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Direct and Indirect Relations Between Reactive and Proactive Aggression, Facial Emotion Recognition, and Polymorphisms in the Monoamine Oxidase A and Serotonin Transporter Genes

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B.A., Duke University, 2010

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Abstract

Direct and Indirect Relations Between Reactive and Proactive Aggression, Facial Emotion Recognition, and Polymorphisms in the Monoamine Oxidase A and Serotonin Transporter Genes

By Devon LoParo

Research has demonstrated that individuals high in antisocial traits tend to have difficulty recognizing fearful and sad facial expressions, though researchers have not attempted to link these deficits to specific forms of aggression, such as reactive and proactive aggression. Two genetic markers frequently studied in association with aggression, a repeat sequence in the promoter region of the monoamine oxidase A gene (*MAOA-uVNTR)* and a polymorphism in the promoter region of the serotonin transporter gene (*5-HTTLPR*). These genes are active in brain regions involved in aggression and facial emotion recognition, such as the amygdala and regulatory prefrontal regions, suggesting that facial emotion recognition deficits or biases may serve as endophenotypes for aggression. In a sample of 180 twins genotyped for the *MAOA-uVNTR* and *5-HTTLPR*, we found that the *MAOA-uVNTR* low-activity allele was associated with a lower proportion of correct fear recognitions, more fear commission errors, and more sad commission errors on a facial emotion recognition task, while the *5-HTTLPR* short allele was associated with a higher proportion of correct sad recognitions and more sad commissions. We also found that fewer correct fear recognitions, more fear commissions, and the *MAOA-uVNTR* risk allele were associated with reactive aggression, while more fear commissions was also associated with proactive aggression. In addition, we found that the proportion of correct fear recognitions, and fear and sad commissions separately mediated the relation between the *MAOA-uVNTR* and reactive aggression. These results suggest that impaired fear recognition is related to both reactive and proactive aggression. Further, the influence of *MAOA-uVNTR* on reactive aggression seems to act in part through impaired fear recognition, indicating that facial emotion recognition may be a useful endophenotype for reactive aggression.

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Direct and Indirect Relations Between Reactive and Proactive Aggression, Facial Emotion Recognition, and Polymorphisms in the Monoamine Oxidase A and Serotonin Transporter Genes

The study of specific genes and psychological traits has begun to transcend simply looking for associations between candidate genes and disorders or personality traits to searching for endophenotypes. Endophenotypes are constructs that are believed to underlie disorders or traits and to be more directly influenced by the genes that have been associated with the disorder or trait than the manifest symptoms (Waldman, 2005). Endophenotype research to this point has focused on a relatively small number of disorders, such as bipolar disorder and schizophrenia (Flint & Munafó, 2007). Though identifying phenotypes that are more closely related to biological processes involved in these disorders is important, the endophenotype model could also help to explain etiological processes in other heterogeneous forms of behavior.

In this study, we are interested in investigating putative endophenotypes for aggression. Aggression is a complex social behavior that is an important research target from several perspectives. It is interesting as both an aspect of normal human interaction and a component of several psychological disorders, such as conduct disorder, antisocial personality disorder, and borderline personality disorder. One line of research has associated aggression with deficits in social cognitive tasks, such as facial emotion recognition (Marsh & Blair, 2008). Researchers have investigated the neurobiological etiology of aggression and facial emotion recognition separately, and found that similar neural regions, such as the amygdala and ventromedial prefrontal cortex (Adolphs, 2006; Nelson & Trainor, 2007), are involved in both traits. Further, genetic markers associated

with aggression, such as a repeat sequence in the promoter region of the monoamine oxidase A gene (*MAOA-uVNTR)* and a polymorphism in the promoter region of the serotonin transporter gene (*5-HTTLPR*), have been shown to affect amygdala and prefrontal cortical reactivity to negative faces (Meyer-Lindenberg et al., 2006; Hariri & Holmes, 2006). Nonetheless, no studies have investigated specific genetic markers in association with deficits or biases in facial emotion recognition. In the current study, we investigated whether these genetic markers were associated with deficits or biases in the recognition of specific facial emotions, as well as whether any deficits or biases we identified served as mediators of the relation between these markers and aggression. Such results would be a first step in identifying facial emotion recognition deficits or biases as endophenotypes for aggression.

Etiology of Facial Emotion Recognition

The recognition of facial displays of emotion is an important aspect of social interaction, as facial emotion plays a crucial role in modulating interpersonal behavior (Corden, 2006). Researchers have found that facial emotion is processed in the brain across a network of structures that includes the occipitotemporal cortex, the anterior cingulate cortex, the amygdala, and the ventromedial prefrontal cortex (Adolphs, 2006). The occipitotemporal cortex, specifically the temporal gyrus and fusiform gyrus, is responsible for processing the geometric configuration of features of the face (Allison, Puce, Spencer, & McCarthy, 1999). After these features have been processed, structures in the temporal lobe link the configuration of facial expressions with stored knowledge about what those features mean (Haxby, Hoffman, & Gobbini, 2002). There is also evidence that beyond this general network, specific facial emotions are processed through

partially distinct neurological regions.

In particular, as evidenced by work involving individuals with bilateral amygdala damage as well as neuroimaging research, the amygdala seems to play a disproportionate role in detection of fearful expressions (Adolphs et al., 1999; Murphy, Nimmo-Smith, & Lawrence, 2003). These findings have led researchers to hypothesize that genetic polymorphisms thought to be involved in stress response may moderate amygdala response to fearful expressions. As mentioned above, studies have shown that individuals with the risk alleles of the *MAOA-uVNTR* and the *5-HTTLPR* tend to display increased amygdala response and decreased response of regulatory prefrontal regions such as the anterior cingulate and occipitofrontal cortices to fearful and angry expressions (Meyer-Lindenberg et al., 2006; Hariri & Holmes, 2006). These activation patterns suggest that these genetic markers may in part be responsible for deficits and biases in facial emotion recognition. Such deficits and biases have been related to several forms of maladaptive behavior and disorders.

Correlates of Deficits and Biases in Facial Emotion Recognition

Given the role that facial emotion recognition plays in modulating interpersonal behavior, researchers have investigated whether deficits in facial emotion recognition are associated with psychiatric disorders characterized by interpersonal deficits. Generalized impairments across facial emotions have been found in disorders such as autism, attention-deficit hyperactivity disorder, schizophrenia, and social anxiety, as well as in antisocial populations (Easter et al., 2005; Gross, 2004; Marsh & Blair, 2008; Singh et al., 1998; Tremeau, 2006). Deficits in facial emotion recognition have also been associated with factors often correlated with psychopathology such as general

intelligence, age, attention, verbal ability, and task-specific motivation (Herba & Phillips, 2004), making it difficult to determine the unique relations between general deficits in facial emotion and psychopathology. Nonetheless, an association of deficits or biases in recognition of specific facial emotions with a disorder or behavior is more informative. For example, there is evidence that individuals with depression, general anxiety, and borderline personality disorder have enhanced sensitivity to negative expressions as indicated by greater accuracy in the recognition of and quicker reaction times to negative faces (Bhagwagar, Cowen, Goodwin, & Harmer, 2004; Masurier, Cowen, & Harmer, 2007; Wagner & Linehan, 1999). Specific deficits have also been identified in association with antisocial behavior.

A recent meta-analysis (Marsh & Blair, 2008) determined that in antisocial populations there are specific deficits in recognizing fearful facial expressions. In particular, the meta-analysis demonstrated that antisocial populations were less accurate in identifying fearful, sad, and surprised expressions, and the deficits in fear recognition were significantly greater than deficits in recognizing any other emotion. Further, Marsh and Blair (2008) demonstrated that these differences were not a result of task difficulty and that there were no reliable differences in patterns of deficits between psychopathic and non-psychopathic antisocial populations. Fearful expressions are thought to be distress cues that elicit empathy and inhibit aggression (Marsh et al., 2005) which act as social reinforcers that punish developing children for engaging in behaviors that elicit these expressions (Blair, 2005). Individuals who do not recognize fear correctly may not realize their behavior is distressing or may not be adequately punished for their behavior, causing them to exhibit aggressive behavior more often or more persistently than children

who accurately perceive such distress cues. Facial emotion recognition deficits and biases seem not only to be associated phenotypically with aggression, but also appear to share etiological pathways.

