprived UK quintile, consistent with the high levels of deprivation in some parts of the Wirral. Forty eight women (3.9%) described themselves as other than White British.

Measures

DNA extraction and genotyping

DNA was extracted from saliva pads using methods from Oragene (http://www.dnagenotek.com/DNA_Genotek_Product_OG250_Lit.html) and using cheek swabs via a standard protocol (Freeman *et al*. 1997). Quantification was performed using a nanodrop 1000 (NanoDrop, Fisher Scientific, Loughborough, UK). *MAOA* VNTR genotyping was performed on an ABI 3130 (Life Technologies, Paisley, UK) capillary electrophoresis machine. DNA (~20 ng) was amplified using FAM labelled primers (IDT, Integrated DNA Technologies, Leuven, Belgium) and a polymerase chain reaction (PCR) and thermocycling protocol taken from Sabol *et al*. (1998). The PCR to amplify the region of the uVNTR was performed in non-skirted 96 well plates (ThermoFisher, Loughborough, UK) using an unlabelled forward primer: 5′-GAA CGG ACG CTC CAT TCG GA-3′ and an 5′ FAM labelled reverse primer 5′-FAM-ACA GCC TGA CCG TGG AGA AG-3′ (IDT). The PCR conditions included 1.5 mM $MgCl_2$ and 1 μM concentrations of each primer in a 10 μl reaction. The products were diluted at 1:20 and subject to capillary electrophoresis using manufacturers' protocols on an Applied Biosystems (Life Technologies) 3130 system. Sizing was performed vs. a labelled standard using the GeneMapper software supplied (ABI Ltd, Applied Biosystems, Life Technologies, Paisley, UK). Genotypes were called using Genemapper V4.0 (Life Technologies) and were independently rechecked. Duplicate samples were tested and both positive and negative controls were included on each 384 well plate (Abgene, Cambridge, UK). Genotyping was performed blind to sex, measures of pregnancy life events and infancy negative emotionality.

Infant negative emotionality

The Neonatal Behavioural Assessment (NBAS) was administered to the intensive sample at 5 weeks after birth. The NBAS is a standardized measure designed to assess orienting, motor and emotion regulatory processes during the first weeks of life (Brazelton & Nugent 1995, Lester *et al*. 1976). It is conducted by a trained administrator who carries out, in a prescribed sequence, a range of manoeuvres, designed to elicit the infant's optimal orienting and motor performance, and emotional responses to mildly aversive procedures such as undressing or being brought from lying to sitting. Ratings are generally made by the administrator from memory immediately after the assessment. This limits the scope for determining inter-rater reliability so in this study the assessments were video recorded using four cameras placed to obtain a comprehensive picture of infant responses. The NBAS coding method yields a total of 27 scores which have been previously reduced in factor analytic studies, yielding variously three to nine factors (McCollam *et al*. 1997). We used only the 'irritability' scale which is a count of the number of occasions on which the infant shows a change of state from calm, of at least three seconds, to fussing or crying in response to seven standard challenges. The count of fuss/cry episodes provided a specific measure of reactivity paralleling maternal report and observational measures of temperament later in infancy, where responses to challenges such as restraint or the unpredictable noises are assessed (Gartstein & Rothbart 2003, Rothbart *et al*. 2001). Three assessors were trained by Dr Joanna Hawthorne, director of the UK Brazelton Centre. Pair-wise agreement (ICC) between independent ratings made from memory and video recordings on 220 infants ranged between 0.81 and 0.89. Video based scores were used in analyses.

Life events

The Life History Calendar (LHC) (Caspi *et al*. 1996) was administered to participants in the intensive sample at 32 weeks gestation.

The LHC is a calendar-based response sheet, which is marked with key events in the respondent's life, such as birthdays. It is administered as a structured interview, and begins with questions regarding life trajectories, for example, where the respondent has lived and worked. This information, serves as memory cues for later questions about the occurrence of specific life events. The LHC has been showed to generate reliable recall of events over periods of up to five years (Caspi *et al*. 1996, Ehrensaft *et al*. 2004) and has been used previously in studies of G × E (Caspi *et al*. 2003). The total number of life events during pregnancy, with values of 0, 1, 2, 3 and '4 or more', was used in analyses.

