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MAOA genotype, social exclusion and aggression: an experimental test of a gene–environment interaction

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In 2002, Caspi and colleagues provided the first epidemiological evidence that genotype may moderate individuals' responses to environmental determinants. However, in a correlational study great care must be taken to ensure the proper estimation of the causal relationship. Here, a randomized experiment was performed to test the hypothesis that the *MAOA* gene promoter polymorphism (*MAOA-LPR*) interacts with environmental adversity in determining aggressive behavior using laboratory analogs of real-life conditions. A sample of 57 Caucasian male students of Catalan and Spanish origin was recruited at the University of Barcelona. Ostracism, or social exclusion, was induced as environmental adversity using the *Cyberball* software. Laboratory aggression was assessed with the Point Subtraction Aggression Paradigm (PSAP), which was used as an analog of antisocial behavior. We also measured aggressiveness by means of the reduced version of the Aggression Questionnaire. The *MAOA-LPR* polymorphism showed a significant effect on the number of aggressive responses in the PSAP ($F_{1,53} = 4.63$, $P = 0.03$, partial $\eta^2 = 0.08$), as well as social exclusion ($F_{1,53} = 8.03$, $P = 0.01$, partial $\eta^2 = 0.13$). Most notably, however, we found that the *MAOA-LPR* polymorphism interacts significantly with social exclusion in order to provoke aggressive behavior ($F_{1,53} = 4.42$, $P = 0.04$, partial $\eta^2 = 0.08$), remarkably, the low-activity allele of the *MAOA-LPR* polymorphism carriers in the ostracized group show significantly higher aggression scores than the rest. Our results support the notion that gene–environment interactions can be successfully reproduced within a laboratory using analogs and an appropriate design. We provide guidelines to test gene–environment interactions hypotheses under controlled, experimental settings.

Keywords: Aggression, antisocial behavior, experiment, *MAOA*, social exclusion

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The first epidemiological evidence that genotype may moderate individuals' responses to environmental determinants was that the *MAOA* gene moderated the impact of maltreatment on the development of antisocial behavior (Caspi *et al*. 2002).

The *MAOA* gene is located on chromosome Xp11.23-11.4, and it contains a 30-base pair motive in its promoter that is polymorphic (*MAOA-LPR*: Levy *et al*. 1989; Ozelius *et al*. 1988). Diverse alleles have been identified for this upstream variable number of tandem repeats (uVTNR) polymorphism. Specifically, in the aforementioned article, the authors showed that maltreated children carrying a low-activity allele of the *MAOA-LPR* polymorphism were more likely to develop antisocial behavior than were maltreated children carrying a high-activity allele (Caspi *et al*. 2002).

Interactions between genetic and environmental determinants have been replicated in some cases (Kim-Cohen *et al*. 2006), although not in all (Monroe & Reid 2008). Several reasons have been put forward to explain this situation (Monroe & Reid 2008). The lack of convergent results in G×E interactions in the domain of abnormal behavior may be due to a number of methodological challenges and pitfalls (for a review, see Rutter *et al*. 2006). However, in a correlational study, great care must be taken to ensure the proper estimation of causality, even when a causal time line exists (Pearl, 2009). One way of achieving this is through experimental manipulation coupled with randomization.

However, for obvious ethical reasons human beings cannot be exposed to real environmental pathogens. Caspi and Moffitt (2006) give several reasons why the use of analogs is needed in order to progress in the study of gene–environment interactions (see Table 1 and Appendix S1, Supporting Information for these adapted guidelines to experimental research). Coupling an experimental approach with psychological genomics would allow uncover causal relationships between the genotype and the phenotype, as it has been done, for instance, in the study of the *COMT* gene (Lonsdorf *et al*. 2009), the *MAOA* gene (McDermott *et al*. 2009), the *SERT* gene (Verona *et al*. 2006) or the *OXR* gene (Israel *et al*. 2009).

