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

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CHAPTER 8

GENERAL DISCUSSION

The development of externalizing behavior in children is highly dependent on the quality of parenting they receive (Miner & Clarke-Stewart, 2008; Stormshak et al., 2000), but appears to be explained best by the interaction of genes with environment ($G \times E$) (e.g., Rutter, 2012). However, $G \times E$ findings have raised concerns about mixed findings and replications, making it difficult to draw conclusions (Dick et al., 2015; Weeland et al., 2015). One challenge lies in extending correlational $G \times E$ studies, by using designs that permit causal inferences and rule out alternative explanations in terms of gene-environment correlations (rGE). Another important challenge lies in understanding how underlying neurobiological processes link genes and environment to child externalizing behavior (Salvatore & Dick, 2015). In this thesis, we sought to clarify $G \times E$ interactions in child externalizing behavior based on multiple genes influencing the dopamine system. First, we conducted a longitudinal study that allowed us to predict the development of $G \times E$ over time, thereby accounting for passive rGE (chapter 2). Second, an intervention study was carried out in which the environment was experimentally manipulated (chapters 3-7).

Longitudinal results

Longitudinal $G \times E$ studies permit the investigation of how externalizing behavior unfolds over time and whether parenting behavior predicts change in externalizing behavior in case of a specific genetic subgroup. In **chapter 2**, we investigated whether the likelihood to develop severe externalizing behavior (delinquency) as a consequence of negative parenting (high psychological control, low parental support), depended in part on genetic variation in two dopamine-related genes (*DRD2*, *DRD4*). We found evidence for $G \times E$ interactions (*DRD2* \times parental support, *DRD4* \times psychological control). The *DRD2* interaction was curvilinear, in that adolescents carrying the A2A2 genotype showed steeper increases in delinquent behavior across early to mid-adolescence followed by quicker decreases by late adolescence, in response to low parental support. The *DRD4* 7-repeat allele interacted with high psychological control – however, when accounting for passive rGE , the interaction was no longer significant, indicating that passive rGE may underlie $G \times E$ effects. This needs further investigation in follow-up studies, specifically with experimental studies that rule out alternative explanations for any detected $G \times E$ by design.

Experimental results

In the following chapters we aimed to resolve concerns about rGE that have plagued longitudinal $G \times E$ work, by conducting an experimental intervention study (see **chapters 3** and **4**). Here, we again considered negative parenting to be a risk factor that contributes to the development of externalizing behavior, but also to be one of the strongest potentially modifiable factors (McCart et al., 2006). We used the behavioral parenting training Incredible Years (IY; Webster-Stratton, 2001) aimed at reducing externalizing behavior in young children. Parents learned to decrease negative child behaviors, by praising, rewarding and coaching the “positive opposite”

of each negative behavior. Thus, IY is focused on reducing negative child behaviors through improving positive parenting strategies and improving parental sensitivity and warmth. However, to show changes in externalizing behavior, children may need to be particularly sensitive to positive parenting strategies, like parental reward and praise. In this study, we tested whether children's sensitivity to experimentally-induced positive parenting was dependent on genetic characteristics. Because most of $G \times I$ (gene-by-intervention) research is limited by the focus on single candidate genes and fails to do justice to the polygenic nature of development, we created a dopaminergic polygenetic index including multiple dopaminergic genes. The cumulative consideration of multiple genes, via polygenetic indices, might collectively account for significant polygenetic effects (Reif & Lesch, 2003). Therefore, rather than focusing on a single dopaminergic polymorphism as a potential moderator, we adopted a systems approach by creating a polygenetic index (Nikolova et al., 2011).

