Association Between Reward-Related Activation in the Ventral Striatum and Trait Reward Sensitivity is Moderated by Dopamine Transporter Genotype

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Abstract: The impact of individual differences on human reward processing has been a focus of research in recent years, particularly, as they are associated with a variety of neuropsychiatric diseases including addiction and attention-deficit/hyperactivity disorder. Studies exploring the neural basis of individual differences in reward sensitivity have consistently implicated the ventral striatum (VS) as a core component of the human reward system. However, the mechanisms of dopaminergic neurotransmission underlying ventral striatal activation as well as trait reward sensitivity remain speculative. We addressed this issue by investigating the triadic interplay between VS reactivity during reward anticipation using functional magnetic resonance imaging, trait reward sensitivity, and dopamine (DA) transporter genotype (40-bp 3'VNTR of *DAT*, *SLC6A3*) affecting synaptic DA neurotransmission. Our results show that *DAT* variation moderates the association between VS-reactivity and trait reward sensitivity. Specifically, homozygote carriers of the *DAT* 10-repeat allele exhibit a strong positive correlation between reward sensitivity and reward-related VS activity whereas this relationship is absent in the *DAT* 9-repeat allele carriers. We discuss the possibility that this moderation of VS-trait relation might arise from *DAT*-dependent

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differences in DA availability affecting synaptic plasticity within the VS. Generally, studying the impact of dopaminergic gene variations on the relation between reward-related brain activity and trait reward sensitivity might facilitate the investigation of complex mechanisms underlying disorders linked to dysregulation of DA neurotransmission. *Hum Brain Mapp* 32:1557–1565, 2011. © 2010 Wiley-Liss, Inc.

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INTRODUCTION

The capacity to process information to detect and predict rewards is key to goal-directed and thus adaptive human behavior. As individual differences in the processing of reward cues are associated with a number of neuropsychiatric diseases including addiction and attention-deficit/hyperactivity disorder (ADHD), understanding the neural mechanisms of reward anticipation both in patients [Scheres et al., 2007; Wrase et al., 2007] and healthy individuals [Knutson et al., 2001] has been a focus of research. In particular, the use of functional magnetic resonance imaging (fMRI) is providing valuable insights into the neural substrate of reward processing, identifying the ventral striatum (VS) as a key component during reward anticipation [for a review see Knutson and Cooper, 2005]. Neural activation in the VS was furthermore shown to be correlated with individual differences in a number of reward-related behavioral measures [Forbes et al., 2007; Hariri et al., 2006; Knutson et al., 2007]. Particularly, activation in this region was reported to be larger in individuals with increased reward sensitivity. For example, Beaver et al. analyzed ventral striatal response to pictures of various foods [Beaver et al., 2006]. They found increased VS activation in response to images displaying appetizing foods in individuals with higher reward sensitivity. Specifically focusing on the phase of reward anticipation, Hahn et al. [2009] confirmed this association between reward sensitivity and ventral striatal activation following a reward cue in a monetary incentive delay (MID) task.

These studies hint at the neural basis of the substantial individual differences in reward sensitivity found in humans [Gray, 1987]. While an important first step, particularly which and how (genetic) factors of dopamine function may impact ventral striatal activation as well as trait reward sensitivity remains speculative. Receiving considerable dopaminergic projections from midbrain neurons that fire in response to cues indicating pleasant events [Schultz, 1998], activation in the VS during reward processing is heavily impacted by individual differences in dopaminergic neurotransmission. Synaptic dopamine (DA) bioavailability in the VS is mainly regulated by the dopamine transporter (DAT), which is abundantly expressed in this region [Ciliax et al., 1999; Egan et al., 2001]. Due to the important role of DAT in maintaining synaptic DA homeostasis and in the action of many (psychostimulant) drugs, this gene has been repeatedly suggested as a candidate gene for ADHD. Meta-analyses of genetic association studies of a functional *DAT* gene (*DAT*, *SLC6A3*) variation identified a small (pooled odds ratio: 1.12–

1.17) but significant association with ADHD [Gizer et al., 2009; Yang et al., 2007]. Specifically, the 10-repeat allele of the 40-bp variable number of tandem repeat (VNTR) polymorphism within the 3' untranslated region of *DAT* [Gizer et al., 2009; Mill et al., 2002; VanNess et al., 2005] was shown to be a risk allele for ADHD [Dreher et al., 2008a]. In support of the central role of dopaminergic neurotransmission in reward anticipation, Dreher et al. showed that this *DAT* variation impacts VS activity during reward anticipation [2009]. However, due to inconsistent in vitro and in vivo findings regarding the functional impact of the most common alleles (9- and 10 repeats) on *DAT* expression, implications for DA neurotransmission have to be cautiously discussed.

