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My dopamine has been busy: Research on gene by environment interactions in child externalizing behavior

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CHAPTER 7

GENETIC MODERATION OF INTERVENTION EFFICACY:
DISTINGUISHING RECEPTOR-, TRANSPORTER-,
AND ENZYME-RELATED DOPAMINERGIC GENES

- KEYWORDS** – Externalizing behavior
Gene-by-environment interaction
Dopaminergic subsystems
Incredible Years
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- ABSTRACT** – **In this report, we build on results of a prior report documenting the genetic moderation of intervention efficacy in the case of boys scoring high on externalizing problems (Chhangur et al., in press). Because prior results revealed that a polygenic index comprised of five dopaminergic genes (DRD4, DRD2, DAT1, MAOA, and COMT) moderated the effect of the Incredible Years (IY) intervention program, we sought to gain insight into which of three dopaminergic subsystems—receptor, transport and/or enzyme—might be principally responsible for the moderational effect discerned. The randomized-controlled-trial involved 190 families with 4-8-year-old boys who had relatively high levels of behavior problems at screening. Results of latent growth modelling in Mplus showed that the originally detected polygenic-moderation effect was specifically caused by the subset of enzyme genes (MAOA, COMT). Boys carrying more enzyme-related plasticity alleles (but not boys carrying fewer of such alleles) decreased significantly in externalizing behavior as a result of their parents' involvement in IY intervention program. This effect remained even when the other dopaminergic genes were controlled for.**

Externalizing behavior problems evident early in life tend to persist if not treated, which stresses the importance of prevention efforts (Campbell et al., 2006, 2000). The etiology of early externalizing problems includes diverse environmental factors among which especially problematic parenting have been shown to play a crucial role (Miner & Clarke-Stewart, 2008; Patterson, 1976). Intervention efforts aimed at reducing externalizing behavior therefore targets parenting (e.g., McCart et al., 2006). A meta-analysis of the effectiveness of one such parenting intervention, the Incredible Years program (IY; Webster-Stratton, 2001), revealed it to be effective in preventing and remediating externalizing problems (Menting et al., 2013). However, given the modest effect size of IY, especially in the context of indicated prevention (i.e., Cohen's $d = .20$), there might be substantial heterogeneity in program efficacy. Because this is true for most child-related interventions, be they preventive, curative or enhancement oriented, insight into the determinants of such heterogeneity might be crucial for boosting effectiveness of these programs (Bakermans-Kranenburg & Van IJzendoorn, 2015; Belsky & Van IJzendoorn, 2015).

Recent research indicates that children's genetic make-up plays a significant role in predicting which children benefit most and least from a variety of interventions (e.g., Brett et al., 2015; Brody, Beach, Philibert, Chen, & Murry, 2009; Plak, Kegel, & Bus, 2015), including the IY program (Chhangur et al., in press). Specifically, a recent meta-analysis showed that there is repeated evidence of such "for-better-and-for-worse" environmental effects in research on gene-by-environment ($G \times E$) interactions (Van IJzendoorn & Bakermans-Kranenburg, 2015). This may not come entirely unexpected as differential-susceptibility theorizing stipulates that the very personal factors associated with children's vulnerability to adversity, may also be associated with these children benefiting more than others from environmental support and enrichment (Belsky et al., 2007; Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005). But most notable for purposes of this report is a meta-analysis showing that a variety of dopamine-related polymorphisms moderate a variety of environmental effects in a differential-susceptibility-related manner (Bakermans-Kranenburg & Van IJzendoorn, 2011).

When it comes to evidence of genetic moderation of environmental effects, studies of gene-by-intervention ($G \times I$) interactions—involving random assignment of participants to treatment conditions—have several advantages over correlational $G \times E$ investigations (Bakermans-Kranenburg & Van IJzendoorn, 2015; Belsky & Van IJzendoorn, 2015). One of the most well-acknowledged limitations of non-experimental $G \times E$ research is that a gene-environment correlation (r_{GE}) can "masquerade" as putative $G \times E$ interactions (Bakermans-Kranenburg & Van IJzendoorn, 2015). Even when a particular G in a longitudinal $G \times E$ design proves unrelated to the E in question, other unmeasured G 's could be influencing the environmental factor under examination and thus confound the interpretation of any detected $G \times E$. Consider in this regard the results of one $G \times E$ study which found that a significant $G \times E$ effect was no longer significant after controlling for parents' genes (Chhangur, Overbeek, et al., 2015).

