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

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GENETIC MODERATION OF INTERVENTION EFFICACY:
DOPAMINERGIC GENES, THE INCREDIBLE YEARS,
AND EXTERNALIZING BEHAVIOR IN CHILDREN

CHAPTER 6

GENETIC MODERATION OF INTERVENTION EFFICACY: DOPAMINERGIC GENES, THE INCREDIBLE YEARS, AND EXTERNALIZING BEHAVIOR IN CHILDREN

KEYWORDS — Externalizing behavior
Gene-by-environment interaction
Dopaminergic plasticity

ABSTRACT — **This study investigated whether children scoring higher on a polygenic plasticity index based on 5 dopaminergic genes (DRD4, DRD2, DAT1, MAOA, and COMT) benefited the most from The Incredible Years (IY) parent program. Data were used from a randomized-controlled-trial including 341 Dutch families with 4-8-year-old children (55.7% boys) showing moderate-to-high levels of problem behavior. IY proved most effective in decreasing parent-reported (but not observed) externalizing behavior in boys (but not girls) carrying more rather than fewer dopaminergic plasticity alleles; this gene-×-intervention effect was most pronounced in the case of boys whose parents' manifested the most positive change in parenting in response to the intervention. These results proved robust across a variety of sampling specifications (e.g., intention to treat, ethnicity).**

Elevated levels of externalizing behavior (e.g., aggression, oppositional behavior, disobedience) in the early years of life might forecast a variety of problems later in childhood (Campbell et al., 2006). Left untreated, externalizing behavior often worsens with age and tends to persist over time (Mesman, Bongers, & Koot, 2001; Vaughn et al., 2013), generating substantial social and economic costs to individuals and society (Raaijmakers et al., 2011; Scott et al., 2001). These observations underscore the importance of early intervention to ameliorate such early emerging problems. Some of the most effective interventions in this regard are designed to increase positive parenting behavior. Such behavioral parent training programs employ parents as change agents, enabling them to deploy more positive parenting practices, thereby reducing problematic child behavior (Forehand, Lafko, Parent, & Burt, 2014).

Not all children benefit equally from intervention-induced changes in positive parenting behavior. Conversely, not all children who appear to be at risk for developing externalizing problems—as a result of exposure to unsupportive, negative and harsh parenting—do so. These differential responses to, respectively, parenting support and risk raise questions about the source of such heterogeneity. For quite some time now it has been presumed that some children are more at risk because of their own characteristics of "vulnerability", be they temperamental, physiological and/or genetic in nature. Indeed, this notion is central to the long-standing diathesis-stress model of person-×-environment interaction which has informed much research (e.g., Zuckerman, 1999). However, this "vulnerability" framework is less adequate in explaining heterogeneity in response to environmental support and enrichment.

Recently, an alternative person-×-environment framework has been advanced which explicitly addresses differential response to both risk and support. This differential susceptibility perspective stipulates that the very personal characteristics that make a child especially vulnerable to adversity may also enable him or her to benefit more so than others from support and enrichment (Belsky et al., 2007; Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005). In so doing, this evolutionary inspired framework implies that it will be children regarded as especially vulnerable to adversity due to their personal characteristics who would benefit the most from efforts to promote well-being, to prevent problems from developing in the first place, and to ameliorate existing problems (Belsky & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015).

Here we test this differential-susceptibility-derived proposition that some children are more susceptible to intervention-induced environmental change than others by focusing on their genetic make-up, Specifically, we test the hypothesis that children manifesting moderate-to-high levels of problem behavior and carrying more of particular variants of a set of dopaminergic genes (i.e., "plasticity alleles") will benefit more than their peers from The Incredible Years (IY) parenting intervention (Webster-Stratton, 2001b). This prediction is also based on recent research on the genetic moderation of intervention efficacy (Belsky & Van IJzendoorn, 2015; Van

IJzendoorn & Bakermans-Kranenburg, 2015) and evidence that dopaminergic genes moderate environmental effects in a differential-susceptibility-related, "for-better-and-for-worse" manner (Bakermans-Kranenburg & Van IJzendoorn, 2011). Because IY seeks to change children by increasing positive parenting, including their use of praise, tangible rewords and other positive reinforcements (e.g., Beauchaine, Webster-Stratton, & Reid, 2005; Gardner, Hutchings, Bywater, & Whitaker, 2010), we also predicted, following Bakermans-Kranenburg and colleagues (2008), that it would be children who carried the most putative plasticity alleles *and* whose parents evinced the most increase in positive parenting in response to the IY program who would benefit the most from the intervention.

Parenting and externalizing behavior

Extensive evidence indicates that parenting behavior is longitudinally associated with child behavior (e.g., Miner & Clarke-Stewart, 2008). Negative parenting strategies in particular are related to elevated levels of externalizing behavior, including inconsistent discipline, disapproval, harshness, physical discipline, lack of positive tone, and coercion (Gershoff, 2002; Pettit & Bates, 1989; Shaw et al., 1994). In contrast, positive parenting strategies that convey warmth and acceptance and provide positive consequences for desirable behavior while enhancing the parent-child relationship can prevent externalizing behavior from persisting and increasing over time (Dishion et al., 2009; Gardner, Shaw, Dishion, Burton, & Supplee, 2007).

The Incredible Years program

Given these observations, it is not surprising that efforts to prevent the development of severe externalizing behavior have targeted parenting, seeking to reduce negative, while promoting positive parenting practices. IY parent training (IY; Webster-Stratton, 2001b) is one such program designed to prevent the development of child externalizing behavior problems or to ameliorate early emerging problems and is therefore the specific focus of this report. IY has been evaluated in over 50 studies and proven effective in both clinical and community samples (Menting, Orobio de Castro, & Matthys, 2013; Webster-Stratton & Hammond, 1997; Weeland, Chhangeur et al., in press). IY intervention effects have been replicated independently (e.g., Scott, Spender, Doolan, Jacobs, & Aspland, 2001) and results include reductions in externalizing behavior that endure several years after exposure to the program (e.g., Jones et al., 2008; Posthumus et al., 2012).