Etiology of Aggression

Researchers have attempted to determine the etiology of aggression, both neurobiologically and genetically. Research on the neurobiological basis of aggression has focused on the neurotransmitter serotonin, as serotonergic functioning is consistently associated with aggression in humans and non-human animals (Nelson & Trainor, 2007). Researchers have found this association when examining neural regions upon which serotonergic neurons synapse, levels of the neurotransmitter itself, and genetic polymorphisms that code for proteins involved in serotonergic functioning (Nelson & Trainor, 2007). Serotonin is active in neural regions associated with aggression, such as the amygdala and hypothalamus, and is inhibited by projections from the prefrontal cortex (Carver et al., 2008; Yang & Raine, 2009). Aggression has also been associated with structural and functional variation within the amygdala, hypothalamus, and periaqueductal gray matter (Blair, 2010; Gregg & Seigel, 2001), and it is hypothesized that these structures may work together as a unified threat-response system in which the PFC inhibits circuits in the hypothalamus and amygdala that promote aggression (Nelson & Trainor, 2007).

Given that roughly 50% of the variance in aggression is due to genetic influences (Rhee & Waldman, 2002), researchers have attempted to identify candidate genes that may be risk factors for aggression. Given the wealth of evidence for significant associations between serotonin and aggression, extant research on the genetic origins of

aggression has concentrated on genes involved in serotonergic functioning (Ficks & Waldman, manuscript in preparation). Two of the most frequently tested genetic markers for association with aggression in humans are the *MAOA-uVNTR* and the *5-HTTLPR* due to findings in human and animal research that their respective genes are involved in aggression (Brunner et al., 1993; Lesch et al., 1996). These two genetic markers are both located in the promoter regions of their respective genes, have been found to regulate levels of expression of their genes (Sabol et al., 1996; Cadoret et al., 2003), are located in genes that are involved in serotonin metabolism in the brain, and have been found to moderate neural responses to emotional stimuli in the amygdala (Meyer-Lindenberg et al., 2006; Hariri et al., 2002) and the PFC (Buckholtz et al., 2007; Heinz et al., 2005). Though associations of these genetic markers with heterogeneous, multidimensional measures of antisocial behavior have been somewhat equivocal to date (Ficks & Waldman, manuscript in preparation), there is emerging evidence that the markers may be more closely associated with specific unidimensional facets of aggression, such as reactive and proactive aggression (LoParo, Ficks, Latzman, & Waldman, manuscript in preparation). Examining common and unique correlates and etiology of reactive and proactive aggression may provide a more consistent and comprehensive understanding of aggressive behavior.

Differentiating Reactive and Proactive Aggression

Reactive aggression is defined as an angry response triggered by negative emotional experiences or perceived threats to the self, whereas proactive aggression is defined as premeditated aggressive behavior that is instrumental in nature (Eisenberger et al., 2007; Craig & Halton, 2009). In general, this distinction has been supported in the

extant literature, as each has shown unique patterns of association with psychological constructs such as disagreeableness, self-control (Latzman, Vaidya, Clark, & Watson, 2011) and social cognitive biases (Crick & Dodge, 1996).There is also emerging evidence across both neurological and genetic research domains for the discriminant validity of these aggressive subtypes. Neurologically, although regulatory regions within the prefrontal cortex, particularly within the orbitofrontal cortex, which is involved in decision-making and emotional processing (Bechara, Damasio, & Damasio, 2000) are thought to play a role in both forms of aggression (Bechara, et al., 2000), regions of the hypothalamus and periaqueductal gray matter have been hypothesized to contribute primarily to reactive aggression (Blair, 2010; Gregg & Siegel, 2001). Further, reactive aggression has been associated with *heightened* amygdala reactivity to emotional stimuli whereas proactive aggression has been associated with *reduced* amygdala reactivity (Blair, 2010). Though reactive and proactive aggression appear to be partially neurologically distinct, more work needs to be done to determine whether these constructs are indeed driven by unique patterns of neurological activity.

Researchers have also used behavior genetics methods to attempt to etiologically distinguish reactive from proactive aggression. In a twin sample, Brendgen et al. (2006) found 39% and 41% of the variance in reactive and proactive aggression, respectively, was due to genetic influences. Though the two forms of aggression shared most of their genetic variance, each form also had unique genetic influences. At the level of specific genetic markers, most researchers have not differentiated or compared associations with reactive and proactive aggression. Nonetheless, a recent study in a clinical sample found that the *MAOA-uVNTR* seems to be associated with reactive but not proactive aggression

(LoParo et al., manuscript in preparation), while the *5-HTTLPR* was not found to be associated with either facet of aggression.

The *MAOA-uVNTR*

MAOA is a mitochondrial enzyme which catalyzes the degradation of the neurotransmitters serotonin, norepinephrine, and dopamine in the brain. Specifically, it resides in the mitochondrial outer membrane and degrades the aforementioned neurotransmitters, leading to their decreased availability. The gene that codes for MAOA is located on the short arm of the X-Chromosome. The *MAOA* promoter region contains a 30 base pair repeat sequence polymorphism (i.e., a VNTR), meaning that individual differences exist in the number of copies (i.e., 2, 3, 3.5, 4, or 5) of this repeat sequence. The 2, 3, and 5 copy variants of the polymorphism transcribe MAOA between 2 and 10 times less efficiently than the 3.5 or 4 copy variants (Sabol et al., 1998). Because of this difference in efficiency, the 2, 3, and 5 copy variants lead to lower MAOA activity (and consequently, higher levels of synaptic serotonin) in the brain than the other variants and have been hypothesized to increase risk for aggression (Nelson & Trainor, 2007).

The *5-HTTLPR*

Another genetic polymorphism that has been examined in relation to aggression is the *5-HTTLPR*, a region of the serotonin transporter gene that contains a 44 base pair insertion/deletion resulting in "long" and "short" variants (Heils et al., 1996). The homozygous short (S/S) genotype has been found to result in decreased serotonin transporter transcriptional efficiency, which in turn decreases both the expression of the serotonin transporter (5-HTT) and serotonin reuptake (resulting in higher levels of synaptic serotonin), in comparison with heterozygous (S/L) and homozygous long (L/L)

genotypes (Cadoret et al., 2003; Heils, et al., 1996).

Current Study

In the current study, we attempted to determine direct and indirect relations between reactive and proactive aggression, facial emotion recognition deficits and biases, and the *MAOA-uVNTR* and *5-HTTLPR*. First, we tested whether the *MAOA-uVNTR* and the *5-HTTLPR* were associated with reactive or proactive aggression. Based on findings in a larger clinically-referred sample (LoParo et al., manuscript in preparation), we predicted that the *MAOA-uVNTR* would be associated with reactive but not proactive aggression, whereas the *5-HTTLPR* would be associated with neither reactive nor proactive aggression.

Second, we examined whether these two genetic markers were associated with facial emotion recognition deficits or biases for angry, fearful, happy, or sad faces on a continuous performance task (CPT) in which participants were asked to view a series of faces and press a button when they saw two examples of a target emotion in a row. Given the literature supporting these genetic markers' effects on amygdala reactivity to negative faces (Meyer-Lindenberg et al., 2006; Hariri & Holmes, 2006), as well as findings linking both markers to aggression in general (Ficks & Waldman, manuscript in preparation), we predicted that both markers would be related to specific deficits or biases in fear, angry, and sad facial emotion recognition.

Third, we attempted to further differentiate between reactive and proactive aggression by determining whether deficits and biases in facial emotion recognition that have been associated with antisocial behavior in general show differential patterns of association with these two specific facets of aggression. Given evidence linking reactive

aggression to increased amygdala reactivity and proactive aggression to decreased amygdala reactivity to negative stimuli (Blair, 2010), we predicted that reactive aggression would be associated with biases toward overidentification of fearful, angry, and sad faces, while proactive aggression would be associated with deficits in fearful, angry, and sad face recognition.

Finally, we tested whether facial emotion recognition deficits or biases mediated the relation between the genetic markers and reactive and proactive aggression. Based on findings that the *MAOA-uVNTR* and the *5-HTTLPR*, fear recognition, and reactive aggression related to amygdala and prefrontal activation (Meyer-Lindenberg et al., 2006; Hariri & Holmes, 2006; Murphy et al., 2003; Blair, 2010), we predicted that deficits and biases in fearful face recognition would mediate the relation between the *MAOA-uVNTR* and reactive aggression.

Method

Participants

Parental ratings of aggressive behavior, facial emotion perceptual deficits and biases, and DNA were collected from a sample of 90 twin pairs drawn from the Georgia Twin Registry, a sample of twins from the general population of Georgia born between 1980 and 1991 and recruited through birth records. The twin families in the current laboratory study had previously participated in two questionnaire studies of child psychopathology and personality. Parents of the twins were recruited by telephone to participate in the current study, and the twin families were assessed on a variety of measures in our laboratory at Emory University for a 3-hour period. Participating twins' parents completed questionnaires assessing the family's demographic characteristics as well as symptoms of common DSM-IV childhood psychiatric disorders and related traits.