It is notable that a variety of very recent $G \times I$ studies document the moderational role of dopaminergic polymorphisms in conditioning experimentally induced environmental effects (Belsky & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015; Chhangur et al., in press). Consider in this regard work by Van den Hoofdakker and colleagues (2012) showing that children carrying 10-repeat alleles of the *DAT1* benefited most from a behavioral parenting intervention designed to treat children with ADHD. Consider, too, research by Brody and associates (2015) demonstrating that a family-based intervention designed to prevent substance use proved effective principally in teenagers that carried the same plasticity allele.

To date, virtually all such $G \times I$ studies have focused on the moderating role of single candidate genes (but see Musci et al., 2015; Thibodeau, Cicchetti, & Rogosch, 2015 as exceptions). It is widely appreciated, however, that multiple genes play a role in virtually every phenotype. This has led to the promotion of system's thinking regarding $G \times E$ interaction research. Such an approach encourages the creation of polygenic scores using multiple genes influencing a particular biological process or pathway, such as the dopaminergic or serotonergic system (Brody, Chen, & Beach, 2013; Conner, Helleman, Ritchie, & Noble, 2010; Nikolova et al., 2011), or neurological endophenotypes, such as hippocampal volume (Whittle et al., 2011), amygdala volume (Yap et al., 2008), and ventral striatum reactivity (Nikolova, Bogdan, Brigidi, & Hariri, 2012). Informed by such system's thinking, we recently documented a moderation effect of a polygenic dopaminergic index (i.e., 7-repeat allele of *DRD4*, the A1 allele of *DRD2*, the 10-repeat allele of *DAT1*, the low-activity allele of *MAOA*, and val allele of *COMT*) on the effect of the IY intervention on the development of children's externalizing problems (Chhangur et al., in press). Specifically, results revealed that IY proved most effective in decreasing parent-reported externalizing behavior in boys (not girls) carrying many dopaminergic plasticity alleles, compared to boys with fewer such alleles. However, the use of polygenic scores does not illuminate which specific dopaminergic processes are principally responsible for the polygenic moderational effect detected. More insight into genetic moderation could be gained by decomposing this polygenic composite further, for example, by distinguishing dopaminergic genes that are (a) receptor, (b) transport, and (c) enzyme related (Chen et al., 2011). Such an approach is very preliminary in character but could nevertheless increase our understanding of what the underlying mechanisms are of the found $G \times I$ interaction. This is thus the focus of this report. To our knowledge, such an effort in $G \times I$ or even $G \times E$ work has ever before been carried out in research on child externalizing behavior problems or child development more generally.

System-level genetic approach: Receptor, transporter, enzyme

Dopamine is an excitatory neurotransmitter involved in motivational, attentional, and reward processes (Padmanabhan & Luna, 2014). A complex balance between the amount of dopamine synthesized, released, stored, recaptured, and metabolized

determines the intensity of dopaminergic signaling (Bonisch & Eiden, 1997; Roth & Elsworth, 1995). The five aforementioned genes play such roles in dopamine availability in the synapse and thus neuro signaling by either affecting the amount of dopamine (1) released (via neural signaling), (2) recaptured or (3) degraded (i.e., metabolized), possibly making children more and less prone to, for example, environmental cues of reward (Matthys et al., 2013; Moore & Depue, 2016).