Important to appreciate, however, is that average effect size of IY and most other parenting interventions are small to moderate in magnitude (McCart et al., 2006), with children varying in the degree to which they benefit from the program. In fact, a recent meta-analysis of IY effectiveness chronicled a substantial effect in treatment studies, but a small effect in indicated prevention research on children identified as having minimal but detectable signs or symptoms (*d* = .20, Menting et al., 2013). Even

the most successful interventions for externalizing behavior might be effective for only about two thirds of children (see Webster-Stratton & Hammond, 1997). Such results highlight the need to illuminate potential moderators of intervention efficacy. It seems plausible that such determinants of variation in response to intervention could be genetic in character, as responsiveness to changes in parenting may depend on reward sensitivity. Specifically, the dopaminergic system would seem to play a pivotal role due to its link with reward sensitivity and reinforcement learning (Bakermans-Kranenburg & Van IJzendoorn, 2011).

Differential susceptibility to environmental influences

Decades of research informed by the diathesis-stress model of person-environment interaction (Zuckerman, 1999) made clear that some individuals are more susceptible to the negative effects of exposure to diverse conditions of contextual adversity (e.g., poverty, depressed mother, harsh parenting); and that this enhanced vulnerability to adversity might be a function of their personal characteristics (i.e., temperamental, physiological or genetic characteristics). Recent theorizing has called attention to the fact that the very individual attributes that appear to make some children more vulnerable to adversity might also make them more likely to benefit from supportive environmental conditions. Indeed, differential-susceptibility theorizing postulates that some children are more developmentally plastic or malleable "for better and for worse" rather than just more likely to succumb to negative rearing conditions (Belsky et al., 2007; Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005).

A growing body of evidence is consistent with this claim (Belsky & Pluess, 2009, 2013). Especially important for purposes of the current study is research documenting the role of dopaminergic genes in moderating a variety of environmental effects in a for-better-and-for-worse, differential-susceptibility-related manner. For example, Foley and colleagues (2004) found that boys with the *MAOA* low-activity allele were more likely than their high-activity counterparts to be diagnosed with conduct disorder when exposed to high levels of childhood adversity, but were less likely to do so when exposed to low levels of adversity. The polymorphisms selected for inclusion in the work reported herein have been found to moderate environmental effects in gene by environment (G × E) research in a similar differential-susceptibility-related manner (Belsky & Pluess, 2013; Van IJzendoorn & Bakermans-Kranenburg, 2015). The current investigation is therefore specifically conceptualized in such terms rather than in terms of vantage sensitivity—which refers to factors that make some individuals more susceptible to positive exposures but does not make them more susceptible to negative ones (Pluess & Belsky, 2013).

Why dopaminergic genes?

Dopamine is an excitatory neurotransmitter involved in motivational, attentional, and reward processes. It is heavily expressed in dopaminergic pathways in the brain

(e.g., the ventral tegmental area, nucleus accumbens, and prefrontal cortex) where it appears to modulate excitatory signaling (Blum et al., 1996). This signaling plays an important role in reward processing. Different dopaminergic polymorphisms are known to alter reward processing—that makes children and adolescents apparently more or less prone to environmental cues of reward by affecting how much dopamine moves into a synapse or how quickly it is reabsorbed or degraded (Matthys et al., 2013; Moore & Depue, 2016). This might influence how susceptible children are to parenting practices based on reward. Here we focus on the *cumulative function* of several polymorphisms that are known to affect levels of dopamine signaling in the brain, and thereby possibly contribute to individual differences in reward processing: the 7-repeat allele of *DRD4*, the A1 allele of *DRD2*, the 10-repeat allele of *DAT1*, the low-activity allele of *MAOA*, and the val allele of *COMT*.

All the just-listed allelic variants have been linked to increased sensitivity to environmental influences in a for-better-and-for-worse manner (Belsky & Pluess, 2009; 2013). Consider in this regard evidence of Laucht and colleagues (2007) showing that adolescents carrying the *DAT1* 10-repeat allele manifested most and least inattention problems when living under high and low aversive conditions, respectively, compared to other children. Relatedly, Kim-Cohen and colleagues (2006) found that boys carrying the *MAOA* low-activity allele were rated by teachers and mothers as having more mental health problems than other boys when experiencing physical abuse, but fewer problems when not mistreated. Notable, in fact, is Bakermans-Kranenburg and Van IJzendoorn's (2011) meta-analysis showing that individual dopamine-related genes moderate diverse environmental effects in a differential-susceptibility-related manner in the case of children under 10 years of age. Just as noteworthy, however, is that G × E results of correlational studies have proven difficult to replicate and the interpretation of G × E effects has not always been straightforward (e.g., Duncan & Keller, 2011).

Gene-×-Intervention interaction

A major step forward in testing G × E builds on an experimental paradigm that involves intervention. As such, G × I (gene-by-intervention) research has several advantages (Belsky & Van IJzendoorn, 2015; Van IJzendoorn & Bakermans-Kranenburg, 2015). First, it eliminates possible gene-environment correlations (*r*GE) that plague interpretation of virtually all G × E work (Bakermans-Kranenburg & Van IJzendoorn, 2015; Chhangur, Weeland, Matthys, & Overbeek, 2015). Second, G × I research provides a means for establishing differential susceptibility due to its focus on environmental enrichment. Third, standardized interventions afford precise and thus reliable measurement of the environment, thereby reducing measurement error. Fourth, G × I designs provide considerably more statistical power due to the dichotomous parameterization of an environmental factor (i.e., experimental/control), as well as a targeted focus on "at risk" samples (Bakermans-Kranenburg & Van IJzendoorn, 2015).