Children ranged in age from 6 to 18 years (mean = 13.7, SD = 2.4 years), and 39.3% were male. The ethnic background of the sample was 96.1% Caucasian, 2.6% African American, and 1.3% Asian ancestry. There were 137 participants for which facial emotion data was complete, 133 participants for which facial emotion and aggression data were complete, and 88 participants for which genotypic data was complete. Demographic characteristics of these reduced samples were similar to the full sample.

Because *MAOA* is a gene on the X-chromosome, there are challenges when comparing its effects between sexes. Although males have only one copy of the gene, females have two copies (as they have two X-chromosomes), and thus it is unclear how the female expression of this gene compares to male expression. Expression is particularly unclear for heterozygous females because of X-inactivation, a process through which one X-chromosome is silenced, equalizing expression between males and females (Van den Veyver, 2001). Some genes escape inactivation, leading to gene expression that is potentially incomparable to male expression (Carrel & Willard, 2005), and there is conflicting evidence whether *MAOA* escapes inactivation (Benjamin et al., 2000; Carrel & Willard, 2005). Thus, heterozygous females may have either intermediate levels of expression or levels closer to the high-expressing or low-expressing male phenotype. Though some previous researchers have excluded heterozygous females (Widom & Brzustowicz, 2006) to avoid this issue, we included heterozygous females and grouped them separately from homozygous/hemizygous low and high activity allele groups.

Genotyping

Buccal cells were collected from subjects via a 30-mL solution of 4% sucrose

held in their mouths for 1 minute. The washes were immediately refrigerated and transported to the laboratory. The buccal cells were pelleted for 10 min at 2000 *g*. Deoxyribonucleic acid (DNA) extraction was performed with a QIAmp Tissue kit (Qiagen, Valencia, California) according to the protocol developed by the manufacturer. Samples were then preserved in TE (10 mmol/L Tris Hcl, 1 mmol/L ethylenediaminetetraacetic acid [EDTA]).

The preserved samples were sent to two laboratories for polymerase chain reactions (PCR) amplification of the *5-HTTLPR* markers: 1) the University of Arizona's Laboratory of Molecular and Systematic Evolution in Tucson, AZ and 2) the Psychiatric and Neurodevelopmental Genetics Unit (PNGU) in the Center for Human Genetic Research at Massachusetts General Hospital (MGH) in Boston, MA. The *5-HTTLPR* polymorphism was genotyped by PCR at MGH, according to the following protocol. Genomic DNA (5 ng) was amplified in a 7-µl reaction using the marker specific primers (0.2 µM), KlenTaq DNA Polymerase (0.2 U), the proprietary KlenTaq Buffer (1X), dNTPs (200 µM each), glycerol (5%), and Betaine (1 M). The *5-HTTLPR* primers were ordered from Integrated DNA Technologies (IDT). Amplification was performed with the following protocol: 13 cycles of denaturation for 30 seconds at 93ºC, annealing for 30 seconds (beginning at 61.5ºC) and dropped 0.5º C every cycle, and primer extension at 72ºC for 30 seconds. This was followed by 37 cycles of denaturation for 30 seconds at 93ºC, annealing for 30 seconds at 55º C, primer extension at 72ºC for 30 seconds, and final extension at 72ºC for 1 hour. Amplified products were pooled and combined with size standard (LIZ-250) before being analyzed on an ABI-3730 (Applied Biosystems). GeneMapper v3.5 software (Applied Biosystems) was used to analyze the raw results

from the ABI-3730. A genotype was not considered final until two PNGU personnel had independently checked (and if necessary, corrected) the GeneMapper results and both individuals were in agreement. After the genotyping procedures were completed at the University of Arizona and MGH, our lab received Microsoft Excel spreadsheets containing the final called genotypes for all samples.

Genotyping of the *MAOA-uVNTR* was performed using the following protocol. Genomic DNA (5 ng) was amplified in a of 7 µl reaction using the marker specific primers (0.2 µM), KlenTaq DNA Polymerase (0.2 U), the proprietary KlenTaq Buffer (1X), dNTPs (200 µM each), glycerol (10%). The *MAOA-uVNTR* primers were ordered from Applied BioSystems and were as follows: MAOA_PR02-F NED ACAGCCTGACCGTGGAGAAG, MAOA_PR02-R GAACGGACGCTCCATTCGGA. The MAOA_PR02-R primer also contains a proprietary tail that helps stabilize the amplified product. Amplification was performed with the following protocol: thirteen cycles of denaturation for 30 seconds at 93ºC, annealing for 30 seconds beginning at 69.5ºC and dropped 0.5º C every cycle and primer extension at 72ºC for 30 second; 37 cycles of denaturation for 30 seconds at 93ºC, annealing for 30 seconds at 63ºC and primer extension at 72ºC for 30; 72ºC for 1 hour.

Parent Report of Reactive/Proactive Aggression

Parents reported on their children's reactive and proactive aggression using a scale described by Dodge and Coie (1987) that was derived through a principle-components analysis of a larger pool of 12 items describing various childhood aggressive behaviors. Reponses to each of these scale items indicate how often these behaviors occur and may range from 1 ("never") to 5 ("almost always"). Items were selected for each

scale based on the factor loadings for teacher-reports obtained in each of two independent samples of children (Dodge & Coie, 1987). For the first factor (reactive aggression) the 3 most strongly associated items ("when teased, strikes back," "blames others in fights," and "overreacts angrily to accidents") yielded factor loadings that ranged from 0.70 – 0.86, and for factor two (proactive aggression) the 3 most strongly associated items ("uses physical force to dominate," "gets others to gang up on a peer," and "threatens and bullies others") yielded factor loadings that ranged from 0.64 – 0.84 (Dodge & Coie, 1987). Items selected for the reactive aggression scale loaded less strongly on the proactive aggression scale (0.31 – 0.45), and the opposite was also true (0.33 – 0.61) (Dodge & Coie, 1987). The remaining 6 "unclassified" items did not show consistent between-factor discrimination (Dodge & Coie, 1987) and were not included in the current study. Despite the small number of items per scale, Cronbach's alphas for reactive and proactive aggression scales in the current sample were high ($\alpha = 0.81$ and $\alpha = 0.78$, respectively).

Facial Emotion Recognition Task

The set of stimuli consisted of grayscale images of adults displaying four different facial expressions (angry, fearful, happy, and sad). There were two intensity states for each emotion stimulus, such that each emotion stimulus had a low and high intensity version. The task required participants to complete 8 blocks of 20 trials. Each block was defined by a different target emotion, with two blocks for each emotion. The order of the blocks was randomized. Before each block, the participant was presented with a target emotion and instructed to press a button as fast as they could when they saw the target emotion displayed twice in a row. Facial emotion stimuli then were presented singly in

the center of the screen. Each face was presented for 1 second, with a 1.5 second interstimulus interval. Participants were free to press the button at any time, and button presses during the interstimulus period were considered to be responses to the most recently presented stimulus. The trials within each block were randomized such that 20 of the 40 trials across the two blocks for each target emotion were target stimuli and the rest were other emotion stimuli at random. This randomization led to between 9 and 11 opportunities for a correct response for each emotion for each participant. Data collected included the number of correct responses, errors of omission, errors of commission, and the current and previously presented emotion when a commission error occurred.

In this study, we used the proportion of correct responses in each emotion condition (the number of correct responses divided by the sum of correct responses and number of omissions) to characterize responses to target emotions, and the total number of commission errors for each target emotion to characterize responses to non-target stimuli.