In summary, reward-related neural activity may be impacted by both, genetically influenced variations in DA neurotransmission and individual differences in reward-related personality traits. The present study simultaneously investigates these three factors and their interactions. Specifically, we predict to find a positive association between activation in the VS during anticipation of reward and individual differences in reward sensitivity in accordance with Hahn et al. [2009]. Investigating the genetic influence on this effect, we particularly hypothesize an interaction of *DAT* variation, VS activation during reward anticipation, and individual differences in reward sensitivity.

MATERIALS AND METHODS

Participants

Data of 53 subjects between 18 and 47 years (mean = 29.0, standard deviation SD = 7.6) who were unrelated European Caucasians were analyzed in this study. This includes data from 20 subjects analyzed previously [Hahn et al., 2009]. All were recruited from the local community through advertisements. The participants (1) had no first-degree relative with a neurologic or psychiatric disorder, (2) had no history of dependence on illicit drugs and alcohol, (3) were currently not taking any psychotropic medication, and (4) had no sensorimotor deficits or other neurological disorders. Additionally, they all showed non-pathological scores in the German versions of Beck depression inventory II [Hautzinger et al., 2005]. Written informed consent was obtained after detailed explanation of the study protocol. The study was approved by the Ethics Committee of the University of Würzburg, and all procedures involved were in accordance with the latest version (fifth revision) of the Declaration of Helsinki.

From the original sample of 58 right-handed, healthy individuals (32 females), four subjects had to be excluded from further analyses due to technical error or excessive head motion (translation larger than 2 mm and/or rotation larger than 1° in any direction), respectively. Additionally, we excluded one subject due to unavailable genotype information, thus arriving at the final sample of 53 subjects.

Psychometric Testing

All participants completed the sensitivity to punishment and sensitivity to reward questionnaire [SPSRQ; Torrubia et al., 2001] in its German version [Hewig and Hagemann, 2002. Der SPSR-Fragebogen von Torrubia, Avila, Molto und Caseras—unpublished German translation. University of Trier; personal communication]. The SPSRQ is a 48-item self-report measure that consists of two scales measuring sensitivity to reward (SR) and sensitivity to punishment (SP). The two scales show 3-month retest reliabilities of 0.87 and 0.89 for the SR and the SP scale, respectively. Orthogonality of the scales has been confirmed, while good construct validity has also been shown [Sava and Sperneac, 2006].

Genotyping

Genomic DNA was extracted from whole blood samples according to standard protocols. Genotyping of the *DAT* 40-bp VNTR was performed by polymerase chain reaction (PCR) and subsequent gel electrophoresis as published previously [Vandenberg et al., 1992]. With this procedure, we identified 25 *DAT* 10-repeat allele homozygotes (56% female) and 28 9-repeat allele carriers (60% female), four of which were homozygotes. The sample was dichotomized comparing 10/10 carriers with carriers of at least one 9-repeat allele for reasons of comparability with studies investigating the VNTRs functional influence on DAT density [van de Giessen et al., 2009; van Dyck et al., 2005]. The groups did not differ in regard to age ($t_{(51)} = -0.321$; $P = 0.75$), smoking status ($X^2_{(1)} = 2.21$; $P = 0.14$) or Beck depression inventory II ($t_{(51)} = -0.591$; $P = 0.56$) scores.

To exclude *DAT* genotype group differences regarding allelic distribution of other genetic polymorphisms related to dopaminergic neurotransmission which may impact VS activation, we performed additional genotyping and statistical testing using Fisher's exact test. The two *DAT* genotype groups (10/10 and 9-repeat allele carriers) did not differ in regard to allele frequencies of *COMT* Val158Met ($P = 0.114$), *DRD4* 48-bp VNTR ($P = 0.775$), and *5-HTT-LPR* ($P = 0.870$) polymorphisms. Furthermore, none of these genetic polymorphisms showed a main effect on VS activation nor an effect of VS-trait correlation ($P > 0.10$, corrected).