Research on genetic moderation of intervention efficacy is growing following a pioneering study showing that video-feedback intervention designed to reduce externalizing behavior by promoting sensitive parenting and positive discipline proved effective only for children carrying 7-repeat alleles of the *DRD4* gene (Bakermans-Kranenburg et al., 2008). More recent research further revealed that children carrying the 7-repeat allele disproportionately benefited from computerized training designed to enhance phonemic awareness (Kegel, Bus, & Van IJzendoorn, 2011) and, separately, text comprehension (Plak, Kegel, & Bus, 2015). Working with older children—African-American adolescents growing up in rural Georgia—Brody, Yu, and Beach (2015) observed that a family-based intervention designed to prevent, among other things, substance use, proved effective principally for teenagers carrying this same putative plasticity allele. Most notably, Van IJzendoorn and Bakermans-Kranenburg (2015) meta-analysis found that genetic moderation of efficacy proved to be the norm, even across interventions of varying intensity (Van IJzendoorn & Bakermans-Kranenburg, 2015). One limitation of virtually all G × I work to date, however, is its focus single candidate genes, thus failing to do justice to the polygenic nature of development.

Current study

The research reported herein evaluates the genetic moderation of the efficacy of the IY program. Rather than focusing on a single candidate gene we employ a systems' approach (Nikolova et al., 2011), creating a dopaminergic polygenetic composite (based on the allelic variants already highlighted). Following Belsky and Beaver (2011), we gave children one point for each polymorphism for which they had at least one putative plasticity allele (i.e., 0/1 scoring, range: 0-5). We predicted that children scoring highest on the polygenic index would show the greatest decrease in externalizing behavior in response to the IY intervention; and that this would be especially so when parents evinced substantial rather than limited improvement in their positive parenting behavior in response to intervention. In other words, it would be children carrying many rather than few plasticity alleles whose parents changed the most who would benefit most from the IY program. After evaluating these predictions with all children, we conducted a series of sensitivity analyses based on appreciation that (1) inclusion of children who vary in their race/ethnicity could be problematic when genetics are a focus of interest (Propper, Willoughby, Halpern, Carbone, & Cox, 2007) and (2) results can differ when only cases with complete data are studied vs. when an intention-to-treat design is employed. In all analyses, G × I effects were tested separately for boys and girls because one of the polymorphisms included in our polygenic index, the *MAOA*, is sex linked (Byrd & Manuck, 2014).

METHODS

The ORCHIDS study

Data for the research reported here comes from the ORCHIDS study (Observational Randomized Controlled Trial on Childhood Differential Susceptibility) conducted in The Netherlands. Data were collected in two cohorts from November 2012/2013 through November 2013/2014. Detailed information about the sample and sampling appears elsewhere (Chhangur, Weeland et al., 2012; Weeland, Chhangur et al., in press). Using a randomized control design, the ORCHIDS study addresses the differential effectiveness—across children with varying temperamental and genetic character-istics—of the IY program in reducing externalizing behavior in 4-8-year-old children showing moderate-to-high levels of such problems by enhancing a warm parent-child relationship through child directed play, coaching of social, emotional, and academic skills, praise and rewards, effective limit setting, and handling (e.g., ignore and time-out techniques).

Sample

Participants were recruited in two cohorts via two Dutch regional health care organ-izations. Parents of 20,084 children aged 4-8 were mailed a questionnaire to assess frequency of externalizing child behavior (i.e., screening stage), resulting in 5,876 questionnaires returned in a timely manner (response rate: 22.5%). Children scoring at or above the 75th percentile on the Eyberg Child Behavior Inventory (ECBI) Intensity Score scale (n = 1,524) were eligible for the RCT. If parents reported moderate-to-high levels of externalizing behavior of multiple children within a family, the child with highest ECBI Intensity score was invited. A total of 1,393 mother-child or father-child dyads were thus invited to participate and 61% of these to-be-recruited families were reached; 46% of the latter (N = 387) agreed to participate. ECBI Intensity scores from screened families (M = 2.65, SD = .52) differed from invited families (M = 3.59, SD = .46; t (5872) = -89.57, p < .001) and from those who agreed to participate (M = 3.64, SD = .47; t(5872) = -28.90, p < .001), though those who agreed to participate scored somewhat higher than those invited but did not participate (t (1522) = -2.54, p = .01). Cheek cells were collected for DNA assaying from 385 children (failed genotyping assay: n = 2), with the primary-analysis sample consisting of 341 of these children and their parents; 44 families randomized to the intervention group are excluded from the primary analysis because they did not attend any of the intervention sessions. Notably, those excluded (M = 3.66) did not differ from those included on the ECBI Intensity score (M = 3.71, p = .56). Most parents participating in the RCT were mothers (89.4%), Caucasian (90.5%), and well educated (23.3% vocational training; 42.9% higher vocational training or university). Intervention and control groups did not differ on sample characteristics (i.e., age, child age, gender, number of siblings, educational level, ethnicity) and baseline externalizing behavior (see Table 1).

Table 1. Sociodemographic Information and Initial Level of Externalizing behavior for Control and Intervention Group by Gender

	Total	(N = 190)	Control	(n = 100)	Intervention (n = 90)	
Boys						
Age child (years)	6.3	1.34	6.4	1.38	6.1	1.29
Age parent (years)	37.9	4.70	38.0	4.63	37.0	4.81
% mother	92.6		94.0		91.1	
Number of children	2.3	0.82	2.3	0.77	2.3	0.89
Education mother[a]						
% low	19.5		19.0		20.0	
% medium	27.4		26.0		28.9	
% high	52.6		45.0		50.0	
Education father[a]						
% low	21.1		21.0		21.1	
% medium	28.4		24.0		33.3	
% high	47.9		51.0		44.4	
% Caucasian mother	91.5		91.0		91.1	
% Caucasian father	90.5		91.0		88.9	
% single parent	9.5		7.0		12.2	
Externalizing behavior	3.8	0.50	3.8	0.51	3.9	0.49
Girls	Total	(N = 151)	Control	(n = 90)	Intervention (n = 61)	
Age child (years)	6.3	1.25	6.2	1.18	6.4	1.35
Age parent (years)	38.4	4.97	39.3	4.99	38.4	4.98
% mother	89.4		88.0		90.2	
Number of children	2.2	0.76	2.2	0.79	2.3	0.73
Education mother[a]						
% low	20.7		18.9		23.3	
% medium	27.3		30.0		23.3	
% high	51.3		50.0		53.3	
Education father[a]						
% low	31.3		32.2		30.0	
% medium	23.3		24.4		21.7	
% high	42.9		41.1		45.0	
% Caucasian mother	93.4		95.6		90.2	
% Caucasian father	92.1		95.6		86.9	
% single parent	8.0		5.6		11.7	

Note. [a]low = completed middle or high school; medium = completed vocational training; high = completed higher vocational training or university. None of the groups differed significantly on sociodemographic characteristics and initial parent-reported externalizing behavior with independent samples t test or χ² -test.