Data Analytic Methods

Generalized linear mixed models (Cohen, Cohen, West, & Aiken, 2003) were used for the primary association analyses conducted. We modeled the proportion of correct responses using a normal distribution, as Kolmogorov-Smirnov tests demonstrated that the distributions of these variables did not differ significantly from a normal distribution (see Figure 1). We modeled the commission errors (Figure 2) and aggression phenotypes (Figure 3) using a negative binomial distribution with a log link function to accommodate overdispersion (i.e., the variance being greater than the mean). Mixed models employing generalized estimating equations (Self & Liang, 1987) also

were required for these analyses, given that the sample had a nested data structure due to each participating family including multiple siblings. In these analyses, we first entered into the model and evaluated for significance a set of covariates that included children's sex, age, age^2, sex X age, and sex X age^2. Following the entry of these covariates into the model and the evaluation of their statistical significance, children's *MAOA-uVNTR* and *5-HTTLPR* genotypes were entered and treated as factors with three levels, corresponding to the three genotypes at each marker. Based on previous findings of the associations of aggression and antisocial behavior with these two genetic markers (Ficks & Waldman, manuscript in preparation), we considered the low-activity allele of the *MAOA-uVNTR* and the short allele of the *5-HTTLPR* the high-risk alleles. As our focal tests of genotype differences, we specified a set of 1-df *a priori* contrasts to test for higher levels of aggression in carriers of the low-activity *MAOA-uVNTR* variant and in carriers of the short allele of the *5-HTTLPR*. The generalized linear modeling analyses yield a Wald's χ^2 statistic that was used in hypothesis testing and converted into the effect size index R^2 (i.e., proportion of variance accounted for) using the formula χ^2 / N, where N = the number of children included in the analysis.

We first tested the effects of the *MAOA-uVNTR* and *5-HTTLPR* on reactive and proactive aggression, as well as on the proportion of correct responses and number of commission errors for each emotion type. We next tested for associations between the facial emotion variables and reactive and proactive aggression. After determining these relations, we carried forward the facial emotion variables that were significantly related to both the genetic markers and the aggression phenotypes into mediational analyses. We then tested whether these facial emotion variables mediated the associations between the

genetic markers and the aggression phenotypes by entering the facial emotion variable into the model used to test the effect of the genetic markers on the aggression phenotypes prior to the genetic marker and reevaluated whether the strength of the genetic association with aggression was weakened or rendered nonsignificant.

Results:

Association of Reactive and Proactive Aggression with the *MAOA-uVNTR* and the *5-HTTLPR*

Results for the analyses of association of reactive and proactive aggression with the genetic markers are shown in Table 1. Children with one or two copies of the low-activity *MAOA-uVNTR* variant showed higher levels of reactive aggression than children homozygous for the high-activity allele (Wald's $\chi^2 = 4.17$, $p = .041$, $R^2 = 4\%$), whereas no differences in reactive aggression were found between children with one or two copies of the low-activity allele (Wald's $\chi^2 = 0.12$, $p = .728$, $R^2 = 0\%$). No significant differences in levels of reactive aggression were found between children with one or two copies of the *5-HTTLPR* short allele as compared with children homozygous for the long allele (Wald's $\chi^2 = 0.35$, $p = .561$, $R^2 = 0\%$), nor were there significant differences between children with one or two copies of the short allele (Wald's $\chi^2 = 3.41$, $p = .065$, $R^2 = 3\%$), though children with two copies tended to have higher levels of reactive aggression than those with only one copy.

Children with one or two copies of the low-activity *MAOA-uVNTR* variant did not differ in their levels of proactive aggression relative to children homozygous for the high-activity allele (Wald's $\chi^2 = 3.08$, $p = .079$, $R^2 = 3\%$), nor were there differences in proactive aggression between children with one or two copies of the low-activity allele

(Wald's $\chi^2 = 2.69$, $p = .101$, $R^2 = 2\%$). Similarly, no differences in levels of proactive aggression were found between children with one or two copies of the *5-HTTLPR* short allele and children homozygous for the long allele (Wald's $\chi^2 = 1.41$, $p = .236$, $R^2 = 1\%$), nor were there differences between children with one or two copies of the short allele (Wald's $\chi^2 = 0.16$, $p = .693$, $R^2 = 0\%$).

Association between Facial Emotion Variables and the *MAOA-uVNTR* and the *5-HTTLPR*

Proportion of Correct Angry Responses

Full results for the association of covariates and the genetic markers with proportion of correct responses in each target emotion condition are presented in Table 2. For proportion of correct angry responses, we found no evidence of sex or age (linear or nonlinear) differences, nor evidence of an interaction between sex and age. There were also no differences in the proportion of correct angry responses between children with one or two copies of the low-activity *MAOA-uVNTR* variant and children homozygous for the high-activity allele (Wald's $\chi^2 = 0.81$, $p = .369$, $R^2 = 1\%$), nor were there differences between children with one and two copies of the low-activity allele (Wald's $\chi^2 = 0.32$, $p = .572$, $R^2 = 0\%$). Furthermore, no differences in the proportion of correct angry responses were found between children with one or two copies versus no copies of the *5-HTTLPR* short allele (Wald's $\chi^2 = 0.24$, $p = .627$, $R^2 = 0\%$), nor were there differences between children with one or two copies of the short allele (Wald's $\chi^2 = 0.46$, $p = .499$, $R^2 = 0\%$).

Proportion of Correct Fear Responses

For the proportion of correct fear responses, we found evidence of an interaction

between sex and age (Wald's $\chi^2 = 4.52$, $p = .034$, $R^2 = 5\%$) such that boys tended to have a lower proportion of correct fear responses than girls at younger ages, though both sexes tended to perform similarly at older ages. There were no differences in the proportion of correct fear responses between children with one or two copies of the low-activity *MAOA-uVNTR* variant and children homozygous for the high-activity allele (Wald's $\chi^2 = 0.41$, $p = .523$, $R^2 = 0\%$), but there were differences between children with one and two copies of the low-activity allele (Wald's $\chi^2 = 7.78$, $p = .005$, $R^2 = 9\%$) such that children with two copies of the low activities allele had a lower proportion of correct fear responses. No differences in the proportion of correct fear responses were found between children with one or two copies versus no copies of the *5-HTTLPR* short allele (Wald's $\chi^2 = 1.63$, $p = .202$, $R^2 = 2\%$), nor were there differences between children with one or two copies of the short allele (Wald's $\chi^2 = 0.46$, $p = .498$, $R^2 = 0\%$).

Proportion of Correct Happy Responses

For the proportion of correct happy responses, we found no evidence of sex or age (linear or nonlinear) differences, nor evidence of an interaction between sex and age. There were also no differences in proportion of correct happy responses between children with one or two copies of the low-activity *MAOA-uVNTR* variant and children homozygous for the high-activity allele (Wald's $\chi^2 = 1.49$, $p = .222$, $R^2 = 2\%$), nor were there differences between children with one and two copies of the low-activity allele (Wald's $\chi^2 = 0.50$, $p = .480$, $R^2 = 0\%$). Furthermore, no differences in the proportion of correct happy responses were found between children with one or two copies versus no copies of the *5-HTTLPR* short allele (Wald's $\chi^2 = 2.00$, $p = .157$, $R^2 = 2\%$), nor were there differences between children with one or two copies of the short allele (Wald's $\chi^2 =$

0.17, p = .678, R^2 = 0%).

Proportion of Correct Sad Responses

For the proportion of correct sad responses, we found a curvilinear relation with age (linear: Wald's χ^2 = 9.79, p = .002, R^2 = 11%; curvilinear: Wald's χ^2 = 3.99, p = .046, R^2 = 5%) such that the proportion of correct responses increased with age at a decreasing rate. There were no differences in the proportion of correct sad responses between children with one or two copies of the low-activity *MAOA-uVNTR* variant and children homozygous for the high-activity allele (Wald's χ^2 = 0.14, p = .706, R^2 = 0%), nor were there differences between children with one and two copies of the low-activity allele (Wald's χ^2 = 0.00, p = .967, R^2 = 0%). There were differences in the proportion of correct sad responses between children with one or two copies versus no copies of the *5-HTTLPR* short allele (Wald's χ^2 = 4.11, p = .043, R^2 = 5%) such that children with at least one copy of the short allele had higher proportion of correct sad responses. There were no differences between children with one or two copies of the short allele (Wald's χ^2 = 0.17, p = .678, R^2 = 0%).

Angry Commission Errors

Full results for the association of covariates and the genetic markers with commission errors in each target emotion condition are presented in Table 3. For commissions involving angry faces, we found no evidence of sex or age (linear or nonlinear) differences, nor any evidence of an interaction between sex and age. There were also no differences in the number of angry commissions between children with one or two copies of the low-activity *MAOA-uVNTR* variant and children homozygous for the high-activity allele (Wald's χ^2 = 0.81, p = .369, R^2 = 1%), nor were there differences

between children with one and two copies of the low-activity allele (Wald's $\chi^2 = 0.10$, $p = .758$, $R^2 = 0\%$). Furthermore, no differences in the number of angry commissions were found between children with one or two copies versus no copies of the *5-HTTLPR* short allele (Wald's $\chi^2 = 0.19$, $p = .665$, $R^2 = 0\%$), nor were there differences between children with one or two copies of the short allele (Wald's $\chi^2 = 0.49$, $p = .483$, $R^2 = 1\%$).