DRD2 and *DRD4* are considered *receptor* genes that modulate dopaminergic neural signaling and dopamine release in the synaptic cleft. In the normal process, dopamine is released by a presynaptic neuron into the synapse, where it binds to autoreceptor proteins on the postsynaptic neuron, sending a signal to that neuron. Dopamine is then released for the autoreceptors to be recaptured by transporter proteins or metabolized by enzymes. Thus, these two genes modulate dopamine receptor availability in the brain and, thereby, dopamine signaling and release (Forbes et al., 2009; Noble et al., 1997). The A1 allele of *DRD2* and the 7-repeat of *DRD4* are treated as plasticity alleles herein (e.g., Bakermans-Kranenburg et al., 2008; Belsky & Beaver, 2011; Chhangur et al., in press; Mills-Koonce et al., 2007) and are functionally associated with lower receptor density and thus with less dopamine signaling/release (Forbes et al., 2009).

DAT1 is considered a *transporter* gene that modulates the *recapturing* of dopamine from the synaptic cleft. Along the presynaptic neuron are transporter proteins which transport the dopamine released by autoreceptors back into the neuron, terminating the dopamine signal. In the normal process, transporters recapture dopamine in the synaptic cleft to terminate ongoing signaling, thereby enabling new dopaminergic neural signals. Thus, this gene modulates dopamine transporter availability and, thereby, the recapturing of dopamine used for new neuro signaling and ending ongoing dopamine signals (e.g., Heinz et al., 2000; Mill, Asherson, Browes, D'Souza, & Craig, 2002; VanNess, Owens, & Kiltz, 2005). Too much transporter availability might lead to too much or too fast recapturing, thereby ending the dopamine signaling too quickly. The 10-repeat allele of the *DAT1* is treated as plasticity allele herein (e.g., Belsky & Beaver, 2011; Chhangur et al., in press; Laucht et al., 2007) and is functionally associated with higher transporter density and thus with higher/faster recapturing, that in turn might result in less (ongoing) dopamine signaling (Felten, Montag, Markett, Walter, & Reuter, 2011; Mill et al., 2002; Yang et al., 2007). However, other studies found the 10-repeat allele relative to the 9-repeat allele to be associated with lower transporter availability and thus less recapturing, thereby increasing the duration of dopamine available at postsynaptic autoreceptors and dopamine signaling (e.g., Van de Giessen et al., 2009).

MAOA and *COMT* are considered *enzyme*-related genes that *degrade* dopamine availability in the brain. The enzymes are present in both the presynaptic neuron and synaptic cleft. In the presynaptic neuron the enzymes break down the dopamine that has been transported here, while the enzymes in the synaptic cleft

break down the remaining dopamine in the synapse. Thus, these two genes affect dopamine enzyme availability and, thereby, the degradation of dopamine in the brain (Boulton & Eisenhofer, 1997; Chen et al., 2004). The low-activity allele of the *MAOA* and the val allele of the *COMT* are treated as plasticity alleles herein (e.g., Belsky & Beaver, 2011; Chhangur et al., in press; Van IJzendoorn et al., 2008). The val (or high-activity) allele of the *COMT* is functionally associated with higher enzyme density and greater degradation and thus might result in less dopamine signaling (Lachman et al., 1996). However contradictory, the precise role of the low-activity *MAOA* allele is unclear, as it presumably functionally associated with lower enzyme density and thus with lower degradation; this would be expected to result in more dopamine signaling (Boulton & Eisenhofer, 1997). Some research on the functional effects of the *MAOA*, however, fails to document significant associations between the low-activity allele and lower enzyme density in the human brain, even if such was discerned when a related haplotype was the focus of inquiry (e.g., Balciuniene, Emilsson, Orelund, Pettersson, & Jazin, 2002). More research is needed to illuminate the precise role of the *MAOA* low-activity allele, especially as this allele has been associated with developmental plasticity (e.g., Belsky & Beaver, 2011; Belsky & Pluess, 2009; Kim-Cohen et al., 2006). Despite the fact that *COMT* and *MAOA* plasticity alleles are suspected of having opposite effects on dopamine signaling, they also have effects on multiple neurotransmitters (Illi et al., 2003; Matthys, Vanderschuren, & Schutter, 2013) and this may why both contribute to greater responsiveness to environmental conditions. To be clear, all other plasticity alleles considered herein have been related to less dopamine signaling (e.g., Bakermans-Kranenburg & Van IJzendoorn, 2011).