Design and procedure

Two months following screening trained research assistants conducted pretest home visits to collect DNA and observational data from the child, questionnaires to measure child externalizing behavior and positive parenting behavior were emailed to parents a week earlier. Subsequently, families were randomly assigned (ratio: 1:1) to control (n = 190) or intervention group (n = 197); recall that 44 families allocated to intervention did not attend any sessions, thus being excluded from the primary

analyses. Approximately four and eight months after the pretest (i.e., posttest, follow-up), parents again completed the questionnaires and observations were made of child externalizing behavior. The Institutional Review Broad in The Netherlands (METC UMC Utrecht, protocol number 11-320/K) approved the study.

Incredible Years parent training

The IY program uses a collaborative group approach; group leaders serve as facilitators rather than experts while seeking to empower parents. Issues and topics addressed include the importance of child-directed play, social-and-emotion coaching, the use of praise to reward and incentives to motivate appropriate behavior, and the importance of consistency in the use of non-corporal disciplinary practices. This trial involved 14 weekly sessions in which parents watched and discussed video-vignettes of parent-child interactions, engaged in role playing, and discussed family experiences in small (sub)groups. More specifically, following each vignette, group leaders asked questions to stimulate discussion about (in)effective parenting behavior and alternative approaches. Before sessions, parents received exercises to practice at home, read relevant literature, and practiced behavior-management skills with their child. A final and 15th session took place a month following the 14th session, serving as a "booster" to consolidate intervention effects by repeating, discussing, and practicing skills. The intervention groups consisted of 8-15 parents. Parents attended an average of 11 of the 15 sessions (*M* = 10.85; *SD* = 3.95). Although the parent who filled out the questionnaires attended the sessions, the other parent was allowed to do so as well. Every group was led by two group leaders. Main leaders had a background in clinical child psychology, had experience running IY groups before the study commenced, and were officially certified group leaders. All leaders received two-hour supervision sessions at least three times across the 14-week period. Besides these, regular inter-session meetings between group leaders took place (see also Weeland, Chhangur et al., in press).

Measures

Parent-reported externalizing behavior

The Eyberg Child Behavior Inventory (ECBI; Eyberg & Pincus, 1999), used at screening, pretest, posttest, and follow-up, assessed child externalizing problems. One of the instrument's two scales is the focus of in this report. This Intensity subscale consists of 36 items tapping frequency of externalizing behavior using a 7-point Likert-type scale (0 = *never* to 7 = *always*). Example items are: 'Does not obey house rules' and 'Whines'. The internal consistencies (Cronbach's alpha) were .85, .86, and .88, respectively, at pretest, posttest, and follow-up.

Observed externalizing behavior

The Dyadic Parent–Child Interaction Coding System (i.e., DPICS) was used to measure observed externalizing behavior (Robinson & Eyberg, 1981; Webster-Stratton, 1989). At all three measurement times, parent-child dyads were observed for 20 minutes, divided into four five-minute episodes: (1) free play (i.e., to get used to being videotaped), (2) child-directed play (i.e., child picked a toy and directed the session) (3) parent-directed play (i.e., parent picked a toy and directed the session), and (4) clean-up (i.e., parent instructed child to clean-up). In the latter three episodes, negative child behavior was coded using 5 categories: indirect command non-compliance, direct command non-compliance, cry-whine-yell, destructive behavior, and physical negative behavior. A total summed score based on these three episodes and reflecting the frequency of these behaviors served as the observational index of externalizing behavior. The inter-episodes correlations among within-episodes composites were significantly correlated at pretest (*r* = .11-.48), posttest (*r* = .25-.40), and follow-up (*r* = .17-.38) measurement occasions. Observations were coded by six trained research assistants blind to condition and measurement wave. Monthly calibration meetings were held to prevent observer drift. A random 20% of observations were independently coded by two coders unaware which observations would be used to assess inter-observer agreement. Interrater reliability, based on intraclass correlation (ICC), was .83, .82, and .70, respectively, at pretest, posttest, and follow-up.

Parent-reported positive behavior

The Parent Practices Inventory (PPI; Webster-Stratton, 2001a) used at all measurement occasions assessed parenting skills and discipline styles. Although positive parenting behavior was also observed, the fact that little variance was detected in the observational data precluded us from using this measure as an outcome. The PPI consists of 15 scales, each containing several items related to how parents typically respond to their child's appropriate and inappropriate behavior (measured using a 7-point Likert-type scale: 0 = *not likely at all/never* to 7 = *very likely/always*). For this report, praise and incentives (11 items) and positive verbal discipline (9 items) were combined to create an index of positive parenting behavior (e.g., 'When my child complete chores I praise him', 'When my child refuses do to something I discuss the problem with him', and 'When my child behaves well, it is important to set up rewards or privileges'). Cronbach's alphas were .73, .76, and .79, respectively, at pretest, posttest, and follow-up.

Genotyping

Genotyping was conducted at *BaseClear* laboratories, Leiden, The Netherlands, using well-established methods. Buccal swabs collected from children were incubated in lysis buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 0,1 mg/ml proteinase K, and 0,5% w/v SDS) until further processing. Genomic DNA was isolated from the samples

using the Chemagic buccal swab kit on a Chemagen Module I workstation (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). All analyses were performed automatically using specialized genotyping software. Each plate's results were checked by a lab-worker (and checked by a second worker); those showing notable deviations or failings were repeated. As a control check, each 96wells plate contained one blank and analyses were continued only if the blank showed a negative result. Overall, genotyping yielded a success rate of approximately 99% or higher for the 5 polymorphisms central to this report.