We first considered the general effectiveness of the IY program in **chapter 5**. The main finding was that the intervention significantly improved positive parenting behavior (reported and observed) and that children whose parents received IY showed greater reductions in externalizing behavior (reported but not observed) compared to those in the control group. Then, in **chapter 6** we evaluated the genetic moderation of efficacy of the IY program by creating a dopaminergic polygenetic index (*DRD2* A1, *DRD4* 7-repeat, *DAT1* 10-repeat, *MAOA* low-activity, *COMT* val allele), based on the prior defined allelic variants in chapter 3 and results listed in chapter 5. As expected, IY was most effective in decreasing reported externalizing behavior in boys carrying more rather than fewer of the dopaminergic polymorphisms. This effect was most evident in boys whose parents' showed the strongest increases in positive parenting in response to the intervention. The fact that this identified $G \times I$ effect was restricted to reported and not observed externalizing behavior is consistent with the general intervention effect for this sample (chapter 5). Finally, in **chapter 7** we built on the results of genetic moderation of intervention efficacy listed in chapter 6, by decomposing the polygenetic index in dopaminergic subsystems (receptors, transporters, enzymes). Results showed that the detected polygenic-moderation effect was specifically caused by the subset of enzyme-related polymorphisms (*MAOA* low-activity, *COMT* val allele).

Different $G \times E$ frameworks in explaining sensitivity to environment

Initially, three major patterns of environmental responsiveness have been suggested, that refer to *diathesis-stress* (e.g., Zuckerman, 1999), *differential susceptibility* (Belsky et al., 2007; Belsky & Pluess, 2009, 2013; Boyce & Ellis, 2005), and *vantage sensitivity* (Pluess & Belsky, 2013; Pluess, 2015). In chapter 2 we found evidence for a diathesis-stress type interaction, whereas in chapters 6 and 7 we found evidence for a vantage sensitivity type interaction. More specifically, the results in chapter 2 illustrated that children carrying the *DRD2* A2A2 genotype and *DRD4* 7-repeat

polymorphism (before accounting for passive *rGE*) were more vulnerable for negative parenting but did not benefit most from positive parenting. One possible explanation might be that children without these specific genetic variants already scored around zero on delinquent behavior, irrespective of parenting behavior (i.e., floor effect). The results chronicled in chapter 6 and 7 were partly consistent with our a priori defined differential-susceptibility hypotheses in chapter 3, but only supported the for-better pattern of change not the for-worse pattern. Thus, rather than finding evidence for differential susceptibility we found evidence for vantage sensitivity. However, since children were screened to show relatively high levels of externalizing behavior at pretest—presumably indicating an at risk group—we expected children of the same genetic subgroup, who benefited most from the intervention, to also develop most externalizing behavior if assigned to a control group. One possible explanation for not finding differential susceptibility might be that despite our screening procedure we were unable to sample a truly “at risk” group, thereby implying that the environmental risk condition (i.e., negative parenting) did not intensify if not treated (Patterson, 1982). Although this remains speculation, perhaps a differential susceptibility type interaction would have been obtained within a clinical sample that included more severe externalizing behavior and/or more severity in environmental context (e.g., lower social-economic-status families). More specifically, such severity could have triggered a coercive exchange between child and parent (e.g., Patterson, 1982; Scaramella & Leve, 2004), leading to increases in externalizing behavior in the control group. A next step in $G \times I$ research could therefore be to oversample high risk families.

Further directions to test differential susceptibility: Nano- and mirco-trials

Future research, particularly experimental within-subject designs, will allow more advanced testing of the “for-better-and-for-worse” differential-susceptibility-related hypothesis. The most important proposition of differential susceptibility is that the very same individuals showing the worst outcomes in negative environments would also benefit the most from supportive or enriched environments. Yet we only compared children exposed to either a positive or negative environment using a between-subject design. Nano- and micro-trials in which the same children are exposed to both positive and negative environmental stimuli would be one way of extending the work presented herein and testing hypotheses of differential susceptibility within individuals (Van IJzendoorn & Bakermans-Kranenburg, 2015). *Nano-trials* are useful for elucidating immediate behavioral or neural responses to a small range of stimuli. To investigate reward-related differential susceptibility, based on genetic variations, children's automatic evaluation towards rewarding or unrewarding stimuli could be examined in a within-subject design. Previous research showed that participants reacted more quickly to positive stimuli when instructed to pull a lever toward them (approach-based reaction) and to negative stimuli when instructed to push the lever away (avoidance-based reaction) (Elliot & Covington, 2001). Since approach and avoidance behavior is linked to dopamine release in the