In the particular case of antisocial behavior, one form of environmental insult that can be easily manipulated and which individuals can be exposed to without great ethical concerns is ostracism or social exclusion (Williams 2007). Experimental induction of ostracism has been shown to increase levels of aggression (Twenge *et al*. 2001; Warburton *et al*. 2006). Numerous tasks have been developed for studying experimental aggressive behavior as an analog of antisocial behavior (Chermack & Giancola 1997; Giancola & Chermack 1998). One of the most widely used and well-validated tasks is the Point Subtraction Aggression Paradigm

Table 1: Guidelines for experimental research on G×E interactions

Moffitt et al. (2005) suggestions for general G×E research	Our criteria for experimental G×E research
Consulting quantitative behavioral-genetic studies	Evidence of G×E from epidemiological genetic research
Identifying a candidate environmental pathogen for the disorder in question	Search for analogs of environmental risk and target behavior
Optimizing environmental risk measurement	Search for independent effects of these analogs upon the dependent variable
Identifying candidate susceptibility genes	Check for plausible effect of the environmental analog on biological systems involved in the task
Testing for an interaction	Check for association of the candidate gene with similar laboratory tasks
Evaluating whether a G×E interaction extends beyond the initially hypothesized triad of gene, environmental pathogen and disorder	Controlling for all possible confounding variables (blocking, covariates, etc.)
Replication and meta-analysis	Testing for G×E interaction Replication and meta-analysis

(PSAP; Cherek 1981, 1992; Cherek *et al*. 2003). Finally, the high-activity alleles of the *MAOA-LPR* polymorphism have been related to individual cooperation in behavioral economics experiments (Mertins *et al*. 2011), while low-activity alleles have been related to reactive aggression within the laboratory (McDermott *et al*. 2009).

On the basis of these determinants, we investigated whether the *MAOA* uVTNR promoter polymorphism moderated the individuals' aggressive responses to ostracism. A 2 (ostracism: inclusion vs. exclusion) × 2 (*MAOA-LPR* genotype: low-activity allele vs. high-activity allele) between-subjects design was used. It was expected that carriers of a low-activity *MAOA-LPR* variant would respond more aggressively in the social exclusion condition than the carriers of a high-activity *MAOA-LPR* variant, regardless of the social condition.

Materials and methods

Participants and procedure

Participants were recruited from a larger pool of subjects who were screened to ensure that they were free of lifetime history of neurological or psychiatric illnesses. The final sample comprised 57 male college students (mean age = 22.77, SD = 4.37) recruited at the University of Barcelona. All participants described themselves as bilingual in Catalan and Spanish, self-reported Caucasian ethnicity and to be of Catalan or Spanish origin. We only recruited males because the *MAOA* gene is located on X chromosome, and therefore are hemizygous for this gene, allowing a straightforward genotyping. All sessions were conducted between 10 a.m. and 4 p.m. and took place in the Individual Differences Laboratory at the University of Barcelona. On arrival at the lab, participants signed the informed consent and filled in the Aggression Questionnaire-Refined (AQ-R). They were then instructed to play Cyberball, which was immediately followed by the PSAP. At the end of the experiment they were given €5 or course credits, irrespective of their score on the PSAP. They also completed a manipulation check questionnaire and were fully debriefed about the procedures and aims of the experiment. Epicentre© Catch-All™ swabs were used to gather DNA samples from inside the participants' cheek, and DNA was extracted using the manufacturer's procedure (Epicentre, Madison, WI, USA). The study was approved by the Institutional Review Board IRB00003099.

Social exclusion

Social exclusion was induced using the Cyberball software (Williams & Jarvis 2006). In the present experiment, included participants received the ball 33% of times it was tossed, while excluded participants only received the ball twice at the beginning of the game. Cyberball was programmed to last 3 min. After playing the PSAP, participants were asked to complete a manipulation check questionnaire (e.g. 'I was ignored' or 'I was excluded') about the Cyberball game that also included questions about feelings and mood. Thirty participants were assigned to the social inclusion group while 27 were socially excluded.