Dopamine modulates the value of rewarding experiences, thereby implying that dopamine signaling is needed to experience rewarding effects (e.g., Pessiglione et al., 2006; Schultz, 2010). It has been appreciated that, in particular, less dopamine signaling may result in: *reduced salience* of rewarding experiences (Buckholtz et al., 2010); *reduced valence* of specifically delayed rewarding experiences (Comings & Blum, 2000); *reduced arousal* in response to rewards (Schultz, 2002); and *reduced effects of reward-based learning* (Comings & Blum, 2000). As such, it might very well be that children with less dopamine signaling are possibly slower in their reward-based learning and, thus, need more immediate and salient rewarding feedback to increase rewarding experiences, reward-based learning, and physiological arousal (Matthys et al., 2012). Therefore, they may be more sensitive/responsive to immediate positive reward than children high on dopamine (Weeland et al., 2015). Since intervention efforts, like IY, are designed to foster skilled parenting by, in part, promoting immediate positive reinforcement to positive child behavior (i.e., reward and praise), children with less dopamine signaling may disproportionately benefit from positive change in parenting behavior, leading to decreases in externalizing behavior. But again in this regard additional work on neurochemistry is needed to address the exact role of *MAOA*, hopefully illuminating why it is that children with low-activity allele, presumably associated with more dopamine signaling, are more developmentally

responsive to environmental conditions (Byrd & Manuck, 2014; Ficks & Waldman, 2014; Taylor & Kim-Cohen, 2007).

In summary, the functional distinctions just made regarding a set of dopamine genes, based on a system-level approach and developmental plasticity, have not been considered in child developmental research on $G \times E$ or $G \times I$ interaction, even in investigations that have relied on polygenic indices (Belsky & Beaver, 2011; Chhangur, et al., in press; Musci et al., 2015; Thibodeau et al., 2015). Therefore, we explore the proposition that one or more of the three dopaminergic subsets of plasticity alleles just highlighted—receptor, transporter, enzyme—might be primarily responsible for the polygenic moderation of IY efficacy already detected in the case of boys (Chhangur et al., in press) about the same data set. This approach can shed light on which biological process(es) might specifically contribute to heterogeneity in program efficacy.

METHODS

Participants

Data for this report comes from the ORCHIDS study (Observational Randomized Controlled Trial on Childhood Differential Susceptibility) being carried out in The Netherlands (Chhangur, Weeland et al., 2012; Weeland, Chhangur et al., in press). ORCHIDS investigates differential effectiveness of IY intervention program aimed at reducing externalizing behavior in 4- to 8-year-old children. The study consisted of a screening phase in a general population sample and a randomized-controlled-intervention phase in a selected subsample of children with moderate-to-high levels of externalizing behavior. For detailed information about CONSORT schedule and sample descriptive, see Weeland, Chhangur et al., in press). Cheek cells were collected for purposes of DNA assaying from 385 children (failed genotyping assay: $n = 2$). Building on the report of Chhangur et al., (in press), the current study investigates only boys and their parents ($n = 212$) with the primary analysis-sample consisting of 190 families (excluding intervention dropouts: $n = 22$).

Design

Two months after the screening trained research assistants conducted pretest home visits to assess DNA and parent-child observations. Also, questionnaires (mailed to parents a week earlier) were collected. After the pretest, families were randomly assigned to either the control group ($n = 190$) or the intervention group ($n = 197$), with 100 boys in the former and 90 in the latter. Approximately four months (i.e., posttest) and eight months (i.e., follow-up) after the pretest, parents again completed child-behavior questionnaires and parent-child observations were assessed. The Institutional Review Board in The Netherlands (METC UMC Utrecht, protocol number 11-320/K) approved the study.