Demographic variables, potential confounders and stratification variable

At recruitment into the extensive sample, mothers' ages, cohabiting status and years of education were obtained, together with the stratification measure which was report of partner psychological abuse. This was assessed using a 20-item questionnaire covering humiliating, demeaning or threatening utterances in the partner relationship during pregnancy over the previous year (Moffitt *et al*. 1997). The scale comprised the total from 20 no–yes (coded as 0 absent, 1 present) items. Participants first rated these items about their own behaviour towards their partner, and then about their partner's behaviour towards them. The measure has been shown to yield large correlations between self and partner informant reports (Moffitt *et al*. 1997). As this variable was used to select women for eligibility for intensive sample membership it was included in the analyses reported here within the weights. The psychological abuse variable used in the analyses was the highest of the partner to participant and participant to partner scores for each family.

Indices of social stress that might be confounded with life events were the UK Index of Multiple Deprivation (Noble *et al*. 2004) and marital satisfaction assessed with the Kansas Marital Satisfaction Scale (Schumm *et al*. 1985) from the extensive sample. Maternal anxiety was assessed at 32 weeks gestation in the intensive sample using the State Anxiety Scale (Spielberger *et al*. 1983) because of reported associations of maternal anxiety in the third trimester with emotional and behavioural disorders in childhood (O'connor *et al*. 2003). We considered the possible mediators, infant birth weight by gestational age and 1 min Apgar scores (threshold 7 or lower), because of their reported associations, respectively, with adolescent depression (Costello *et al*. 2007) and externalizing disorders in childhood (Baillargeon *et al*. 2011). These were obtained from hospital birth records on the extensive sample. Maternal depression scores at the time of the NBAS were obtained using the Edinburgh Postnatal Depression Scale (Cox *et al*. 1987) because post-natal depression is commonly associated with adverse developmental outcomes in infancy (Murray *et al*. 1996).

Statistical analysis

The two-phase stratified sample design allows estimates to be reported for the general population by applying weights. Inverse probability weights were constructed using the sample stratification factor, mother's cohabiting status, age and years of education, the deprivation index for her home neighbourhood, and the infant's sex, birth weight and 1 min Apgar score. Test statistics for weighted means, correlations and regression estimates were based on survey adjusted Wald tests [*t*-tests if single degrees of freedom (df) or *F*-tests if multiple df], using the robust 'sandwich' estimator of the parameter covariance matrix (Binder 1983), except for testing Hardy–Weinberg equilibrium where a weighted bootstrap *P*-value was constructed for the D statistic (Hartl & Clark 2006). Models used for inference analysed the count of fuss/cry episodes using Poisson regression, with inverse probability weights and robust parameter covariance matrix estimator for valid inference from probability weighted data, and to account for possible overdispersion in the count. For models with covariates, a form of stabilized weight was used that removed weight variability associated with the conditioning covariates. Analyses were undertaken in Stata 11 (2009).

Results

Allele frequencies

Four alleles of the 30 bp u-VNTR were observed: 3- (36.1%), 3.5- (1.4%), 4- (59.6%) and 5-repeats (2.9%) in agreement with frequencies in other studies (Kim-Cohen *et al*. 2006). All studies agree on the functional classification of the two most common alleles, that is, the 3-repeat as low activity and the 4-repeat as high activity. Of rare alleles, both Sabol *et al*. (1998) and Deckert *et al*. (1999) assayed the 3.5- repeat with the same result (high activity). We used the classification of Sabol *et al*. (i.e. 5-repeat equals low *MAOA* activity) in line with other studies (Kim-Cohen *et al*. 2006). Hardy–Weinberg equilibrium (HWE) was observed for genotypes in the females ($n = 105$, D-statistic unweighted $P = 0.527$; population weighted $P = 0.523$) while allele frequencies in males were also consistent with HWE ($P = 0.618$).

MAOA genotype, prenatal risk factors and infant negative emotionality

Table 1 gives means and percentages by *MAOA-LPR* variant for the target, confounder and possible mediator variables of this study. All variables were standardized except ages, binary variables and the variables of primary focus, namely the counts of life events (treating 4 or more as 4) and of fuss/cry episodes. There was little evidence of direct effects, the only nominally significant effect being the association with relationship satisfaction. In particular, there was a non-significant trend for the *MAOA-LPR* low activity variant to be associated with more life events in pregnancy.