brain (Ledoux, 1995), it would be interesting to examine whether genetic variations in dopaminergic genes moderate these reaction-times to positive and negative stimuli in a differential-susceptibility-related manner. *Macro-trials* use a manipulation of a broader aspect of the environment. For instance, parents could be assigned to different conditions (enriched vs. negative) in which they are either instructed to add stickers to a chart and provide help (positive rewarding) or to remove stickers and provide no help (negative unrewarding). The child could be instructed to solve puzzles that are quite difficult to complete alone in these different circumstances (e.g., Tangram-puzzle, Wiggly-block). Indeed, less ability to solve problems and less appropriate interpersonal conflict management-skills have been related to early-onset externalizing behavior (Asarnow & Callan, 1985; Mize & Cox, 1990) and could be thoroughly assessed during an experiment (e.g., Webster-Stratton, Reid, & Hammond, 2001). Interestingly, we could test the proposition whether some children are more sensitive to both a positive and negative parenting environment than others based on genetically induced qualities related to the dopaminergic system.

From single genes to a systems approach

In chapter 2, we investigated longitudinal effects of $G \times E$ between parenting behavior and the *DRD2* and *DRD4* genes on delinquent behavior in a subsample of the *Family and Health* study (Harakeh et al., 2005). However, rather than finding the *DRD2* A1 polymorphism to be moderating parenting effects, it was the *DRD2* A2 allele that caused changes in externalizing behavior. Indeed, there are mixed findings regarding the *DRD2* "risk" variant, especially in case of severe externalizing behavior in adolescence (Eisenberg et al., 2007; Guo et al., 2007; Ratsma et al., 2001; Vasilyev, 2011). Notably, because the results were opposite to what normally would be predicted (e.g., Ratsma et al., 2001), the creation of an index based on a priori expectations would have produced uninterpretable results. However, since several dopaminergic polymorphisms might moderate the association between parenting and child behavior (Bakermans-Kranenburg & Van IJzendoorn, 2011), it has been suggested that complex traits, like externalizing behavior, might be polygenetic in character (Reif & Lesch, 2003). In chapters 6 and 7, we created a polygenetic index based on prior differential-susceptibility-related $G \times E$ and $G \times I$ research findings, particularly found in younger children showing less severe externalizing behavior. The polygenetic index based on the dopamine system (chapter 6) and dopamine-subsystem (chapter 7) moderated externalizing behavior in response to intervention-induced positive parenting. These findings indicated that it is likely that complex behavioral traits are indeed influenced by multiple rather than single loci (Nikolova et al., 2011; Reif & Lesch, 2003).

Beyond selecting only single candidate genes, Genome-Wide Environmental Interaction (GWEI) analyses could be used to select (novel) dopamine-related polymorphisms located across the genome on an independent one-by-one basis (Aschard et al., 2012). Polygenetic indices could be created based on significant proven

polymorphisms. Also, to reduce the high number of polymorphisms independently tested, generalized multifactor dimensionality reduction software could be used in so-called Genome-Wide Association gene-gene Interaction (GWAII) analyses (Kim & Park, 2015). Such an approach would improve statistical power by reducing multiple testing and yet would include multiple theoretically (biologically) related genes based on dopaminergic functioning. However, GWEI and GWAII studies are hypothesis-free and thus it is important to only include hypothesis-driven novel polymorphisms in the polygenetic index (e.g., related to less dopaminergic signaling).