Fear Commission Errors

For fear commissions, we found evidence of an interaction between sex and the nonlinear effects of age (Wald's $\chi^2 = 5.98$, $p = .014$, $R^2 = 7\%$) such that boys tended to make more fear commission errors than girls at younger ages, though both sexes tended perform similarly at older ages. We found differences in fear commissions between children with one or two copies of the low-activity *MAOA-uVNTR* variant and children homozygous for the high-activity allele (Wald's $\chi^2 = 5.49$, $p = .019$, $R^2 = 6\%$) such that children with at least one copy of the low activity allele tended to make more fear commission errors. There were no differences between children with one and two copies of the low-activity allele (Wald's $\chi^2 = 0.14$, $p = .707$, $R^2 = 0\%$). No differences in fear commissions were found between children with one or two copies versus no copies of the *5-HTTLPR* short allele (Wald's $\chi^2 = 0.99$, $p = .319$, $R^2 = 1\%$), nor were there differences between children with one or two copies of the short allele (Wald's $\chi^2 = 1.01$, $p = .315$, $R^2 = 1\%$).

Happy Commission Errors

For happy commissions, we found no evidence of sex or age (linear or nonlinear) differences, nor evidence of an interaction between sex and age. There were also no differences in happy commissions between children with one or two copies of the low-

activity *MAOA-uVNTR* variant and children homozygous for the high-activity allele (Wald's $\chi^2 = 0.64$, $p = .424$, $R^2 = 1\%$), nor were there differences between children with one and two copies of the low-activity allele (Wald's $\chi^2 = 0.08$, $p = .780$, $R^2 = 0\%$). There was a trend toward significant differences in happy commissions between children with one or two copies versus no copies of the *5-HTTLPR* short allele (Wald's $\chi^2 = 3.68$, $p = .055$, $R^2 = 4\%$) such that children with at least one copy of the short allele tended to make more happy commission errors. There were no differences between children with one or two copies of the short allele (Wald's $\chi^2 = 0.16$, $p = .692$, $R^2 = 0\%$).

Sad Commission Errors

For sad commissions, we found a significant linear effect of age (Wald's $\chi^2 = 14.67$, $p = .0001$, $R^2 = 17\%$) such that the number of sad commissions decreased with increasing age. We found differences in fear commissions between children with one or two copies of the low-activity *MAOA-uVNTR* variant and children homozygous for the high-activity allele (Wald's $\chi^2 = 5.24$, $p = .022$, $R^2 = 6\%$) such that children with at least one low-activity allele tended to make more sad commissions. There were no differences between children with one and two copies of the low-activity allele (Wald's $\chi^2 = 0.94$, $p = .332$, $R^2 = 1\%$). There were also differences in sad commissions between children with one or two copies versus no copies of the *5-HTTLPR* short allele (Wald's $\chi^2 = 5.16$, $p = .023$, $R^2 = 6\%$) such that children with at least one copy of the short allele had tended to make more sad commissions. There were no differences between children with one or two copies of the short allele (Wald's $\chi^2 = 0.17$, $p = .678$, $R^2 = 0\%$).

Associations of Facial Emotion Variables with Reactive and Proactive Aggression

Full results for association analyses between covariates and facial emotion

variables are available in Table 3. For reactive aggression, there were significant sex differences (Wald's $\chi^2 = 5.21$, $p = .023$, $R^2 = 4\%$) such that boys had higher levels of reactive aggression than girls. There was also a curvilinear age trend (Wald's $\chi^2 = 4.66$, $p = .031$, $R^2 = 4\%$) such that children's reactive aggression increased to age 10, then decreased thereafter. Furthermore, there was an interaction between sex and age (Wald's $\chi^2 = 4.62$, $p = .032$, $R^2 = 4\%$) such that girls tended to remain stable in reactive aggression over time while boys displayed the age trend described above. The proportion of correct fear responses was significantly related to reactive aggression (Wald's $\chi^2 = 3.85$, $p = .050$, $R^2 = 3\%$) such that children with a lower proportion of correct fear responses tended to have higher levels of reactive aggression. In contrast, the proportion of correct angry (Wald's $\chi^2 = 0.01$, $p = .934$, $R^2 = 0\%$), happy (Wald's $\chi^2 = 0.03$, $p = .865$, $R^2 = 0\%$), and sad (Wald's $\chi^2 = 0.70$, $p = .403$, $R^2 = 1\%$) responses were not significantly related to reactive aggression. The number of fear commissions was significantly related to reactive aggression (Wald's $\chi^2 = 4.51$, $p = .034$, $R^2 = 3\%$) such that children that made higher numbers of fear commission errors tended to have higher levels of reactive aggression. There also were statistical trends in the relations between reactive aggression and happy (Wald's $\chi^2 = 3.171$, $p = .075$, $R^2 = 2.4\%$) and sad commissions (Wald's $\chi^2 = 3.37$, $p = .067$, $R^2 = 3\%$) such that children that made higher numbers of happy or sad commissions tended to have higher levels of reactive aggression. Number of angry (Wald's $\chi^2 = 0.46$, $p = .497$, $R^2 = 0\%$) was not significantly related to reactive aggression.

We found no evidence of sex or age (linear or nonlinear) differences, nor evidence for an interaction between sex and age affecting proactive aggression. The

proportion of correct angry (Wald's $\chi^2 = 1.12$, $p = .290$, $R^2 = 1\%$), fearful (Wald's $\chi^2 = 0.12$, $p = .726$, $R^2 = 0\%$), happy (Wald's $\chi^2 = 0.13$, $p = .723$, $R^2 = 0\%$), and sad (Wald's $\chi^2 = 0.13$, $p = .723$, $R^2 = 0\%$) responses were not significantly related to proactive aggression. In contrast, the number of fear commissions was significantly related to proactive aggression (Wald's $\chi^2 = 6.60$, $p = .010$, $R^2 = 5\%$) such that children that made higher numbers of fear commissions tended to have higher levels of proactive aggression. Numbers of angry (Wald's $\chi^2 = 2.38$, $p = .123$, $R^2 = 2\%$), happy (Wald's $\chi^2 = 0.73$, $p = .394$, $R^2 = 1\%$), and sad (Wald's $\chi^2 = 0.32$, $p = .570$, $R^2 = 0\%$) commissions were not significantly related to proactive aggression.

Evaluation of Facial Emotion Variables as Mediators between Genetic Markers and Aggressive Phenotypes

As noted above, the *MAOA-uVNTR* was associated with reactive but not proactive aggression, while the *5-HTTLPR* was associated with neither reactive nor proactive aggression. Therefore, only the facial emotion variables that were associated with both the *MAOA-uVNTR* and reactive aggression were tested as mediators. These variables included the proportion of correct fear responses and number of fear commissions. Number of sad commissions was also tested as a mediator due to it being significantly related to the *MAOA-uVNTR* and showing a statistical trend for its relation to reactive aggression. The relation between the *MAOA-uVNTR* and reactive aggression (Wald's $\chi^2 = 4.17$, $p = .041$, $R^2 = 4\%$) was rendered nonsignificant when the proportion of correct fear responses (Wald's $\chi^2 = 2.08$, $p = .149$, $R^2 = 2\%$), the number of fear commissions (Wald's $\chi^2 = 0.08$, $p = .777$, $R^2 = 0\%$), and the number of sad commissions (Wald's $\chi^2 = 2.99$, $p = .084$, $R^2 = 3\%$) were entered prior to the *MAOA-uVNTR* in the model. These

results indicate that proportion of correct fear responses, fear commissions, and sad commissions are mediators of the relation between the *MAOA-uVNTR* and reactive aggression.

Discussion

The current study examined direct and indirect associations between the *MAOA-uVNTR* and the *5-HTTLPR,* facial emotion recognition deficits and biases, and reactive and proactive aggression. We found that the *MAOA-uVNTR* low-activity allele was associated with higher reactive (but not proactive) aggression, a lower proportion of correct fear responses, a higher number of fear commission errors, and a higher number of sad commission errors. The *5-HTTLPR* short allele was associated with a higher proportion of correct sad responses and a higher number of sad commission errors, but was not associated with reactive or proactive aggression. We found that a lower proportion of correct fear responses and a higher number of fear commission errors were in turn associated with higher levels of reactive aggression. A higher number of fear commission errors was also associated with higher levels of proactive aggression. Finally, we found that the proportion of correct fear responses, fear commission errors, and sad commission errors separately mediated the association between the *MAOA-uVNTR* and reactive aggression. These findings mainly confirm our predictions regarding the associations of these genetic markers with facial emotion recognition and reactive and proactive aggression.