Measures

In view of the fact that measures used in the current report have been described in detail in the prior report documenting the polygenic moderation of IY efficacy in the case of boys (Chhangur et al., in press) on which the current study builds, they are only listed here in the interest of saving space: parent-reported externalizing behavior (ECBI; Eyberg & Pincus, 1999) and the five polymorphisms now scored in terms of the subsets to which they belong: receptors (*DRD2*, *DRD4*), enzymes (*MAOA*, *COMT*), and transporters (*DAT1*). Following Chhangur et al., (in press), scoring involved assigning one point whenever a boy carried at least one of the putative plasticity alleles; these values were then summed to create a plasticity index with the receptor and enzyme ranging from 0-2 and transporter 0-1. As in the prior work (Chhangur et al., in press), we only found a genetic moderation effect on reported, but not observed, externalizing behavior; thus, the latter measure was not used here. The distribution of boys scoring 0, 1 or 2 in the *receptor-subset* was, respectively, 46.0% ($n = 46$), 47.0% ($n = 47$), and 7.0% ($n = 7$) in the control group and 38.9% ($n = 35$), 48.9% ($n = 44$), and 12.2% ($n = 11$) in the IY intervention group. For the *enzyme-subset*, respectively, 15.0% ($n = 15$), 58.0% ($n = 58$), and 27.0% ($n = 27$) in the control group and 15.6% ($n = 14$), 55.6% ($n = 50$), and 28.9% ($n = 26$) in the intervention group. Finally, for the *transporter-subset*, respectively, 9.0% ($n = 9$) and 91.0% ($n = 91$) in the control group and 6.7% ($n = 6$) and 93.3% ($n = 84$) in the intervention group. Chi-square statistics showed no significant differences in distribution across groups.

RESULTS

Latent growth curve modeling (LGCM) in Mplus (Muthén & Muthén, 2008-2015) was performed to assess the development of externalizing behavior for males at the time of pretest, immediate-posttest (following four months of intervention) and follow-up (four months later). In three separate analyses, one for each dopamine-polymorphism subset (i.e., receptor, transporter, enzyme), a first model tested main effects of condition and genotype and a second the interaction of condition and genotype (with main effects included). In each set of analyses, the main effect of condition proved significant, but not the main effect of genotype (see Table 1). More importantly, the $G \times I$ interaction also proved significant for slope in the case of enzymes ($B_1 = -.348$, $p = .04$) ($\chi^2 [df = 4, n = 190] = 12.82$, CFI = .97, RMSEA = .11, partial $\eta^2 = .026$), though not for either the receptor subset ($B_1 = .035$, $p = .53$) ($\chi^2 [df = 4, n = 190] = 13.16$, CFI = .96, RMSEA = .11) or the transporter subset ($B_1 = -.015$, $p = .91$) ($\chi^2 [df = 4, n = 190] = 13.02$, CFI = .96, RMSEA = .11).

Inspection of Figure 1 reveals that for boys with the most enzyme-related plasticity alleles, the intervention was most effective in decreasing externalizing behavior, particularly by time of follow-up (partial $\eta^2 = .29$). Further, a set of planned comparisons revealed that boys with the most enzyme-related plasticity alleles in the

Table 1. G × E interactions Between Condition and Decomposed Dopaminergic Subsystems in Development of Reported Externalizing Behavior

Note. *df* = degree of freedom; CFI = comparative fit index; RMSEA = root mean square error of approximation; Condition; 0 = control group; 1 = intervention group. * $p < .05$.

Predictor	Intercept	Slope	χ^2 (<i>df</i>)	CFI	RMSEA
Receptor subset					
Condition	-.040 (0.08)	-.039 (0.02)*	4.82 (3)	.99	.06
Polygenetic plasticity alleles	-.004 (0.06)	.017 (0.01)			
Condition x Receptor Plasticity Index	-.110 (0.11)	.035 (0.06)	13.16 (4)	.96	.11
Transporter subset					
Condition	-.043 (0.08)	-.041 (0.02)*	5.11 (3)	.99	.07
Polygenetic plasticity alleles	.032 (0.13)	-.012 (0.02)			
Condition x Transporter Plasticity Index	-.040 (0.26)	.015 (0.13)	13.02 (4)	.96	.11
Enzyme subset					
Condition	-.039 (0.08)	-.042 (0.02)*	6.35 (3)	.99	.07
Polygenetic plasticity alleles	-.049 (0.05)	-.006 (0.01)			
Condition x Enzyme Plasticity Index	-.052 (0.11)	.113 (0.05)*	12.82 (4)	.97	.11

intervention group, in comparison to all other boys, (1) did not differ on externalizing behavior at pretest ($F(1, 184) = .91$, $p = .36$), but (2) scored significantly lower at follow-up ($F(1, 178) = 4.58$, $p = .03$) and thus (3) evinced significantly greater reduction from pretest to follow-up ($F(1, 178) = 12.95$, $p < .001$).