Neurobiological foundation of genetic moderation of intervention efficacy

The findings chronicled in this thesis confirmed that the dopaminergic polymorphisms were responsible for genetic moderation, making some children seemingly more sensitive to parenting behavior than others (chapter 2, 6, and 7). However, the specific underlying neurobiological process that mediated the effect of parenting behavior on child externalizing behavior remains unclear. Recently, Moore and Depue (2016) introduced a "threshold model" stipulating that neurobiological sensitivity to environment can be conceptualized as a threshold that is influenced by the function of (1) the level of neurotransmitter activation and (2) the magnitude of environmental stimuli. This theorizing is consistent with our claim that less dopamine signaling might especially result in reduced reward salience and slower reward-based learning, that relate to difficulties in turning attention to or encoding rewarding stimuli (Comings & Blum, 2000; Schultz, 2002). As such, children with less dopaminergic signaling may have a higher threshold value to be sensitive to reward-based parenting and, importantly, the range of effective rewarding stimuli to prevent/reduce externalizing behavior may be much smaller for parents. More specifically, these children may especially be in need for powerful and immediately positively rewarding stimuli in their environment due to genetically induced qualities of the dopaminergic system, responsible for impaired reward processing (Matthys et al., 2012). Indeed, chapter 6 confirmed that children carrying more dopaminergic polymorphisms benefited most from their parent's involvement in the intervention program. But most notable, this genetic moderation effect was most pronounced when parents increased a lot in positive parenting, thus implying that their improved parenting quality now fell above rather than below the child's sensitivity threshold value.

Complexity of differentiating dopaminergic subsets

Even though research on $G \times E$ has tended to consider dopamine related genetic variability under a single denominator, in theory different genes might impact different aspects of the dopamine system (Chen et al., 2011). However, in reality distinguishing dopaminergic subsets seems to be complex. For example, both the *DRD2* A2 (chapter 2) and *DRD2* A1 (chapter 3, 6, and 7) variants are related to increased sensitivity to parenting behavior. In addition, the *COMT* polymorphism is linked to *higher* dopamine

degradation and the MAOA polymorphism to lower dopamine degradation (chapter 7). Notably, especially the MAOA is also involved in the degradation of serotonin and norepinephrine. Specifically, higher serotonergic and norepinephrine functioning is associated with increased sensitivity to environmental context in general (Homberg & Lesch, 2011). Although these results complicate the straightforward interpretation of polymorphisms roles in the dopamine system, this might explain why the MAOA polymorphism—regardless of its contradictory dopaminergic function—relates to increased responsiveness to parenting behavior. That is, the MAOA polymorphism may be more involved in serotonin and norepinephrine functioning (i.e., lower degradation, higher serotonin/norepinephrine) than in dopamine functioning (i.e., lower degradation, higher dopamine). This involvement in serotonin and norepinephrine functioning, might explain why the originally detected polygenic-moderation effect (chapter 6) was specifically caused by the subset of enzyme-related genes (chapter 7).

Our findings did by no means cover all dopaminergic brain processes and thus other dopaminergic genes that are yet neglected merit consideration, including the *Dopamine Receptor D1/2/5 (DRD1, DRD2, DRD5)* (Dichter et al., 2012), *DOPA decarboxylase (DCC)* (Ma et al., 2005), *Ankyrin repeat and kinase domain containing 1 (ANKK1)* (Ponce et al., 2009), *Vesicular monoamine transporter 1/2 (VMAT1, VMAT2)*, *Brain Derived Neurotrophic Factor (BDNF)* (Berton et al., 2006), *Dopamine beta-hydroxylase (DBH)* (Barkley, Smith, Fischer, & Navia, 2006).