All analyses included as background factors the sex and age of the infant and maternal depression score at the time of the NBAS assessment. We considered life-events in pregnancy and potential confounding effects of neighbourhood deprivation, single cohabiting status, and, at 32 weeks of pregnancy, maternal anxiety score and poor marital satisfaction as our set of prenatal risk factors that were external to the baby. Associations among these measures are shown in Table 2.

Since *MAOA* is an X linked gene, and in view of the uncertainty regarding the activity status of heterozygous females (Kim-Cohen *et al*. 2006), primary analyses were performed in males and female homozygotes combined ($n = 163$). We first tested whether the effects of the risk set as a whole were moderated by infant genotype. When all risk factors and their interactions with *MAOA-LPR* variants were included, the overall test for the interaction terms was significant, $\chi^2(5) = 12.30$, $P = 0.031$, with individual interactions being life-events ($P = 0.017$), deprivation ($P = 0.028$), marital satisfaction ($P = 0.577$), prenatal anxiety ($P = 0.682$) and marital status ($P = 0.648$). Retaining the life-events and deprivation interaction effects and the main effects of the other risk factors gave the model displayed in Table 3. Coefficients reflect the proportional change in the number of NBAS manoeuvres which evoked a fuss/cry response, with coefficients above one implying a proportional increase and those below a decrease for each unit change in the covariate. *MAOA-LPR* low activity individuals comprised the reference group for the interaction so that the table shows the main effect of life events in the *MAOA-LPR* low activity

Table 1: Estimated population means and proportions of life events, neighbourhood deprivation and potential confounders by *MAOA* status

		MAOA variant						Group differences *P*-values	
		Low/Low $N_f = 16$ $N_m = 42$		Low/High $N_f = 46$		High/High $N_f = 43$ $N_m = 62$			
Measure	*N*	Mean %	SD	Mean %	SD	Mean %	SD	Three group 2df test	Two group (LL vs. LH + HH) controlling for sex
Maternal age at consent (years)	209	27.5	5.90	26.1	5.30	28.5	6.15	.104	.746
Most deprived quintile (%)	209	41.9		52.3		33.9		.214	.779
Single non-cohabiting (%)	209	27.3		21.0		15.7		.352	.294
Partner psychological abuse (std)	209	0.20	1.17	−0.01	0.93	−0.10	0.93	.165	.110
Maternal prenatal (32 week) anxiety (std)	206	0.25	1.07	−0.11	0.99	−0.07	0.95	.175	.080
Prenatal (32 week) marital satisfaction (std)	191	−0.30	1.25	−0.21	1.29	0.27	0.49	.007	.048
Life-events in pregnancy (0,1,2,3,4+)	209	1.91	1.36	1.72	1.52	1.38	1.35	.116	.167
Birthweight by gestation (std)	209	−0.02	1.11	−0.12	0.75	0.05	0.95	.590	.844
Low APGAR at 1 min (%)	209	8.2		3.4		9.7		.422	.872
Maternal depression at 4 weeks (std)	207	−0.08	0.85	0.17	1.20	−0.04	0.97	.618	.550
Childs age at NBAS (days)	209	38.9	8.7	34.7	6.0	36.6	8.00	.179	.196
Count of NBAS cry/fuss episodes	209	1.73	1.58	1.24	1.09	1.48	1.28	.402	.544

Population weighted estimates and ordinary/logistic regression *t* and *F*-tests of group difference. N_m and N_f are male and female frequencies.

Table 2: Estimates of population correlations among prenatal life-events and observed infant negative emotionality at 5 weeks, and potential confounders and mediators

	Dep	Mar. Stat.	Psy. Abu.	Mat Anx.	Mar Satis.	LE preg.	Bwt/gest.	Apgar 1 min	Mat dep.
Marital status	**.15**								
Psychological abuse	.09	**.26**							
Maternal anxiety	−.08	**.33**	**.29**						
Relationship satisfaction	**−.17**	**−.57**	**−.26**	**−.40**					
Life-events in pregnancy	.00	**.27**	−.**20**	**.21**	**−.40**				
Birth weight/gestational age	−.08	−.05	−.07	.06	.02	−.01			
Apgar (1 min)	.01	−.01	.02	.02	−.06	**−.17**	.11		
Maternal depression	.00	**.19**	**.16**	**.40**	**−.32**	**.28**	.03	.08	
Infant negative emotionality	−.08	**.24**	.06	**.17**	**−.19**	**.17**	.01	−.08	−.01