MAOA genotyping

The polymorphism that was genotyped is a uVTNR of the motive ACCGGCACCGGCACCAGTACCCGCACCAGT, which is 30-bp long, known as *MAOA-LPR*. Primer sequences were based on the sequence 'Embl/M89636/HSMAOAB Human monoamine-oxidase A (*MAOA*) gene', yielding the oligonucleotides MAO_-Forward 5′-ACAGCCTGACCGTGGAGAAG-3′ and MAO_Reverse 3′-GAACGGACGCTCCATTCGGA-5′. PCR conditions are described in Garpenstrand *et al*. (2001). This yields different alleles that include 2, 3, 3.5, 4 and 5 repeats for this polymorphic site that have been grouped in two groups according to their transcriptional activity, which in turn results in high or low levels of expression of MAOA (Guo *et al*. 2008; Sabol *et al*. 1998). These groups of alleles were classified into low-activity alleles (2, 3 and 5 repeats) and high-activity alleles (3.5 and 4 repeats). A total of 21 participants were carriers of the three-repeat allele (10 in the inclusion group and 11 in the exclusion group), while 36 participants were carriers of the four-repeat allele (20 in the inclusion group and 16 in the exclusion group). These allele frequencies did not differ significantly from other Catalan samples [(Gutiérrez *et al*. 2004); $\chi^2(4) = 0.86$, $P = 0.93$] or other Caucasian samples [(Caspi *et al*. 2002); $\chi^2(4) = 2.57$, $P = 0.63$].

Point Subtraction Aggression Paradigm

The dependent variable was the number of aggressive responses in the second PSAP session. The PSAP is a computer game originally designed by Cherek (Cherek *et al*. 2003) and it is a well-validated measure of aggressive behavior. Briefly, it consists of a computer game in which participants may make three different behaviors in response to a fictitious opponent, one of which is retaliatory or aggressive. In two consecutive 25 min sessions with 3 min interval between sessions, participants played the three-button version of the task. Pressing the A' button made the participants earn points exchangeable for money, pressing the 'B button stole points from the opponent's counter, and pressing the 'C' button protected participants' counters of steals from the opponent. The 'A button', which corresponds to the reinforcing response, was set to be pressed at a fixed ratio of hundred presses, while the 'B button', which is the retaliatory one, was set at a fixed ratio of 10 presses. Participants also had the chance to protect their earnings against opponent subtractions and start a provocation-free interval by pressing the 'C button' 10 times. The program was set to provoke participants at random, unless they press the 'C' button and started a provocation-free interval of 250 s. If participants stole points after this provocation, then this behavior is considered as reactive aggression.

Aggression Questionnaire-Refined
In order to control for baseline levels of aggressiveness that may confound our results, we applied the AQ-R before exposure to social inclusion or exclusion. It is a 12-item questionnaire intended to assess different aspects of aggression. It provides a general score of aggression with a good reliability score (Chronbach's $\alpha = .78$). Further details can be found elsewhere (Gallardo-Pujol *et al*. 2006).

Statistical analysis
We performed a two-way analysis of variance (ANOVA) in order to detect possible pre-manipulation differences in aggressiveness levels across groups. In order to ensure that ostracism manipulation succeeded, *t*-tests between included and excluded participants were performed on the manipulation check questionnaire items. Finally, a two-way ANOVA was then performed to identify the effects of the genetic factor (the *MAOA* gene), the environmental factor (ostracism) and their interaction on the participants' aggressive behavior. PASW version 18.0 for MacOS (SPSS Inc, Chicago, IL, USA) was used for statistical computations.

Results

The results of the analysis of pre-manipulation aggressiveness scores showed that there were no differences in initial aggressiveness that could affect responses in the PSAP between the four groups obtained by crossing the two *MAOA-LPR* genotypes and two group assignment in the social exclusion task ($F_{3,53} = 1.46$, $P = 0.24$). Table 2 shows the means and standard deviations of aggressiveness scores and PSAP responses distributed across groups (see below and Supporting Information for further information on distributional aspects of the data).

In order to ensure that the social exclusion manipulation had actually worked, we examined the differences between the two types of participants (included/excluded) on several statements of the manipulation check questionnaire. They differed on practically all statements (almost all *P*-values < 0.05), and notably, socially excluded participants felt more ignored and more excluded ($P < 0.001$) than did socially included participants showing that ostracism manipulation was successful (see Supporting Information). Ostracized participants also felt worse, unhappy, sad, unfriendly and tense than did non-ostracized subjects. We also tested whether these changes in mood could mediate the relationship between social exclusion and aggression, but we did not find evidence of this mediation.