Interpretation of *MAOA-uVNTR* Findings

The results involving the *MAOA-uVNTR,* facial emotion recognition deficits and biases, and reactive aggression suggest that low MAOA activity may contribute to higher

levels of reactive aggression indirectly by causing difficulties in recognizing fearful and sad facial expressions and differentiating them from other emotions. Specifically, individuals with the *MAOA-uVNTR* low-activity allele were worse than individuals with two copies of the high-activity allele at identifying fearful faces (as indicated by a lower proportion of correct fear responses) as well as at discriminating non-fearful faces from fearful faces (as indicated by a higher number of fear commission errors). This pattern suggests general difficulties with fear recognition in individuals with the low-activity allele. This allele was also found to be associated with more sad commission errors, but was not associated with any differences in correct sad responses. This pattern may indicate that while low-activity allele carriers do not have trouble identifying sad faces when they are presented, they have a tendency to incorrectly identify other emotions as sadness when prompted to detect sad faces. Thus, *MAOA-uVNTR* low-activity allele carriers may have difficulty distinguishing sad faces from other negative faces may simply be responding when they see two negative faces in a row.

These deficits in fearful and sad facial emotion recognition in low-activity *MAOA-uVNTR* allele carriers are consistent with findings that the low-activity allele is associated with higher amygdala and lower prefrontal reactivity to negative faces (Meyer-Lindenberg et al., 2006). In accordance with this literature, we predicted that the low-activity allele would also be related to anger recognition deficits or biases, but our results did not support this hypothesis. It is possible that *MAOA-uVNTR* low-activity allele carriers have differential neural reactivity to angry faces that is unrelated to any subsequent differences in accuracy identifying or differentiating them, or that this aspect of the task used in this study was too easy to yield reliable individual differences.

We also replicated previous findings (LoParo et al., manuscript in preparation) that the *MAOA-uVNTR* low-activity allele is associated with reactive but not proactive aggression. This finding is consistent with literature indicating that both reactive aggression (Blair, 2010) and the low-activity allele (Meyer-Lindenberg et al., 2006) are related to heightened amygdala reactivity to negative stimuli.

Given that we found that the proportion of correct fear responses, the number of fear commission errors, and the number of sad commission errors were significantly related to both the *MAOA-uVNTR* low-activity allele and reactive aggression, we tested and found that these three aspects of facial emotion recognition separately mediated the relation between the *MAOA-uVNTR* and reactive aggression. These results suggest that low MAOA activity may create differential neural responses to fearful and sad faces, causing fear and sadness to be under-recognized or misidentified. These emotions are considered distress cues that elicit empathy or punish aggression (Marsh et al., 2005; Blair, 2005). Recognition inaccuracies of these distress cues could potentially cause higher levels of reactive aggression due to lower empathy or recognition of negative social reinforcement.

Interpretation of the *5-HTTLPR* Findings

We found that *5-HTTLPR* short-allele carriers tended to have a higher proportion of correct sad responses as well as more sad commission errors. While this combination may seem contradictory, it could be that short allele carriers have a lower threshold for perceiving sadness in facial emotions, causing them to correctly identify sad faces when presented but also to falsely identify sadness when another emotion is present. The *5-HTTLPR* has been associated not only with aggression but also internalizing

psychopathology symptoms such as negative affect (Munafó et al., 2003), and elevated anxiety reactivity (Gunthert et al., 2007). It could be the case that short allele carriers perceive the facial emotions of those around them more negatively than long allele carriers, thus leading to negative affect or depressive symptoms, or vice versa. This would be consistent with findings that individuals with depression and general anxiety have enhanced sensitivity to negative expressions, as indicated by greater accuracy in the recognition of and quicker reaction times to negative faces (Bhagwagaret al., 2004; Masurier et al., 2007). Exploring this hypothesis empirically could clarify the etiological pathway between the *5-HTTLPR* and these aspects of internalizing psychopathology.

As we predicted based on previous in a clinical sample (LoParo et al., manuscript in preparation), we found that the *5-HTTLPR* was unrelated to reactive and proactive aggression. Nevertheless, meta-analytic data has demonstrated that this genetic marker is weakly related to antisocial behavior (Ficks & Waldman, manuscript in preparation), and the current results do not necessarily contradict that finding. It could be the case that the *5-HTTLPR* is related to antisocial behavior broadly with an effect size too small to detect in studies of specific facets of aggression. It could also be the case that the *5-HTTLPR* is related to other aspects of antisocial behavior, but not reactive or proactive aggression specifically. Further, given that the *5-HTTLPR* was found to be related to biases in facial emotion recognition in this study, it is possible that these biases mediate the relation between the *5-HTTLPR* and antisocial behavior. Further research is needed to determine the specific etiological pathways between the *5-HTTLPR* and antisocial behavior, and to determine whether facial emotion recognition is an important component of that pathway.

Interpretation of the Findings for Reactive and Proactive Aggression

Our results implied that reactive and proactive aggression have unique patterns of association with facial emotion recognition deficits and biases. While reactive aggression was associated with a lower proportion of correct fear responses, more fear commission errors, and more sad commission errors, proactive aggression was associated with only increased fear commission errors. This pattern implies that misidentification of fearful faces is a deficit common across multiple forms of aggression, which is consistent with meta-analytic findings of associations between fear recognition deficits and antisocial behavior not being moderated by psychopathy levels (Marsh & Blair, 2008). Reactive aggression seems to have some additional unique facial emotion correlates that are unrelated to proactive aggression. Given that reactive aggression is more closely related to oversensitivity to or misinterpretation of threat cues than proactive aggression (Craig & Halton, 2009), it is perhaps unsurprising that reactive aggression is related to a larger set of facial emotion recognition deficits and biases. Further research is needed to determine the etiological sources of the common and unique facial emotion recognition deficits and biases between reactive and proactive aggression, and to determine the extent to which this pattern characterizes a more general set of partially differentiable social cognitive difficulties.

Limitations

Though our results largely match our predictions, are consistent with the literature, and replicate our previous findings in a clinical sample, there are several limitations that should be taken into account when interpreting them. First, with 88 participants for whom genetic and facial emotion data was available, our sample size was small for a genetic association study. The effects of a smaller sample size caused several

putative associations with relatively large effect sizes to not meet our threshold for significance. For example, the association between the *5-HTTLPR* short allele and happy commission errors had an R^2 value of 4.2%, but only achieved a p-value of .055, which we interpreted as a trend toward significance despite the relatively large effect size. Given that Marsh and Blair's (2008) meta-analysis found that antisocial behavior was associated with deficits in recognition across almost all facial emotions, with fear deficits being by far the strongest, it may be the case that a sufficiently-powered study would find that the *MAOA-uVNTR* has a small effect across all emotion types with the effect on fear recognition being the largest. A second, related limitation is that because this study was somewhat exploratory in nature, we did not correct for multiple tests despite the relatively large number of statistical tests performed. Future research with a larger sample size would be sufficiently powered to apply appropriate corrections and to determine whether our results reflect the true pattern of association between these genetic markers, facial emotion recognition, and reactive and proactive aggression.

A third limitation is that although the *MAOA-uVNTR* and the *5-HTTLPR* are both in linkage disequilibrium to some degree with other markers within their respective genes (Sabol et al., 1998; Heils et al.,1996), testing only one marker characterizes only a small proportion of the genetic variation in a gene. Most candidate gene studies of psychiatric disorders and psychological traits have focused on only one or very few polymorphism(s) to operationalize the candidate gene of interest, a situation that renders most findings very hard to interpret. In the case of negative findings, one does not know whether there truly is no association between the disorder and the gene, or whether one has simply made an unfortunate choice in the marker(s) selected for study. In the case of positive findings,

one does not know whether the associated marker is the only associated marker in the gene, and typically does not know whether that marker is functional, making it virtually impossible to estimate the association's effect size. In addition, most genetic markers studied to date have been in genes proposed as candidates solely based on their function and hypothesized etiological relevance to disorders or traits, rather than based on the findings of large, unbiased genome-wide association scans. Although this situation is changing, as studies begin to genotype multiple SNPs in candidate genes, most candidate gene studies have heretofore genotyped and tested for association only very few markers across the gene, and have selected those markers in a haphazard, non-systematic fashion. This poses the additional difficulty that the information content (or percent of variation) within the gene captured by the selected markers is unknown. Researchers should begin employing a strategy in which bioinformatics procedures are used to select an efficient set of 'tagging' SNPs that capture the majority of genetic variation in the candidate gene of interest (deBakker et al., 2005), and use omnibus multimarker tests of association that effectively control Type I error while maximizing statistical power (Chapman et al., 2004).