Two secondary analyses were undertaken to evaluate the robustness of the results just reported. In a first robustness check, the analysis was re-run including, as covariates, the other two gene subsets and the interaction of each of their component genes with treatment condition. This enabled us to evaluate the *unique* moderational effect of dopaminergic enzyme-related genes. Yet again the two-way interaction between the enzyme subset and condition proved significant for slope ($B_1 = -.166$, $p = .03$) ($\chi^2 [df = 8, N = 190] = 15.01$, CFI = .97, RMSEA = .07).

In the second robustness check, we repeated the LGCM analysis which originally included all genes in the polygenic index, but this time eliminating one subset of genes at a time from the index (i.e., first transporter and then receptor genes). This allowed us to examine changes to model fit or parameter estimates upon comparing the original polygenic index and these revised versions. The overall interaction between experimental condition (i.e.,

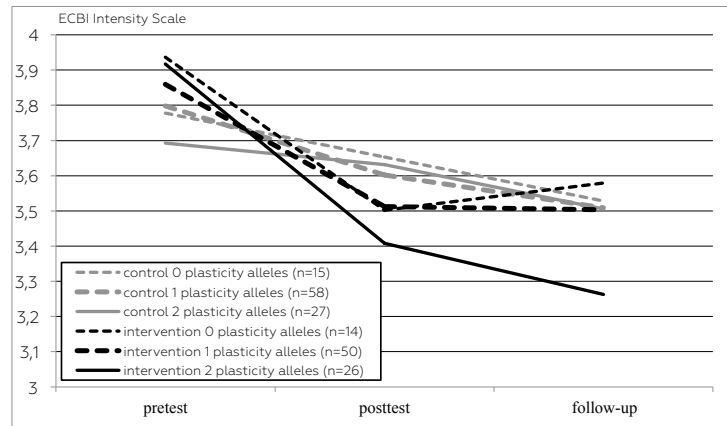


Figure 1. Development of reported externalizing behavior (as indicated by scores on the Eyberg Child Behavior Inventory [ECBI] Intensity Scale) across pretest, posttest, and follow-up for control and intervention group with 0, 1 or 2 putative enzyme-related plasticity alleles.

intervention vs. control) and the original polygenic plasticity index (0-5) proved significant for slope ($B_1 = -.091$, $p = .02$) ($\chi^2 [df = 4, N = 190] = 12.78$, $CFI = .97$, $RMSEA = .11$). After excluding the transporter genes, the interaction again proved significant ($B_1 = -.105$, $p = .02$) ($\chi^2 [df = 4, N = 190] = 12.54$, $CFI = .97$, $RMSEA = .11$). After excluding receptor genes (while retaining transporter genes), the interaction was no longer significant ($B_1 = -.098$, $p = .05$) ($\chi^2 [df = 4, N = 190] = 13.22$, $CFI = .96$, $RMSEA = .11$). These results indicate that even though the model fit did not change significantly, transporter genes nevertheless exerted a less significant influence on the effect of the polygenetic index than receptor or enzyme genes.

DISCUSSION

In this study, we built on the results of a prior report documenting the moderation of intervention efficacy in the case of boys scoring high on externalizing problems (Chhangur et al., in press). We endeavored to gain insight into which of three dopaminergic subsystems—receptor, transport or enzyme—might be principally responsible for the polygenic moderation effect of the IY effects in the development of externalizing problems and in turn, which biological process contributes to differential responsiveness to the intervention efforts. To our knowledge, no such functional distinctions based on a system-level approach have been pursued to date in either $G \times E$ or $G \times I$ research, including studies using polygenic indices (Belsky & Beaver, 2011; Musci et al., 2015; Thibodeau et al., 2015).