DRD2 and COMT

To determine the SNP's of the *DRD2* rs1800497 and COMT rs4680 polymorphisms, 1ul of the isolated samples were analyzed using TaqMan® chemistry (Cat. # 4351379, Applied Biosystems). Samples were run on an ABI-7500 Real-Time PCR instrument and data were analyzed using 7500 System SDS software. *DRD2* genotypes (n = 247 A2/A2, n = 122 A2/A1, n = 14 A1/A1) were in Hardy–Weinberg equilibrium (HWE), χ^2 (1, n = 383) = .05, p = .82 (n = 4 no genotyping); *COMT* genotypes (n = 90 met/met, n = 185 met/val, n = 108 val/val) were in HWE, χ^2 (1, N = 383) = .04, p = .84 (n = 4 no genotyping).

DRD4

For all VNTR polymorphisms (i.e., *DRD4*, *DAT1*, and *MAOA*) one microliter of PCR product was mixed with 0.3 ml LIZ-500 size standard (Applied Biosystems) and 11,7 ml formamide (Applied Biosystems) and run on a AB 3730 genetic analyser set up for fragment analyses with 50 cm capillaries. Results were analysed using GeneMarker software (Softgenetics).The region of interest from the *DRD4* gene was amplified by PCR using the a FAM-labelled primer 5'- GCGACTACGTGGTCTACTCG-3' and a reverse primer 5'- AGGACCCTCATGGCCTTG-3'. Typical PCR reactions contained between 10 and 100 ng genomic DNA templates, 10 pmol of forward and reverse primer. PCR was carried out in the presence of 7.5% DMSO, 5x buffer supplied with the enzyme and with 1.25U of LongAmp *Taq* DNA Polymerase (NEB) in a total volume of 30 ml using the following cycling conditions: initial denaturation step of 10 min at 95oC, followed by 27 cycles of 30 sec 95oC, 30 sec 60oC, 60 sec 65oC and a final extension step of 10 min 65oC. Genotypes (n = 248 no7-repeat/no7-repeat, n = 119 no7-repeat/7-repeat, n = 8 7-repeat/7-repeat) were in HWE, χ^2 (1, N = 375) = 2.11, p = .15 (n = 12 no genotyping).

DAT1

The region of interest from the *DAT1* gene was amplified by PCR using the FAM-labelled primer 5'-TGTGGTGTAGGGAACGGCCTGAG-3' and a reverse primer 5'-CTTCCTGGAGGTCACGGCTCAAGG-3'. Typical PCR reactions contained between 10 and 100 ng genomic DNA templates, 10 pmol of forward and reverse primer. PCR was carried out in the presence of 3.3% DMSO with 1.25U of LongAmp *Taq* DNA Polymerase (NEB) in a total volume of 30 ml using the following cycling conditions: initial denaturation

step of 5 min at 95oC, followed by 29 cycles of 30 sec 95oC, 30 sec 68oC, 60 sec 65oC and a final extension step of 5 min 65oC. Genotypes (n = 31 no 10-repeat/no10-repeat, n = 148 no10-repeat/10-repeat, n = 203 10-repeat/10-repeat) were in HWE, χ^2 (1, N = 382) = .03, p = .86 (n = 5 no genotyping).

MAOA

The region of interest from the *MAOA* gene was amplified by PCR using the FAM-labelled MR primer (5'-GGATAACAATTTCACACAGG-3'), forward primer (5'-ggataacaatttcacacag-gACAGCCTGACCGTGGAGAAG-3') and a reverse primer (5'-GGACCTGGGCAGTTGTGC-3'). Typical PCR reactions contained between 10 and 100 ng genomic DNA templates, 1 pmol of forward primer, and 10 pmol of labelled MR and reverse primers. PCR was carried out in the presence of 5% DMSO with 1.25U of LongAmp *Taq* DNA Polymerase (NEB) in a total volume of 30 ml using the following cycling conditions: initial denaturation step of 5 min at 94oC, followed by 38 cycles of 30 sec 94oC, 30 sec 55oC, 30 sec 72oC and a final extension step of 4 min 72oC. Genotypes for boys were n = 80 low/low and n = 123 high/high (n = 11 no genotyping). Since boys have only one X chromosome, only girls were included in the HWE calculation. Genotypes for girls (n = 23 low/low, n = 75 low/high, n = 96 high/high were in HWE, χ^2 (1, n = 167) = 0.13, p = .73 (n = 6 no genotyping).

Polygenic scoring

Each polymorphism was assigned a point if the child was carrying at least one of the putative plasticity alleles; these values were then summed to create a polygenetic plasticity index ranging from 0-5. To create groups of reasonable size for statistical analysis, children scoring low (0-2) and high (3-5) on this polygenic index were distinguished. The distribution of those scoring low or high on the polygenic index of plasticity was, for boys, respectively, 23.2% (n = 44) and 29.5% (n = 56) in the control group and 17.9% (n = 34) and 29.5% (n = 56) in the intervention group. The comparable distribution for girls was, respectively, 19.9% (n = 30) and 39.7% (n = 60) in the control group and 12.6% (n = 19) and 27.8% (n = 42) in the intervention group.

RESULTS

Latent growth curve modeling (LGCM) in Mplus (Muthén & Muthén, 2008-2015) was performed initially on all children, irrespective of their race/ethnicity and separately for boys and girls, to assess the development of externalizing behavior across pretest, posttest, and follow-up assessments. Because individual growth is estimated for each child, LGCM is an excellent approach for examining variation in the development of externalizing behavior while considering whether certain predictors are associated with differential trajectories. Full information maximum likelihood (FIML) was used to treat missing data. Experimental condition (i.e., intervention vs. control) and

Table 2. G × E Interactions Between Condition and Polygenetic Plasticity Index in Development of Reported Externalizing Behavior by Gender

Note. df = degree of freedom; CFI = comparative fit index; RMSEA = root mean square error of approximation. As $\chi^2 <$ df, the CFI is set to 1.0 and RSMEA to <.001, which makes it sufficient to read off whether the p value is not significant; Condition; 0 = control group; 1 = intervention group; *p < .05. **p < .01.