The main analyses revealed that the genetic factor and experimental manipulation had jointly a significant effect on the observed aggressive responses ($F_{3,53} = 5.14$, $P < 0.01$, $R^2 = 0.23$). In particular, the genetic factor main effect on experimentally induced aggression was statistically significant ($F_{1,53} = 4.63$, $P = 0.04$, partial $\eta^2 = 0.08$): carriers of the low-activity *MAOA-LPR* allele (M = 555.24, SE = 106.71) showed significantly more aggressive responses than did carriers of the high-activity *MAOA-LPR* allele (M = 342.56, SE = 42.41). Social exclusion also had a significant effect on the number of aggressive responses ($F_{1,53} = 8.03$, $P < 0.01$, partial $\eta^2 = 0.13$): socially excluded subjects (M = 539.59, SE = 83.94) behaved more aggressively than did socially included participants (M = 314.10, SE = 47.71). Most importantly, however, the interaction between genotype and social condition was also significant ($F_{1,53} = 4.42$, $P = 0.04$, partial $\eta^2 = 0.08$; see Figure 1). In the social exclusion condition, carriers of the low-activity allele produced more than twice as many aggressive responses (M = 771.73, SE = 170.40) than did carriers of the high-activity allele (M = 380.10, SE = 55.97).

ANOVA relies on two assumptions: (1) normality of the residuals and (2) equality of variances of the residuals between groups. We assessed the normality of the two-way ANOVA residuals where aggressiveness (AQ-R) scores and aggression (PSAP) scores were used as dependent variables by means of the Kolmogorov–Smirnov test with a Lilliefors correction. We found that both the AQ-R aggressiveness residuals ($P = 0.20$) and PSAP aggression residuals ($P = 0.09$) do not statistically differ from a normal distribution. We assessed the equality of the residual variances across groups using Levene's test. The assumption

Table 2: Descriptive statistics for aggressiveness scores and PSAP responses

	Aggressiveness scores		PSAP responses	
Group	Mean	SD	Mean	SD
Socially included, low-activity *MAOA* ($n = 20$)	27.90	7.45	317.10	237.21
Socially included, high-activity *MAOA* ($n = 16$)	23.05	7.25	312.60	278.56
Socially excluded, low-activity *MAOA* ($n = 10$)	25.82	3.71	771.73	565.15
Socially excluded, high-activity *MAOA* ($n = 11$)	24.20	5.60	380.00	223.86

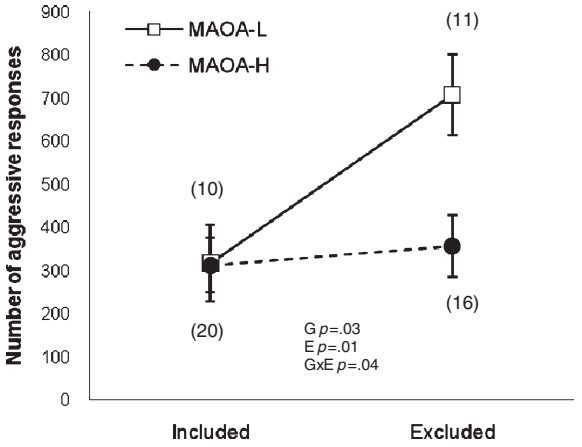

Figure 1: Number of aggressive responses in the PSAP as a function of social exclusion and MAOA genotype. Note: Group sample size in brackets. G, *MAOA* genotype: E, Social exclusion; G × E, Gene–environment interaction.

of equal variances could not be rejected for aggressiveness, $F_{3,52} = 0.74$, $P = 0.54$, but is rejected for PSAP aggression, $F_{3,53} = 4.60$, $P = 0.01$. Therefore, the conclusions drawn from the ANOVA on PSAP aggression are suspect, but not for AQ-R aggressiveness. The robustness of the ANOVA results for PSAP aggression was investigated by performing again the ANOVA analysis this time with confidence intervals obtained using non-parametric bootstrapping with 1000 replicates. In the original ANOVA analysis reported above (under normality assumptions) the estimate of the interaction effect is −0.387 with 95% confidence interval (−0.787; −0.018). Because the interval does not include 0 we concluded that there is a statistically significant interaction effect at the 5% level. The bias corrected bootstrapped confidence interval (Efron & Tibshirani 1993) is (−0.778; −0.022). This interval does not include 0 either and we conclude that the results reported are robust to violations of the assumptions.