Results revealed that the originally detected polygenic-moderational effect was due to the subset of enzyme genes, and not the subset of genes encoding dopamine receptor or transporter functions. Boys carrying many enzyme-related plasticity alleles decreased significantly more in externalizing behavior as a result of their parents' involvement in the IY program, compared to boys carrying fewer such alleles. Moreover, this effect remained even when those of the other dopaminergic subsets were controlled. And notably, when adding these subsets as covariates the model fit improved significantly, from being suboptimal ($RMSEA = .11$) to optimal ($RMSEA = .07$). Important to appreciate, however, is that we conducted three separate tests of $G \times I$. Accordingly, the traditional Bonferroni correction for multiple testing or even the less stringent Benjamini-Hochberg False Discovery Rate correction (i.e. Benjamini & Hochberg, 2005) used in our report on the overall IY effectiveness (see Weeland, Chhangur et al., in press), would have resulted in a nonsignificant effect. This observation highlights the need for replication of the results reported herein.

The $G \times I$ effects detected herein and when using the original polygenic index (Chhangur et al., in press) are consistent with differential susceptibility thinking (Belsky et al., 2007; Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005) in that children with the most enzyme-related plasticity alleles were more sensitive to positive environmental change, induced by IY, with the (untested) presumption being that they would also be more susceptible to the adverse effects of negative contextual conditions (e.g., Belsky & Pluess, 2009; Kim-Cohen et al., 2006). However, as children were screened to have relatively high levels of behavior problems at pretest—presumably indicating an at risk group—we expected the same genetic subgroup that reaped the most benefit from the intervention to also show most externalizing behavior if assigned to the control group (Chhangur, Weeland et al., 2012). This is because environmental risk conditions (i.e., negative parenting) might remain stable or intensify if not treated (Patterson, 1982). This, however, did not prove to be the case.

Enzyme-related dopaminergic genes, relative to receptor- and transporter-related ones, may be especially influential because—apart from their degradation of dopamine—these enzymes are also involved in the degradation of norepinephrine and serotonin (Illi et al., 2003; Matthys et al., 2013). Thus, because these polymorphisms impinge on multiple neurotransmitter systems, they may have an especially strong role in children's susceptibility to environmental signals, and as such may be contributing most directly to the differential-susceptibility effect chronicled in this paper. Ultimately, further work is required to gain insight into specific endophenotypic processes that could explain how the two enzyme genes in question influence dopamine signaling in the brain and, thus, make some boys seemingly more susceptible to the beneficial effects of IY than others. Also, more research is needed to better understand the relation between the functioning of the neurotransmitter systems (e.g., dopaminergic, serotonergic, and noradrenergic) and differential susceptibility processes based on reward and punishment (Matthys et al., 2012). Readers are referred in this regard to a recent paper by Moore and Depue (2016) for a most insightful

description of neurobehavioral foundations of environmental reactivity.

Our initial strategy for decomposing the originally composited dopaminergic genes—each of which had individually been implicated in moderating environmental effects in a differential-susceptibility-related manner—was based on the view that the five genes have different functions that influence dopamine signaling and that it is the additive effect of receptor, transporter, or enzyme that makes some children especially susceptible to the changes in parenting that are presumed to follow from parental participation in the IY program (Nikolova et al., 2011). However, the precise role of the low activity *MAOA* allele remains unclear as this allele is presumably functionally associated with higher enzyme density and thus more dopamine signaling in the brain (Boulton & Eisenhofer, 1997), while the *COMT* val allele is functionally associated with lower enzyme density and thus less dopamine signaling (Chen et al., 2004). Thus, although we gained more insight into $G \times I$ using a subsystem-level approach based on the functioning of particular dopaminergic genes, more research is needed at an endophenotypic level to illuminate mechanisms of influence. Also, since this is the first study in child-research on $G \times E$ that made a distinction between dopaminergic subsets, replication is clearly needed.