Predictor	Intercept	Slope	χ^2 (df)	CFI	RMSEA	p value
Male (n = 190)						
Condition	-.036 (0.08)	-.041 (0.02)*	6.70 (3)	.99	.08	
Polygenetic plasticity alleles	-.030 (0.07)	.010 (.00)				
Condition × Polygenetic Plasticity Index	-.022 (0.15)	-.183 (0.07)**	2.24 (4)	1.00	<.001	.84
Female (n = 151)						
Condition	-.001 (0.08)	-.055 (0.03)*	6.46 (3)	.98	.09	
Polygenetic plasticity alleles	-.162 (0.07)	-.005 (0.02)*				
Condition × Polygenetic Plasticity Index	.038 (0.17)	.085 (0.10)	6.82 (4)	.98	.07	

the polygenetic plasticity index (i.e., more vs. few) served as predictors in the primary model. Because the intervention was focused on inducing positive parenting behavior, and presumed to affect children by changing parenting, we used a Parallel Process LGCM to evaluate whether genetically moderated intervention effects were more pronounced when parents increased more rather than less in positive parenting behavior (i.e., gene-×-slope positive parenting on slope child externalizing behavior) (Cheong, MacKinnon, & Khoo, 2003). Model fit is considered good if the Root Mean Square Error of Approximation (RMSEA) is < .08 and mediocre if <.10. Confirmatory Fit Index (CFI) values should be >.95 (Hu & Bentler, 1999).

Genetic, intervention, and gene-×-intervention effects

Parent-reported child externalizing behavior

With regard to specific effects, we consider first effects on problem behavior at pretest before turning attention to change over time (i.e., slope). Inspection of Table 2 indicates that for both boys and girls there were no significant main effects of treatment condition (i.e., IY vs. control) on the pretest intercept (boys: B_0 = -.036, p = .65; girls: B_0 = -.001, p = .99), thereby indicating that the randomization process proved effective in equating groups for initial levels of problem behavior. For girls there was a significant main effect of the polygenic index of plasticity on the pretest intercept (B_0 = -.163, p = .03), indicating that girls who scored high on the index had fewer problems initially. Turning to the prediction of slope, treatment condition proved significant in the case of both boys (B_1 = -.041, p = .04) and girls (B_1 = -.055, p = .05), revealing that parent-reported externalizing behavior decreased more in the intervention than control group. The models of

Table 3. G × E Interactions Between Condition and Polygenetic Plasticity Index in Development of Observed Externalizing Behavior by Gender

Note. df = degree of freedom; CFI = comparative fit index; RMSEA = root mean square error of approximation. As $\chi^2 <$ df, the CFI is set to 1.0 and RSMEA to <.001, which makes it sufficient to read off whether the p value is not significant; Condition; 0 = control group; 1 = intervention group. *p < .05. **p < .01.

Predictor	Intercept	Slope	χ^2 (df)	CFI	RMSEA	p value
Male (n = 190)						
Condition	.196 (0.08)**	-.108 (0.05)*	5.51 (5)	.95	.02	
Polygenetic plasticity alleles	-.045 (0.08)	-.004 (0.05)				
Condition × Polygenetic Plasticity Index	.005 (0.15)	-.003 (0.10)	5.53 (6)	1.00	<.001	.48
Female (n = 151)						
Condition	.020 (0.09)	-.016 (0.05)	0.01 (3)	1.00	<.001	.99
Polygenetic plasticity alleles	.002 (0.08)	-.028 (0.05)				
Condition × Polygenetic Plasticity Index	.117 (0.17)	-.101 (0.10)	1.12 (4)	1.00	<.001	.89

intercept and slope showed a relatively good fit for boys (χ^2 [df = 3, n = 190] = 6.70, CFI = .99, RMSEA = .08) but a mediocre fit for girls (χ^2 [df = 3, n = 151] = 6.45, CFI = .98, RMSEA = .09).

In a second phase of modeling the condition-×-polygenic index interaction term was included. This two-way interaction proved significant for slope for boys (B_1 = -.183, p = .01) (χ^2 [df = 4, n = 190] = 2.21, CFI = 1.00, RMSEA < .001, partial η^2 = .04), though not for girls (B_1 = .085, p = .37) (χ^2 [df = 4, n = 151] = 7.06, CFI = .98, RMSEA = .07, partial η^2 = .00). Thus, the intervention was most effective in decreasing parent-reported externalizing behavior for boys with high polygenic scores, particularly by time of follow-up (partial η^2 = .20) (see Figure 1). A series of planned comparisons testing the hypothesis that the high-polygenic-index boys in the intervention group would benefit most from the intervention, revealed that in comparison to all other boys, this hypothesized highly-susceptible subgroup of children assigned to the intervention (1) did not differ from all other boys on parent-reported externalizing behavior at pretest (F (1, 180) = 1.00, p = .40), but (2) scored significantly lower at follow-up (F (1, 180) = 3.78, p = .01) and thus (3) evinced significantly greater reduction (i.e., change) from pretest to follow-up than all other boys (F (1, 180) = 5.29, p = .001).