Implications/Future Directions

Our findings that deficits and biases in fear and sadness recognition mediate the relation between *MAOA-uVNTR* and reactive aggression imply that these difficulties may serve as an endophenotype for reactive aggression. Nonetheless, mediation is only one of several criteria used to evaluate a particular trait as an endophenotype (Waldman, 2005). Other such criteria include the endophenotype being expressed among unaggressive relatives of aggressive children, being associated with aggression within families, and

moderating the association between the genetic marker and aggression (Waldman, 2005). Future research should focus on evaluating these and other endophenotype criteria to determine whether fear and sadness recognition difficulties truly are the mechanisms through which the *MAOA-uVNTR* affects levels of reactive aggression.

Reactive and proactive aggression are also differentially associated with social cognitive deficits and biases other than facial emotion recognition, such as hostile attributional biases and intention-cue detection deficits (Dodge & Coie, 1987; Waldman, 1996). Determining the degree to which this mediational pathway is unique to facial emotion deficits and biases or if other social cognitive deficits and biases play a similar role could help to elucidate the specificity of our findings. Further, other candidate genes for aggression, such as other serotonin genes, neuropeptides genes, or GABA genes (Nelson & Trainor, 2007) should be examined in association with both facial emotion recognition deficits and biases and other social cognitive mechanisms previously associated with aggression. As in this study, identifying areas of overlap in neural correlates of candidate genes or neurotransmitters and the social cognitive mechanisms can help to formulate hypotheses about the nature of the putative relations.

In addition to its theoretical contributions, this study has a number of broader implications. Aggression is a primary symptom of several disorders, such as conduct disorder and antisocial personality disorder, as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (*DSM-IV-TR*), though reactive and proactive aggression are never explicitly named or separated in the DSM-IV. Finding that reactive and proactive aggression are at least partially etiologically distinct contributes to the argument set forth by Dodge et al. (1997) that the type of aggression that a child displays

predominantly should be included as part of their diagnosis. This specificity in diagnosis could be essential in choosing the most appropriate intervention strategy, especially in combination with the current facial emotion recognition findings (Coie, Underwood, & Lochman, 1991). For example, a child with primarily reactive aggression could benefit from interventions targeted at building tolerance to threatening stimuli or accuracy in determining facial emotions, whereas a person with primarily proactive aggression may not gain as much from such a treatment. More generally, understanding both common and unique etiological pathways to reactive and proactive aggression is essential to identifying primary treatment targets and developing interventions with strategies tailored to a wider range of behavioral profiles.

Conclusion

In summary, we present evidence that the *MAOA-uVNTR* risk allele is related to deficits and biases in fear and sad facial emotion recognition, while the *5-HTTLPR* is related to biases in sad facial emotion recognition. Further, deficits and biases in fear and sad facial emotion recognition seem to mediate the relation between the *MAOA-uVNTR* and reactive aggression. This pattern of results indicates that facial emotion recognition may be a valid endophenotype for reactive aggression. More generally, deficits and biases in social cognition may not only be correlates of aggression but may play an essential role in its etiology.

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Figure 1:
Distributions of Proportion Correct for Each Emotion