As a further check on the robustness of the results, we assessed whether any observation could have distorted the results obtained using Cook's distance. The largest distance observed in this sample is 0.352. Its percentile using a $F_{57,4}$ distribution is 3%. Should its percentile be 50% or above, it had been considered as influential. Any value whose percentile is <10% is considered not influential (Kutner *et al*. 2005). Therefore, we conclude that there are no influential cases in this sample and that the results reported in the body of the article are robust.

Discussion

Our results support the notion that gene–environment interactions can be successfully reproduced within a laboratory using analogs and an appropriate design. Our findings show that increases in aggressive behavior occur after social exclusion and are moderated by the *MAOA-LPR* polymorphism. According to our hypothesis, carriers of the low-activity allele of the *MAOA-LPR* polymorphism responded more aggressively following social exclusion than carriers of the high-activity allele. No differences between carriers of both alleles were found in the inclusion group. Remarkably, these effects were independent of baseline aggressiveness levels and are attributable to experimental manipulation.

These findings experimentally confirm findings from the epidemiologic literature (Beitchman *et al*. 2004; Caspi *et al*. 2002; Ducci *et al*. 2008; Frazzetto *et al*. 2007; Haberstick *et al*. 2005; Widom & Brzustowicz 2006) that have been confirmed via meta-analysis (Kim-Cohen *et al*. 2006; Taylor & Kim-Cohen 2007). Given the evidence, it is unlikely that previous literature on gene–environment interactions and antisocial behavior and other aggression-related behavior was spurious, but experimental replication was needed (Caspi & Moffitt 2006), according to the correlational nature of the designs employed. So far, no study had attempted to directly test this hypothesis from experimental neuroscience following an *ad hoc* procedure, including analogs of environmental adversities and also analogs of target behaviors. Hence, this study sought to parallel the conditions of observational studies in a controlled laboratory setting.

The contribution of this study is threefold. Firstly, it replicates findings from psychiatric epidemiology experimentally, offering a plausible link between exposure to acute social stressors and accumulation of these stressors that has already been found in longitudinal studies from the point of view of experimental neuroscience. Precisely, evidences for gene–environment interactions have to rely on four basic pillars: (1) observational studies in humans, (2) experimental neurosciences studies, (3) studies in non-human primates and (4) experimental studies in rodents (Caspi *et al*. 2010).

Secondly, it offers a methodological framework for developing deeper knowledge about the mechanisms by which genetic and environmental factors affect actual behavior in specific situations. Hence, we suggest that experiments like this can reveal both potential genetic and environmental contributions to daily behavior. Although a number of advances have been made in this regard (Lonsdorf *et al*. 2009; McDermott *et al*. 2009; Verona *et al*. 2006), this study offers a comprehensive framework in which to develop new experiments to test epidemiological findings and further advance our knowledge of gene–environment interactions. McDermott *et al*. (2009) also explored how carriers of the low-activity *MAOA-LPR* allele responded after a provocation in a behavioral economics task, but they did not explore the effects of social exclusion on antisocial behavior. However, our results are consistent with theirs in the line that only carriers of the low-activity allele respond more aggressively when environmental conditions are not favorable.

Thirdly, this study contributes into the field of social psychology providing evidence that there are genetic mechanisms that moderate the individual's responses to social phenomena, such as social exclusion. Some researchers suggested that social exclusion might lead to self-regulatory deficits and thus increase an increase in aggressive behavior, emotional dysfunction and cognitive overload (Twenge *et al*. 2002). However, there has been only one attempt to understand the possible links between genes and responses to social exclusion (Eisenberger *et al*. 2007). In fact, the *MAOA* gene influences the magnitude of brain response to social exclusion. Not without some controversies (Gallardo-Pujol *et al*. 2008), this was the first evidence of genes moderating responses in a field that traditionally moved further away of genetics and neuroscience (Williams 2007).