Reward processing beyond dopamine

Other neuromodulators than the dopamine ones may also modulate children's differential responsiveness to rewarding stimuli. This complex interaction of the dopaminergic system with other neuromodulators is not yet fully elucidated and needs further investigation (Moore & Depue, 2016). However, it may very well be that specific neuromodulators, like oxytocin, β -endorphin, and testosterone elicit or inhibit dopaminergic signaling in response to reward. *Oxytocin* is a neuropeptide that—beyond its involvement in endocrine function—modulates responses to prosocial behavior, like positive parenting behavior and social bonding (Carter, 2014). Indeed, a recent review on the effects of oxytocin administration revealed enhanced social cognitions in encoding and remembering socially relevant emotional formation (Graustella & MacLeod, 2012). β -endorphin is an endogenous opioid peptide that modulates, among others, the experience of rewards and reward-based learning. A review on the relation between β -endorphin and drugs-induced reward and reinforcement, illustrated an important mediating role of β -endorphin in reward processing of alcohol/cocaine (Roth-Deri, Green-Sadan, & Yadid, 2008). Less is known about the neurosteroid *testosterone* that modulates sensation-seeking, sensitivity to reward, and motivation to act (Bos, Panksepp, Bluthé, & Honk, 2012; Eisenegger, Haushofer, & Fehr, 2011). However, neuroimaging studies demonstrated that testosterone administration acts on reward-seeking processes and enhanced reward dependency (e.g.,

Van Honk et al., 2011). As such, research combining molecular genetics, neuroimaging, and endocrinology is required to investigate variants in dopaminergic genes and in hormone levels that presumably modulate overall reward processing (Caldu & Dreher, 2007). Ultimately, we need to join forces with other disciplines to enable the investigation of “brain reward-processes-x-environment interactions” more broadly defined (chapter 4).

Neurotransmitters that modulate general neural activity

Besides neuromodulators that elicit/inhibit dopaminergic signaling in response to reward, there are ones that modulate neural activity in any type of environmental condition. These neuromodulators might affect children's sensitivity to environmental context in a general manner. Moore and Depue (2016) refer to this as a “neural constraint” thereby implying that increases (hyperactivity) or decreases (rigidity) in overall neural activity would result in, respectively, a lower or higher threshold value to rewarding stimuli. Hyperactivity relates to easy elicitation of attention by stimuli and the ability to discriminate relevant from irrelevant stimuli, whereas rigidity relates to difficult elicitation of attention, a vigilant state, and difficulties in discriminating relevant from irrelevant stimuli. As such, children with less dopaminergic signaling may have a higher threshold value to be sensitive to reward-based parenting, but this threshold may raise or lower depending on general neural activity. Consider in this regard, *Glutamate* and *GABA* that are the most plentiful neurotransmitters in the brain and modulate many processes. Glutamate is an excitatory neurotransmitter and GABA an inhibitory neurotransmitter that work together to balance stimulating and tranquilizing overall neural activity in the brain (Petroff, 2002). *Serotonin* is another inhibitory neurotransmitter that plays a role in many brain processes and modulates overall incoming emotionally-related stimuli (Cools, Roberts, & Robbins, 2008). *Norepinephrine* is an excitatory neurotransmitter that modulates the overall valence of incoming stimuli, alertness, and attentional focus (Kravets, Reyes, Unterwald, & Van Bockstaele, 2015). Conceivably, these neurotransmitters—of which we only listed a few—modulate overall neural activity and might relate to hyperactivity or rigidity in dopamine signaling. In fact, this overall neural activity might have caused the MAOA polymorphism to interact with increased sensitivity to parenting in predicting children's externalizing behavior (chapter 6), irrespective of its direct link with more dopamine signaling (chapter 7).

Developmental perspective on reward sensitivity

Molecular genetic studies illustrate that environmental experiences can modify the expression of genes that in turn influence neural reactivity to environmental experiences in the future (Mitchell et al., 2013; Szyf et al., 2007). DNA methylation differences across the *DRD4* and *MAOA* gene were found in identical twins, suggesting that environmental experiences modified the expressions of genes (Wong et al., 2010). This indicates that epigenetic processes might change dopaminergic