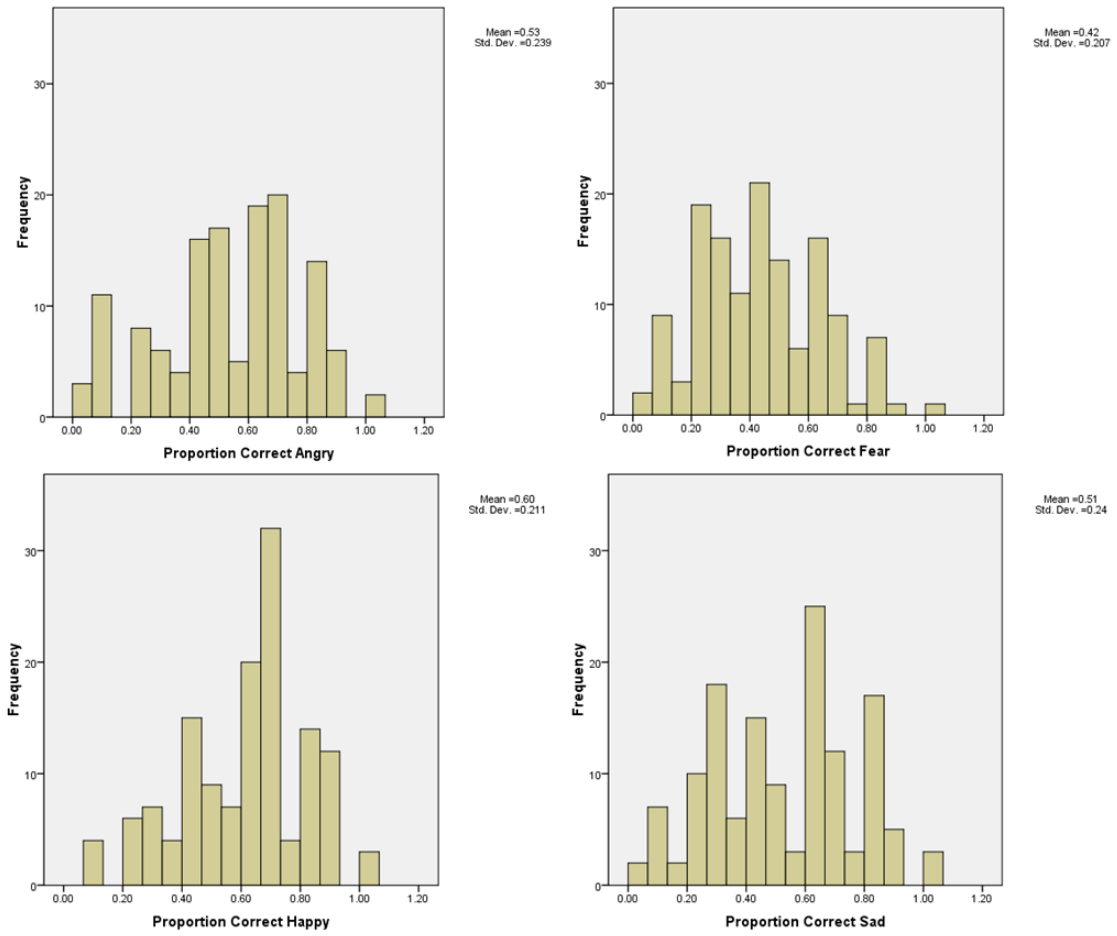

Figure 2:
Distributions of Number of Commissions Errors for Each Emotion

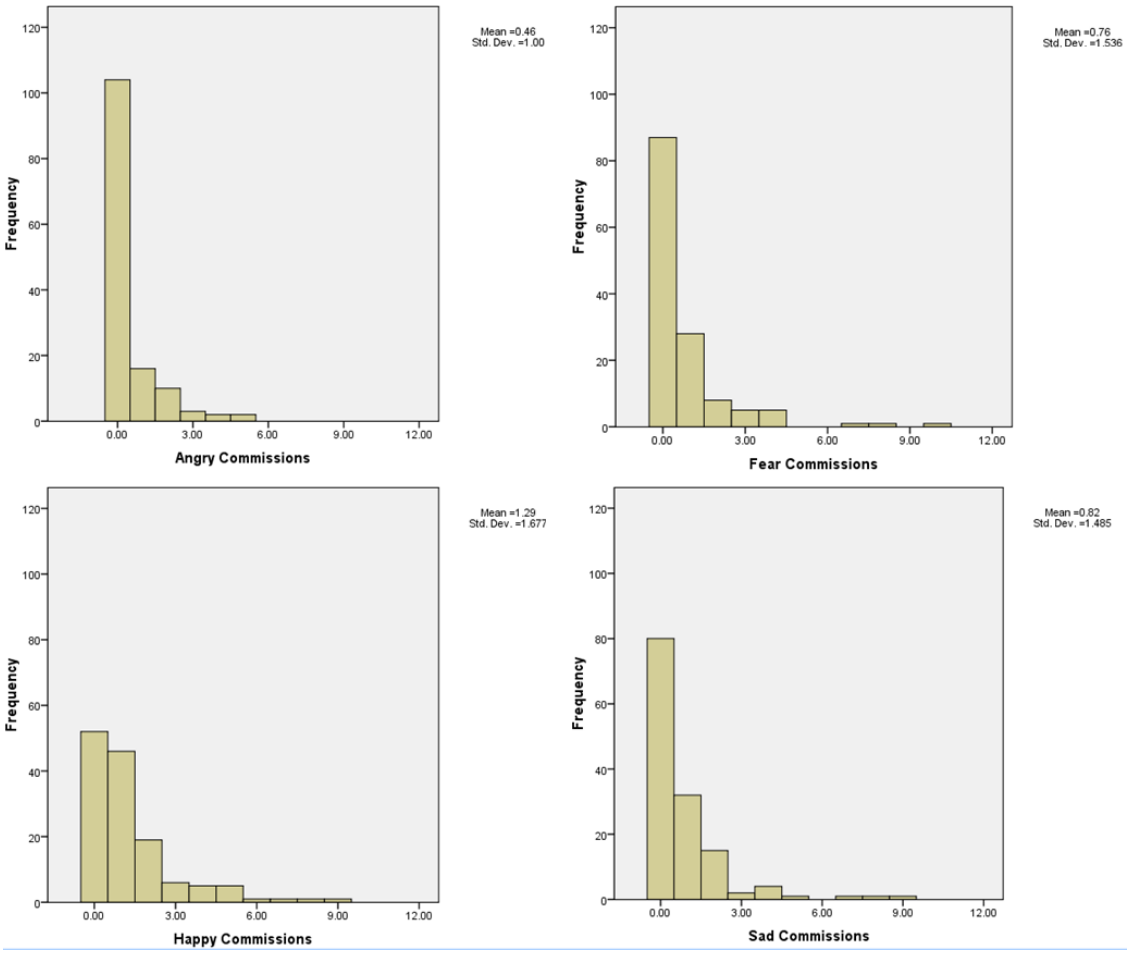

Figure 3:
Distributions of Reactive and Proactive Aggression

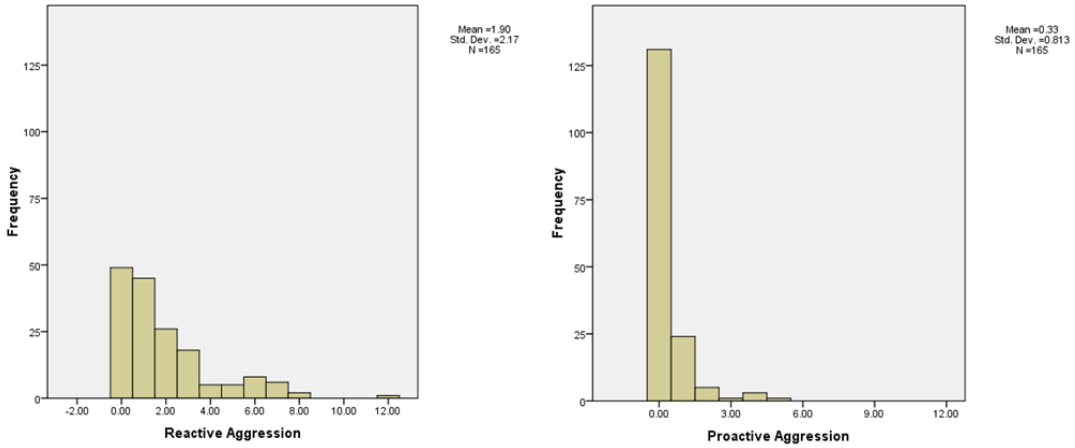

Table 1

Association analyses for Reactive and Proactive Aggression *(Modeled with Negative Binomial with Log Link)*

	Reactive Aggression			Proactive Aggression		
Predictor	Wald's χ^2	*p*	R^2	Wald's χ^2	*p*	R^2
Sex	5.206	.023*	3.9%	2.754	.097	2.1%
Age	0.087	.769	<0.1%	0.381	.537	0.3%
Age^2	4.655	.031*	3.5%	0.730	.393	0.5%
Sex*Age	4.624	.032*	3.5%	0.783	.376	0.6%
Sex*Age^2	0.282	.595	0.2%	0.239	.625	0.2%
Angry Correct	0.007	.934	<0.1%	1.120	.290	0.8%
Fear Correct	3.848	.050*	2.9%	0.123	.726	0.1%
Happy Correct	0.029	.865	<0.1%	0.126	.723	0.1%
Sad Correct	0.698	.403	0.5%	0.126	.723	0.1%
Angry Commissions	0.461	.497	0.3%	2.376	.123	1.8%
Fear Commissions	4.510	.034*	3.4%	6.598	.010*	5.0%
Happy Commissions	3.171	.075	2.4%	0.726	.394	0.5%
Sad Commissions	3.365	.067	2.5%	0.322	.570	0.2%
MAOA LL/HL vs HH	4.174 0.121	.041* .728	3.7% 0.1%	3.084 2.689	.079 .101	2.8% 2.4%
MAOA LL vs HL	0.339	.561	0.2%	1.407	.236	1.3%
5-HTTLPR SS/SL vs LL	3.411	.065	3.0%	0.156	.693	0.1%
5-HTTLPR SS vs SL						

Note: Covariates were entered for every analysis, while facial emotion variables and genetic markers were analyzed separately from each other.

* Indicates significant association.

Table 2
Association analyses for Proportion Correct
(Modeled with Normal)

	Angry Proportion Correct			Fear Proportion Correct			Happy Proportion Correct			Sad Proportion Correct		
Predictor	Wald's χ^2	*p*	R^2	Wald's χ^2	*p*	R^2	Wald's χ^2	*p*	R^2	Wald's χ^2	*p*	R^2
Sex	1.857	.173	2.1%	0.460	.498	0.0%	3.812	.051	4.3%	1.139	.286	1.3%
Age	2.757	.097	3.1%	3.059	.080	3.5%	0.682	.409	0.1%	9.790	.002*	11.1%
Age^2	1.613	.204	1.8%	0.871	.351	0.9%	.0140	.907	0.0%	3.992	.046*	4.5%
Sex*Age	2.252	.133	2.6%	4.519	.034*	5.1%	1.173	.279	1.3%	0.191	.662	0.0%
Sex*Age^2	0.459	.498	0.0%	0.427	.513	0.0%	0.524	.469	0.1%	0.001	.980	0.0%
MAOA LL/HL vs HH	0.806	.369	0.1%	0.408	.523	0.0%	1.494	.222	1.7%	.142	.706	0.0%
MAOA LL vs HL	0.320	.572	0.0%	7.783	.005*	8.8%	0.499	.480	0.1%	.002	.967	0.0%
5-HTTLPR SS/SL vs LL	0.236	.627	0.0%	1.631	.202	1.9%	2.000	.157	2.3%	4.114	.043*	4.6%
5-HTTLPR SS vs SL	0.458	.499	0.0%	0.460	.498	0.0%	0.172	.678	0.0%	2.001	.157	2.3%

* Indicates significant association.

Table 3

Association analyses for Commissions

(Modeled with Negative Binomial with Log Link)

	Angry Commissions			Fear Commissions			Happy Commissions			Sad Commissions		
Predictor	Wald's χ^2	*p*	R^2	Wald's χ^2	*p*	R^2	Wald's χ^2	*p*	R^2	Wald's χ^2	*p*	R^2
Sex	0.588	.443	0.6%	0.666	.414	0.8%	2.781	.095	3.2%	1.886	.170	2.1%
Age	0.090	.764	0.1%	2.881	.090	3.3%	0.648	.421	0.7%	14.671	<.001*	16.7%
Age^2	0.745	.388	0.8%	1.727	.189	2.0%	0.012	.914	0.0%	0.062	.803	0.0%
Sex*Age	0.238	.626	0.3%	0.441	.507	0.5%	2.007	.157	2.2%	2.512	.113	2.9%
Sex*Age^2	0.525	.525	0.6%	5.979	.014*	6.8%	3.161	.075	3.6%	0.877	.349	1.0%
MAOA LL/HL vs. HH	0.808	.369	0.9%	5.487	.019*	6.2%	0.639	.424	0.7%	5.235	.022*	5.9%
MAOA LL vs. HL	0.095	.758	0.1%	0.141	.707	0.2%	0.078	.780	0.0%	0.940	.332	1.1%
5-HTTLPR SS/SL vs. LL	0.188	.665	0.2%	0.993	.319	1.1%	3.683	.055	4.2%	5.159	.023*	5.9%
5-HTTLPR SS vs. SL	0.491	.483	0.6%	1.011	.315	1.1%	0.157	.692	0.2%	0.048	.826	0.0%

* Indicates significant association.