Bold values are significant at nominal $P < 0.05$. Marital status is the binary variable for not being married or cohabiting; psychological abuse is the highest of the partner to participant and participant to partner scores; deprivation is the binary variable for being in the most deprived quintile; education is the binary variable for left education before age 19: all other variables as defined in methods section.

group. The coefficient of 1.34 reflects that among those with the *MAOA-LPR* low activity variant, each increase of one life event was associated with a 1.34 proportional increase in the number of times that infants responded with fussing or crying. Thus, compared with infants whose mothers experienced zero life events during pregnancy, exposure to four or more was associated with $1.34^4 = 3.22$, proportional increase in the number of NBAS fuss/cry episodes. The association between pregnancy life events and infant fuss/cry in the *MAOA-LPR* high activity group is given by the product of the *MAOA-LPR* low activity and the interaction term coefficients, i.e. $1.34 \times 0.77 = 1.03$, a small and non-significant effect. Figures 1 and 2 display simple sub-population means illustrating the two interactions identified. Infants with *MAOA-LPR* low activity variant display more negative emotionality with increasing levels of both of the risk factors. With increasing risk, the *MAOA-LPR* high activity infants appear to be either insensitive (life-events) or to show less negative emotionality (deprivation).

Adding birth weight (adjusted for gestation) and low Apgar score as additional covariates to the model of Table 3 made almost no change to the estimated interaction coefficients

Table 3: Summary of Poisson regression estimates of *MAOA* by prenatal environment interactions predicting observed infant negative emotionality at 5 weeks

	Relative risk	*P*-value	95% CI
Female infant	0.87	.394	0.63, 1.20
Infant age at NBAS (weeks)	0.74	.001	0.63, 0.88
Maternal depression at NBAS	1.00	.963	0.96, 1.04
Prenatal maternal anxiety	1.16	.065	0.99, 1.37
Cohabiting status	1.01	.950	0.62, 1.68
Partnership satisfaction	1.01	.897	0.83, 1.23
Deprivation	1.55	.102	0.91, 2.62
Prenatal LEs	1.34	<.001	1.18, 1.53
MAOA-LPR high activity	2.26	.004	1.30, 3.95
MAOA-LPR high activity × deprivation	0.44	.030	0.21, 0.92
MAOA-LPR high activity × prenatal LEs	0.77	.016	0.63, 0.95

Analyses are weighted to provide estimates for the general population by accounting for sample attrition and sample stratification. All covariates except ages and categorical variables are standardized, and *MAOA-LPR* low activity, male infants and non-deprived neighbourhood are the reference categories of binary dummy variables.
LEs, life events.

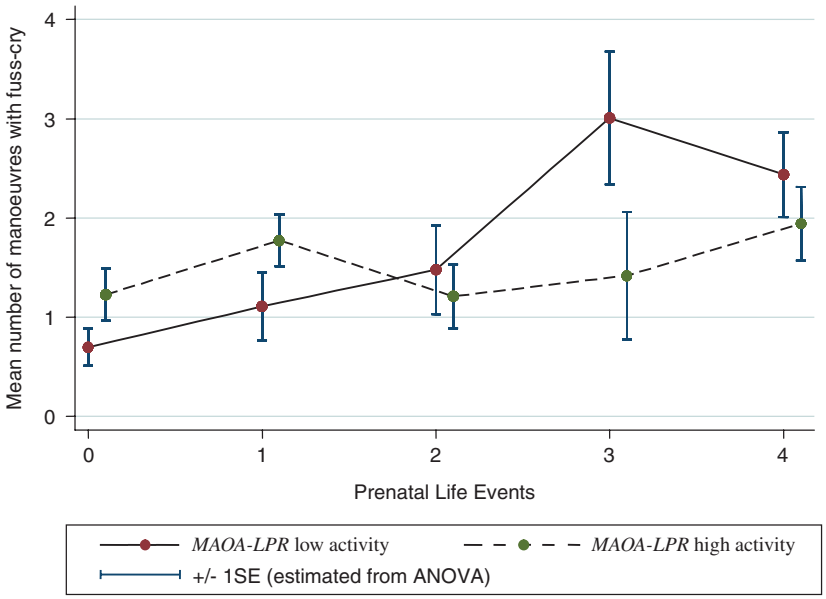

Figure 1: Infant negative emotionality by number of life events in pregnancy, comparing *MAOA-LPR* low activity and *MAOA-LPR* high activity infants. The measure of negative emotionality is the number of fuss-cry episodes. Mean fuss-cry episodes and their standard errors were derived from analysis of variance.