Observed child externalizing behavior

Inspection of Table 3 indicates that the main effect of condition on the pretest intercept of observed externalizing behavior proved significant (B_0 = .196, p = .01), as did that for slope (B_1 = -.108, p = .03), but only for boys (χ^2 [df = 5, n = 188] = 4.92, CFI = 1.00, RMSEA = .02, partial η^2 = .05), not girls (intercept: B_0 = .020, p = .81; slope: B_1 = -.028, p = .56) (χ^2 [df = 3, n = 151] = .001, CFI = 1.00, RMSEA = .<.001, partial η^2 =
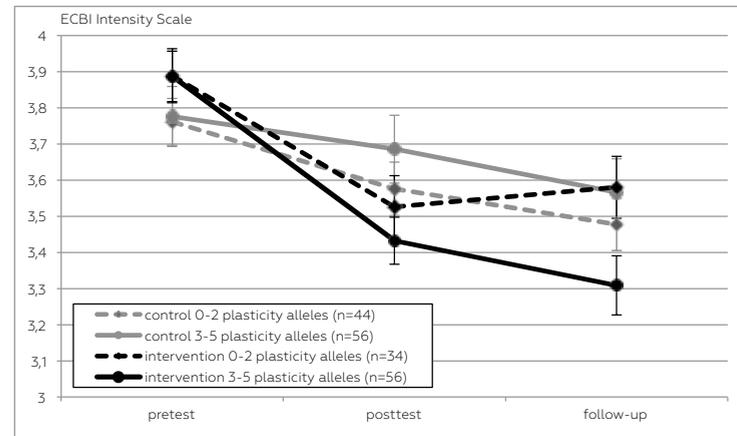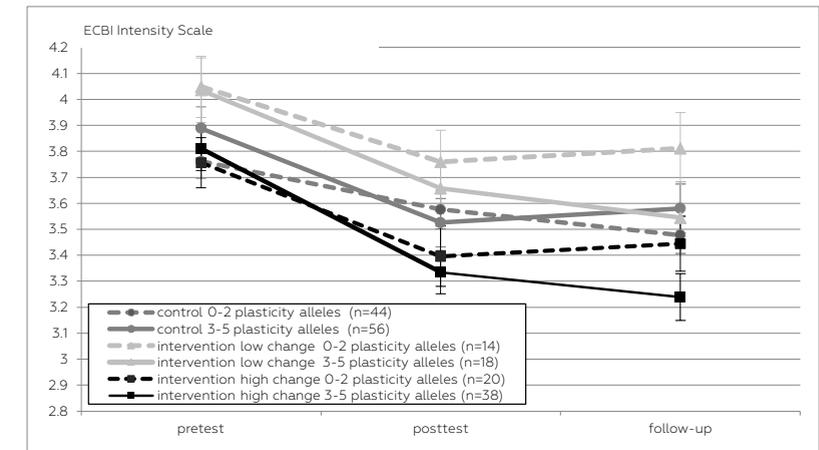
Figure 1. *Development of parent-reported externalizing behavior (as indicated by scores on the Eyberg Behavior Inventory [ECBI] Intensity Score scale) across pretest, posttest, and follow-up for control and intervention group with 0-2 putative polygenetic plasticity alleles or 3-5 such alleles.*

Figure 2. *Development of parent-reported externalizing behavior (as indicated by scores on the Eyberg Child Behavior Inventory [ECBI] Intensity Score scale) across pretest, posttest, and follow-up for intervention group with low or high increase in positive parenting (as indicated by slope scores on the construct of Positive Parenting derived from the Parent Practices Inventory [PPI] and boys with 0-2 putative polygenetic plasticity alleles or 3-5 such alleles.*

.00). Compared to the control group, IY boys showed more externalizing behavior at pretest, but also the steepest decrease over time when controlling for baseline differences. Both the main effect of the polygenic index and the condition-×-polygenic index interaction term proved non-significant for both boys and girls (boys: B_1 = -.003, p = .98, χ^2 [df = 6, n = 188] = 4.92, CFI = 1.00, RMSEA = <.001, partial η^2 = .00); girls: B_1 = -.101, p = .30, χ^2 [df = 4, n = 151] = 1.12, CFI = 1.00, RMSEA <.001, partial η^2 = .00) (see Table 3). These latter results indicate that children's genetic make-up neither predicted their observed externalizing behavior nor moderated the effect of the intervention on this behavior.

The complementary Figures for observed externalizing behavior in boys and parent-reported and observed externalizing behavior in girls are shown in Supplementary Material.

Gene-×-Positive-parenting change

As a preliminary step before evaluating whether the G × I effect would prove most pronounced when parents evinced the most increase in positive parenting, we evaluated whether, in general, parents assigned to the experimental group increased more in positive parenting than those assigned to the control group. This expectation was confirmed. Although condition proved non-significant for pretest intercept of reported positive parenting behavior (B_0 = .131, p = .13), it was significant for slope (B_1 = .141, p = .001) (χ^2 [df = 12, n = 190] = 32.42, CFI = .96, RMSEA = .10). Thus, compared to the control group, IY parents showed more improvement in positive parenting behavior over time.

To examine whether the effect of this change in positive parenting on change in boys' externalizing behavior was moderated by the polygenetic dopaminergic index, we used the continuous parenting slope variable reflecting change over time in positive parenting behavior to formulate a two-way interaction term involving it and the polygenetic index. This two-way interaction proved significant in predicting change (i.e., slope) in reported externalizing behavior (B_1 = -.881, p = .04). To graphically depict this interaction, we created two control subgroups (high and low polygenic index) and four intervention subgroups, the latter reflecting whether boys had a high or low polygenic score combined with whether their parent increased a lot or a little in positive parenting behavior: low polygenic – low positive parenting increase (n = 14), low polygenetic – high positive parenting increase (n = 18), high polygenic – low positive parenting increase (n = 20), and high polygenic – high positive parenting increase (n = 38). Inspection of Figure 2 reveals that boys with high scores on the polygenic plasticity index whose parents increased most in positive parenting evinced the greatest decline in parent-reported externalizing behavior; note, though, that the individual slope for boys scoring high on the polygenetic index whose parents showed less improvement in parenting was also significant.