The question regarding the mechanisms that link *MAOA-LPR* polymorphism and aggression arises from these findings. A deficiency in MAOA activity as seen in low-activity allele carriers may lead toward increased reactivity of certain brain areas to threatening stimuli (Caspi *et al*. 2002). Actually, it has been confirmed that the low-activity *MAOA-LPR* allele is associated to a hyperreactive amyglada and hypo-reactive prefrontal regulatory functions (Meyer-Lindenberg *et al*. 2006), which in turn results into an even more hyperreactive amygdala. Although it has been found that *MAOA* genotype does not correspond to brain MAOA activity (Fowler *et al*. 2007), the same authors found that brain MAOA activity correlates inversely with trait aggression (Alia-Klein *et al*. 2008). Conversely, *MAOA-LPR* genotype negatively correlates with self-reported trait aggression (Eisenberger *et al*. 2007). The reasons of this discrepancy may be diverse and may include differences in sampling methods,

questionnaires and others that go beyond the scope of this article. Moreover, *MAOA-LPR* genotype inversely correlates with self-reported interpersonal hypersensitivity after social exclusion and also with activation in dACC areas during social exclusion, being the low-activity allele carriers the more hyperresponsive (Eisenberger *et al*. 2007). Notably, these areas (and others) were associated to *MAOA* genotype in an emotional threatening task (Meyer-Lindenberg *et al*. 2006). Hence, it may be plausible that social exclusion may elicit threats that in turn arouse hyperresponsivity in carriers of the low-activity *MAOA-LPR* allele.

However, the mechanisms by means of which environmental adversity might contribute to the hyperreactive brain remain unclear. Recent developments from the field of epigenetics may offer some clues on that and explain how maltreatment or other environmental adversities contribute to a hyperreactive brain. The neuron-specific glucocorticoid receptor gene (NR3C1) appears to be hypermethylated, and therefore less expressed, in victims of childhood abuse (McGowan *et al*. 2009). Deficits in the regulation of the Hypothalamic-pituitary-adrenal axis (HPA) may contribute to dampened response to stress and environmental insults. Recent research on social exclusion has shown that transient increases of cortisol and testosterone predict aggression after ostracism (Geniole *et al*. 2011) and it may well be the cumulative effect of repetitive exposure to environmental insults which leads the organism to respond aggressively to new stimuli.

Like any other study, this research has certain limitations, including: (1) a small sample size; (2) the use of college students as participants, which may have led to range restrictions and (3) the use of analogs, which may lack apparent external validity. However, these limitations would have hampered, rather than enhanced, our ability to find statistically significant results. Nor is there any evidence that our sample differed from the general population on any of the phenotypic attributes measured (Wiecko 2010). At the genotypic level, some sort of population stratification could affect our results (Bauchet *et al*. 2007). However, our sample was quite homogenous in terms of language and self-reported ethnic origin, so we would not expect a major variation in stratification dimensions reported by Bauchet *et al*. (2007). At all events, these limitations should not distract from the fact that we have experimentally replicated a gene–environment interaction in human beings using an *ad hoc* procedure.

All in all, in this study, we combined the work drawn from the genetics epidemiology literature regarding gene–environment interactions, with the work drawn from molecular psychiatry and also with the work drawn from the field of abnormal behavior. Our experiment also tapped into the growing neuroscience literature, integrating basic social psychology research with molecular research.

In conclusion, we have experimentally replicated the interaction between *MAOA* genotype and social adversity and its relationship with antisocial behavior, and we propose this framework as a primer for future replications and hope it will stimulate many more experimental investigations of G×E interactions in the future and comprehend the underpinnings behind the gene–environment interactions phenomena.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Adapted guidelines for experimental research on G×E interactions.

Figure S1: Histogram of the AQ-R scores for the whole sample.

Figure S2: Histogram of the AQ-R scores across subgroups.

Figure S3: Histogram of the PSAP scores for the whole sample.

Figure S4: Histograms of the PSAP scores across subgroups.

Table S1: Differences between ostracized and included participants in the manipulation check questionnaire.