and both remained significant (life events $P = 0.019$, deprivation $P = 0.033$). Thus the G × E effects identified did not appear to be mediated by these variables.

Sex differences

We examined evidence for sex differences firstly by examining each 3-way interaction (sex by risk factor by *MAOA* variant) in the presence of the 2-way interactions. In neither case was the 3-way interaction significant (deprivation $P = 0.561$, life-events $P = 0.563$). Also in both cases, the two 2-way interactions involving sex of infant were also non-significant ($P = 0.928$ life events, $P = 0.062$ deprivation).

Since the power of the test of the 3-way interaction was not large, we made further checks of the homogeneity of effects across boys and girls, fitting the model with just the basic control variables at the time of infant assessment, that is, infant age and mother's depression score. In boys, for the G × E with life events in pregnancy, the relative risk was 0.79, very similar to that for the sample as a whole (0.77), although non-significant ($P = 0.065$), and for deprivation it was 0.47 ($P = 0.096$) compared with 0.44 for the whole sample. We fitted the same model to girls, but allowing 3-categories for *MAOA* to include the heterozygous participants previously excluded from the analysis. Compared to the *MAOA-LPR* low activity variant, the heterozygous variant showed less sensitivity to life events (relative risk 0.65; CI 0.49, 0.87; $P = 0.004$) and similar to that for the homozygous *MAOA-LPR* high activity variant (relative risk compared to low 0.72; CI 0.52, 1.00; $P = 0.053$). The same pattern was seen for deprivation with the heterozygous group showing less sensitivity (relative risk 0.32; CI 0.12, 0.89; $P = 0.028$) and

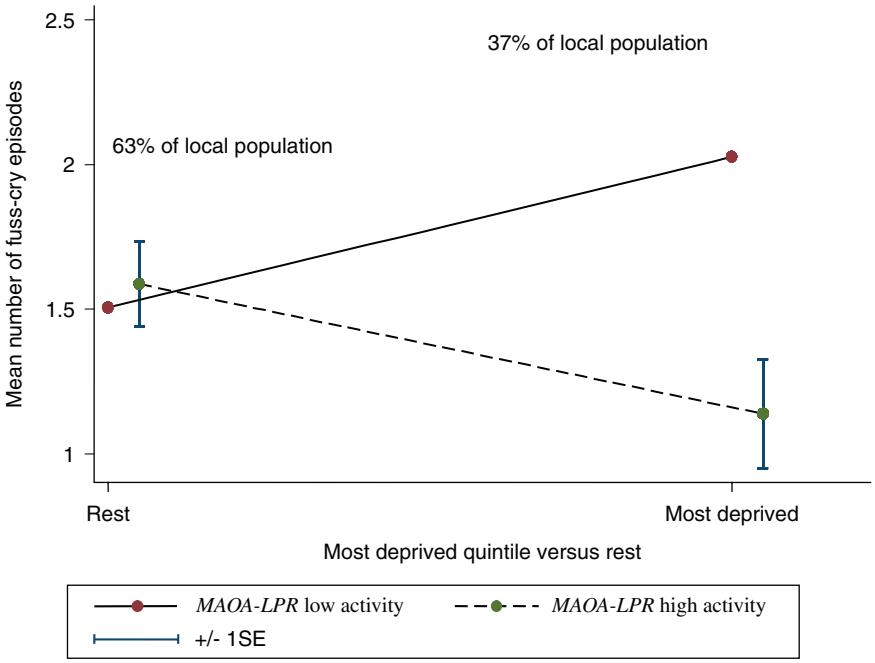

Figure 2: Infant negative emotionality by neighbourhood deprivation, comparing *MAOA-LPR* low activity and *MAOA-LPR* high activity infants. The measure of negative emotionality is the number of fuss-cry episodes. Mean fuss-cry episodes and their standard errors were derived from analysis of variance.