Sensitivity analyses

As a robustness check, a final series of analyses were undertaken (see Table 4). These sought to determine whether the significant results reported pertaining to the genetic moderation of intervention efficacy in the case of boys would hold under
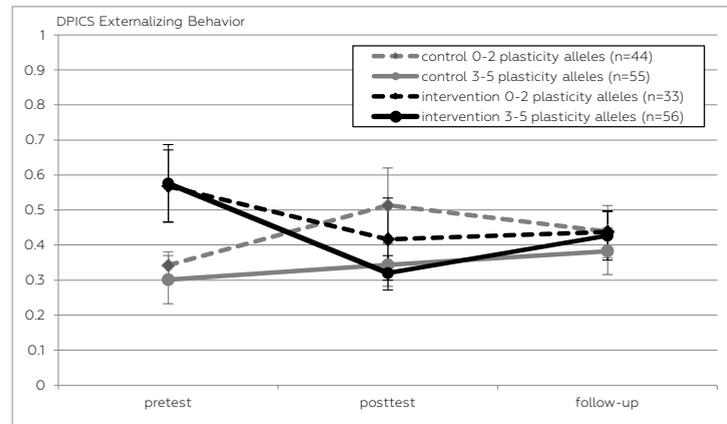
Figure 1a. *Development of observed externalizing behavior in boys (as indicated by scores on the Dyadic Parent–child Interaction Coding System [DPICS] Child Negative Behavior Scale) across pretest, posttest, and follow-up for control and intervention group with 0-2 putative polygenetic plasticity alleles or 3-5 such alleles.*

Figure 1b. *Development of parent-reported externalizing behavior in case of girls (as indicated by scores on the Eyberg Child Behavior Inventory [ECBI] Intensity Score scale) across pretest, posttest, and follow-up for control and intervention group with 0-2 putative polygenetic plasticity alleles or 3-5 such alleles.*

(see Weeland, Chhangur et al., in press). It seems plausible that the null G × I findings reported here for observed behavior could be due to the limited sampling period (i.e., 20 minutes) and highly structured observation context. After all, parental reports reflect, presumably, extensive opportunity to observe child behavior across days, weeks, and months diverse situations.

Although the overall effects of the IY parent program (on boys and girls together) were more pronounced at the immediate posttest relative to the delayed follow-up (see Weeland, Chhangur et al., in press), inspection of Figures 1 and 2 makes clear that in the case of the more genetically susceptible boys, treatment effects were not especially evident immediately after the intervention, but became so by about four months later. This observation suggests that it may take time, in the case of more genetically susceptible boys, for increases in positive parenting induced by the IY intervention to become consolidated and thus influence child behavior. Parenting interventions are designed to change the well-established, coercive and repetitive cycle of aversive parent-child interactions that induces and maintains antisocial behavior (coercive interaction, Patterson, 1982). Thus, it may take a while before increases in parenting-based reinforcement processes become established as a result of the IY program, thereby down-regulating externalizing behavior.

Although the boys scoring lower on the polygenic index in the experimental group changed less than those with higher polygenic scores, the question arises whether this would have proven to be the case had the intervention lasted longer. Had more time been allowed for parents to improve their parenting, via more intervention
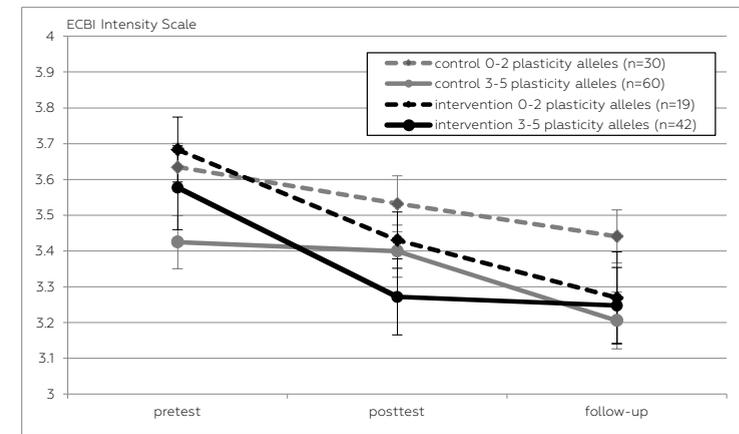
sessions and/or as a result of a later occurring follow-up evaluation, it is possible that the intervention children with fewer plasticity alleles could have caught up with those who responded more quickly to the IY intervention. Also, a very different intervention—or even one administered at an earlier age—might have proven more effective with children with fewer plasticity alleles. Thus, even if we get to the point where we could confidently conclude that a particular program—like IY—does not work equally well for all, this would not lead to the conclusion that the unaffected children are entirely non-susceptible to intervention effects, but rather that different children may benefit from different approaches (e.g., Chorpita & Daleiden, 2009).

It remains unclear why the hypothesized G × I effects emerged only in the case of boys. Importantly, this was not a statistical artifact of there being greater vari-ance in boys' than girls' externalizing behavior. In fact, the overall main effect of the intervention was similar in both subsamples in case of reported externalizing behavior. Nevertheless, we are not the first to document polygenic moderation of an environmental effect that is restricted to boys. Belsky and Beaver (2011) observed the same when investigating effects of parenting on adolescent self-regulation in their non-experimental research. Such results led them to speculate that girls may be more easily socialized, which could account for why girls carrying few putative plasticity alleles proved as subject to the parenting effects as those carrying many. It will take additional research to determine whether the Belsky and Beaver (2011) proposal indeed explains the variation in G × I findings across boys and girls chron-icled herein. Further work is also called for to gain insight into the processes that could explain how the individual genes included in our polygenic index influence

dopaminergic functioning in the brain and, thus, make some boys seemingly more susceptible to the beneficial effects of IY than others.

In addition to raising intriguing issues for future research, the current inquiry had multiple strengths. Most notably, it involved an experimental research design and focused on multiple rather than single candidate genes known to play a role in the functioning of dopaminergic neurotransmitter system. Genes selected were based on prior differential-susceptibility-related G × E and G × I findings. These strengths do not obviate limitations that must be acknowledged. Perhaps the most important, which applies to almost all RCTs, is that the generalization of results might be limited to families willing to be randomized, with equal chance of being assigned to an experimental and control group. Whereas some of the families that did not enroll may have been put off by the demand of attending 14 weekly sessions lasting two hours, others may have not been willing to take the chance of receiving no intervention service. Not to be overlooked is the relatively modest sample size. Even though an experimental design increases power relative to an observational study (Bakermans-Kranenburg & Van IJzendoorn, 2015), especially when testing a moderated intervention effect, it is certainly possible that a larger sample might have revealed significant intervention effects even in girls or boys carrying fewer putative plasticity alleles.

SUPPLEMENTARY MATERIAL

MY DOPAMINE HAS BEEN BUSY   —   CHAPTER   6