gene expression, linked to changes in reward sensitivity. Although, not directly related to underlying epigenetic processes, it has also been found that high levels of testosterone during fetal development are associated with heightened neural reward sensitivity in 8-to-11-years-old children (Lombardo et al., 2012). In addition, children growing up in an adverse environment (e.g., poverty) developed a preference for immediate rather than long-term rewards (Griskevicius, Tybur, Delton, & Robertson, 2011). Moreover, heroin self-administration increased reward sensitivity in nondependent rats, but gradually decreased reward sensitivity in dependent rats (Kenny, Chen, Kitamura, Markou, & Koob, 2006). Thus, it might be that children's reward sensitivity to environmental experiences is not a static "trait" but rather a dynamic feature, fluctuating across developmental phases and experiences. Different (reward) experiences might mold children's sensitivities to subsequent environmental circumstances, resulting in different patterns of environmental sensitivity over time. Therefore, reward sensitivity could be seen as a continuous state-like, rather than as a categorical and static susceptibility factor, in that some children may be more malleable than others depending on their current degree of dopaminergic functioning and chains of environmental experiences they had before.

Recommendations for further research and clinical implications

The central finding in this thesis is that some children, based on genetic variations, benefited more from intervention efforts than others (chapters 6 and 7), but this finding may also raise questions about clinical implications (chapter 4). Some scholars predict that genetic testing for specific polymorphisms will become increasingly important as a guide to prevention and clinical management (Burke et al., 2002; Van Goozen & Fairchild, 2008). Indeed, $G \times I$ research might help to differentiate forms of sensitivity to parenting behavior that are important to tailor personalized intervention and to boost the currently modest effectiveness of interventions. However, there is ample reason to doubt that the relations between genetic markers and intervention effectiveness in the psychological domain can be straightforward enough to inform treatment decisions for individual clients. We illustrated that the surge of interest in dopamine- \times -environment interactions raises concerns about the complex interrelations between different brain systems that are currently not fully understood. For one, reward sensitivity might be domain specific and be fluctuating across development. Without exact knowledge about genetic susceptibility across domains and developmental phases, genetic screening for inclusion in interventions, for instance, would lead to too many false decisions (Ross, Saal, David, Anderson, & American Academy Pediatrics, 2013). Ultimately, we need to join forces with other disciplines to enable the investigation of "brain reward-processes- \times -environment interactions" more broadly defined. *GWAS studies* to find novel dopaminergic polymorphisms, *neuroimaging studies* to investigate the dopaminergic underlying mechanisms in the brain, *molecular genetics* and *endocrinology studies* to investigate the complex interactions with other neurotransmitters and hormones, *epigenetic studies*

to investigate environmental experiences modifying the expression of (dopaminergic) genes, and *social and behavioral studies* to investigate the environmental factors and underlying cognitive and behavioral mechanisms that influence child behavior. Until then, an important consideration is to examine behavioral rather than genetic markers, like "behavioral reward-processes- \times -environment interactions".

Conclusion

In this thesis, we sought to clarify $G \times E$ interactions in child externalizing behavior based on multiple genes influencing the dopamine system. In a longitudinal study, we found that that low parental support longitudinally predicted delinquent behavior, in a diathesis-stress-related manner, but only for those carrying the *DRD2 A2A2* genotype. In an experimental intervention study, we found that a dopaminergic polygenetic index (*DRD2 A1*, *DRD4 7-repeat*, *DAT1 10-repeat*, *MAOA low-activity*, *COMT val*) moderated the intervention efficacy of the IY parent program, in a vantage-sensitivity-related manner. Specifically, boys carrying many dopaminergic polymorphisms decreased significantly in externalizing behavior as a result of their parents' involvement in the IY intervention. In a follow-up study, we found that this detected polygenic-moderation effect was specifically caused by the subset of enzyme genes (*COMT*, *MAOA*) rather than by the subset of receptor genes (*DRD2*, *DRD4*) or subset of transporter genes (*DAT1*). Taken together, the findings chronicled in this thesis demonstrated that the dopaminergic system can be considered to be an important neurobiological system that might explain the association between children's sensitivity to parenting behavior and thereby the development of externalizing behavior. However, because the dopaminergic polymorphisms impinge on multiple other neurotransmitters, more research is needed to better understand specific endophenotypic processes that could explain how the complex dopamine network made some children seemingly more sensitive to parenting behavior than others.