similar to the homozygous high activity variant (relative risk compared to low 0.44; CI 0.15, 1.26; $P = 0.125$). Pooling the heterozygous girls with the homozygous high gave relative risks for girls of 0.68 (CI 0.52, 0.89; $P = 0.004$) for life events and 0.37 (CI 0.14, 0.96; $P = 0.041$) for deprivation. Thus heterozygous girls behaved much like those with the homozygous-high variant and the effect of the genetic variant was similar among boys and girls.

Discussion

This is the first study to report that social adversity during pregnancy modifies the association between *MAOA-LPR* activity variants and early infant negative emotionality. Using a prospective design with a general population sample, we showed that in the presence of the low activity, but not the high activity, variant of the *MAOA* gene, increasing life events were strongly associated with higher infant negative emotionality. Among infants possessing the *MAOA-LPR* low activity variant, reporting four or more life events in pregnancy, compared with none, was associated with a more than three times increase in the likelihood of infants reacting to a low key challenge with fussing or crying at 5 weeks of age. This association was absent in the presence of the *MAOA-LPR* high activity variant. There was a similar interaction with neighbourhood deprivation.

The findings are consistent with previous studies that have found a wide range of adverse environments to be associated with antisocial outcomes only in individuals with the *MAOA-LPR* low activity genotype. Many studies have reported only on males because of uncertainties regarding *MAOA* activity in female heterozygotes (Caspi *et al*. 2002; Fergusson *et al*. 2012; Kim-Cohen *et al*. 2006). We report the findings first omitting female heterozygotes to deal with this problem, but we also found that the outcome associated with heterozygous *MAOA-LPR* was indistinguishable from that of homozygous *MAOA-LPR* high activity variant. Some studies of females have found that, as in males, the *MAOA-LPR* low activity genotype confers vulnerability to maltreatment (Ducci *et al*. 2008; Widom & Brzustowicz 2006), while in others it has been the *MAOA-LPR* high activity variant that is associated with antisocial outcomes in interaction with the environment (Aslund *et al*. 2011). In this study the direction and size of effect in males and females were the same.

Strengths of this study include that the sampling method ensured that findings pertain to the general population, the G × E effect was examined prospectively, plausible confounding variables were examined, and multiple testing was minimized by targeted candidate gene selection. The outcome variable was developmentally relevant, based on available findings on early vulnerability to antisocial and violent behaviours. In contrast to the standard method for rating the NBAS by the assessor from memory, we used video recordings so that inter-rater reliability could be checked. As far as we are aware we are the first to report on agreement between memory and video based ratings.

Although the sample size was adequate to detect two G × E interactions, others of smaller effect may have been missed. We showed that there were consistent effects across males and females, however, analyses of females were limited by the small number of female infants homozygous for *MAOA-LPR* low activity ($N = 16$). Life events in pregnancy were associated with other measured indices of social stress, such as marital dissatisfaction and single parent status, and so may also have been associated with unmeasured indices. It is therefore possible that life events were a proxy for other environmental risks and do not

themselves have a causal role. Furthermore there are genetic contributions to life events (Vinkhuyzen *et al*. 2010), so we cannot rule out the possibility that the apparent G × E in fact represents an interaction between maternal and infant genetic variations. The finding of an effect on early infant negative emotionality does not yet constitute direct evidence that *MAOA* G × E *in utero* influences behavioural or antisocial outcomes in childhood or adult life. Whether this is the case requires longitudinal study over many years.

A complex interplay of etiological, maintaining and ameliorating factors give rise to antisocial disorders in early childhood and contribute to their persistence or desistance (Hill 2002). Against this background, current available evidence on *MAOA* G × E effects, while impressive, shows risk for disorder without yet elucidating how they may influence specific developmental pathways. This requires study of potential biological or psychological mediators of vulnerability or resilience. There are numerous pathways from infancy to conduct disorders in young children, however, evidence converges on the centrality of negative emotionality, either as a main effect or in interaction with environmental factors (Hill 2002; Lahey *et al*. 2008). In particular indices of proneness to irritability or fussiness, or of distress to limitations taken as an index of anger proneness, have been shown to predict later externalizing symptoms (Lahey *et al*. 2008; Smeekens *et al*. 2007).

The study reported here is the first to show effects on infant negative emotionality of *in utero MAOA* G × E. The findings have the potential to inform two key connected questions, how do G × E processes operate over time, and what might be the mechanisms? Little is known about timing and developmental sequences of G × E. Most studies finding effects of maltreatment during childhood do not provide evidence regarding timing because the maltreatment variables cover the whole of childhood. They may reflect effects of sustained maltreatment over time, or more discrete episodes occurring at different times, or specifically timed effects. The report by Kim-Cohen *et al*. (2006) of *MAOA* G × E with physical abuse in seven year olds suggests there are effects in early childhood, as do findings from the UK Avon Longitudinal Study of Parents and Children (ALSPAC) in which life events experienced during infancy and up to 3.5 years, in interaction with *MAOA-LPR*, predicted hyperactivity at age 7 years (Enoch *et al*. 2010).

A possibility opened up by this study is that early G × E contributes to a temperamental vulnerability which in interaction with subsequent adverse environments, for example of physical abuse or partner violence, develops into disorder. Equally there could be successive G × E processes in which *MAOA-LPR* low activity individuals either accumulate risk as they are exposed to a series of adverse environments, which through passive, active or evocative GE correlations, may be disproportionately adverse; or reduce risk by taking advantage of supportive environments in which they may be particularly well adapted (Pickles & Hill 2006). We have recently reported *MAOA-LPR* G x E at 7 months consistent with this possibility. In the presence of the *MAOA-LPR* low activity variant, decreasing maternal sensitivity at 7 months was associated with increasing anger proneness at 14 months (Pickles *et al*. In Press).

Mechanisms of G × E remain speculative. After birth they may for example occur from the interplay between genetically influenced behavioural traits and the environment. In the case of the *MAOA* gene, *MAOA-LPR* low activity individuals show greater threat sensitivity, poor emotion control and enhanced fear memory, which are likely to confer vulnerability in unfavourable environments. Thus the interaction is thought to arise because the individual's *MAOA* influenced 'socioaffective scaffold' is more or less adaptive depending on the characteristics of the environment (Buckholtz & Meyer-Lindenberg 2008). *In utero* the interaction may arise from neurotransmitter, hormonal or gene expression effects. In animal studies effects of prenatal stressors on post-natal behavioural outcomes are mediated via effects of maternal glucocorticoids on foetal physiology (Weinstock 2008). Specifically the offspring of prenatally stressed rats show elevated anxiety and stress induced hypothalamic-pituitary axis (HPA) reactivity which is abolished by adrenalectomy of their mothers. Glucocorticoid mediated mechanisms in the prediction of infant temperament (Davis *et al*. 2007) and associations between prenatal stress and later antisocial behaviour disorders (O'connor *et al*. 2003) have also been described in humans. The *MAOA-LPR* low activity variant has been shown to be associated with increased HPA reactivity to a stressor (Bouma *et al*. 2012), and hence the combination of *MAOA-LPR* low activity status and prenatal exposure to life events could account for increased emotional reactivity found in this study mediated via altered HPA axis reactivity. Alternatively the interaction could occur at the level of *MAOA* gene expression. Mechanisms for glucocorticoid regulation of *MAOA* expression have been identified (Ou *et al*. 2006), so that the effect of life events could arise from altered *MAOA* expression resulting from effects of glucocorticoid exposure. In order for this to explain the interaction, the behavioural consequences of the gene expression must differ between the *MAOA-LPR* low activity and *MAOA-LPR* high activity individuals. Genotype dependent effects of environments on gene expression have rarely been investigated although they have been predicted using bioinformatic approaches (Shumay & Fowler 2010), and empirical support for the phenomenon in the *MAOA* gene has recently been described (Philibert *et al*. 2011).

Several studies have reported associations between prenatal stress and antisocial behaviour problems and this study adds to that work by showing that extent of the effect may be modified *in utero* by genetic variations. If the finding is replicated, and effects on antisocial behaviour disorders in childhood and adult life can be showed, this would provide a basis for the development of prenatal prevention programs targeted at mothers with *MAOA-LPR* low activity foetuses.

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