Task Description and Procedures

We conducted a modified version of the MID task developed by Knutson et al. [2001] which has been used previously [Hahn et al., 2009]. To familiarize subjects with the task, each participant completed 10 practice trials prior to

data acquisition. During each trial, participants saw one of three different cue shapes (presentation time 2,000 ms each) followed by a fixation cross as they waited a variable interval (2,250–2,750 ms). Thereafter, they responded with a button press to a white target square which appeared for a variable length of time depending on the subject's performance. Specifically, the mean reaction time obtained from the 10 practice trials was used as the initial target duration. It was increased by 30 ms if the subject failed to respond fast enough on more than one out of the last three consecutive trials. Likewise it was decreased by 30 ms if the subject succeeded on more than two out of the last three consecutive trials. This approach ought to ensure participants' success on an average of 66% of the trials, thereby yielding a proportion of hits and misses comparable with that reported by Knutson et al. [2001]. Additionally, target duration was set as to never decrease below 100 ms and never exceed 1,000 ms. As this adaptive algorithm was used to alter target durations, reaction times cannot be meaningfully interpreted and are therefore excluded from further analysis. Feedback (2,000 ms), which followed the disappearance of the target, informed participants of whether they had reacted in time during that trial and indicated their cumulative total win in Euros at that point.

fMRI volume acquisition was synchronized with the presentation of the cue and thus was acquired during the anticipatory delay period. Cues signaled the possibility of winning 0.05 € ($n = 20$; a circle with one horizontal line) or 1.00 € ($n = 20$; a circle with three horizontal lines). The third cue ($n = 20$; a triangle) indicated that no money could be won during this trial. The three trial types were randomly ordered within the experiment.

MRI Acquisition

Imaging was performed using a 1.5 T Siemens Magnetom Avanto TIM-system MRI scanner (Siemens, Erlangen, Germany) equipped with a standard 12-channel head coil. In a single session, 24 4-mm-thick, interleaved axial slices (in-plane resolution: $3.28 \times 3.28 \text{ mm}^2$) oriented at the AC-PC transverse plane were acquired with 1-mm interslice gap, using a T2*-sensitive single-shot EPI sequence with following parameters: repetition time (TR; 2,000 ms), echo time (TE; 40 ms), flip angle (90°), matrix (64×64), field of view (FOV; $210 \times 210 \text{ mm}^2$), and number of volumes (310). The first six volumes were discarded to account for magnetization saturation effects. Stimuli were presented via MRI-compatible goggles (VisuaStim; Magnetic Resonance Technologies, Northridge, CA) using presentation (Neurobehavioral Systems; <http://www.neurobs.com>). Head movements were minimized by using a cushioned head fixation device.

fMRI Analysis

Data were preprocessed and analyzed using statistical parametric mapping software (SPM5, Wellcome Department

of Cognitive Neurology, UK, implemented in Matlab 7.0, Math Works, Natick, MA). Slice-time correction was applied and images were realigned. The mean image of the scans was computed and used as the source image for spatial normalization of the data. Data were then spatially smoothed, using an 8-mm FWHM Gaussian isotropic kernel. The time series in each voxel were high pass-filtered to 1/128 Hz to remove low-frequency noise and corrected for temporal autocorrelation using an autoregressive model with a lag of 1.

Then, single subject fixed-effect analyses were performed. Three types of events were defined corresponding to the neutral and the two reward conditions (0 €, 0.05 €, and 1 €). Events for each of these regressors were modeled by convolving onset times with a canonical hemodynamic response function. Parameters of each regressor (beta weights) were estimated using the general linear model, with movement parameters in the three directions of motion and three degrees of rotation included as confounds. The contrast images obtained by subtracting the beta images for the neutral condition from the beta images for the 1 € reward anticipation were then entered into second-level analysis treating subjects as random effects. As previous studies have identified the contrast between 1 € reward anticipation and the neutral condition as the most reliable [Hahn et al., 2009; Knutson et al., 2001], only this contrast is considered for the purpose of this study. On the second level, the subjects' contrast images were tested against 0 using a one-sample *t*-test. For all other analyses we included centered SR scores, centered *DAT* variation data, and their interaction term as regressors in a general linear model. As it was shown to affect dopaminergic neurotransmission and thus reward processing [Dreher et al., 2008b], we also included age as a covariate in all analyses. To analyze the interaction of *DAT* 40-bp VNTR and SR scores, the interaction-term was tested for significance. In the correlation analyses done for descriptive purposes, psychometric data were correlated with mean contrast image beta values over those voxels, which were significantly activated in the respective analysis. In accordance with Forbes et al. [2001], all analyses were conducted within the VS using a spherical voxel mask with a radius of 20-mm centered on the Talairach coordinates of $x = 0$, $y = 10$, and $z = -10$. Correction for multiple comparisons within this region was realized by utilizing a Monte Carlo simulation approach running AlphaSim (B.D. Ward; provided with AFNI software) with a single voxel *P* value of 0.05. The spatial intercorrelations between the voxels as modeled by the FWHM of a Gaussian kernel were calculated using the 3dFWHMx AFNI software routine (for details of the procedure see <http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf>). With this procedure, ROI-specific cluster-sizes corresponding to a corrected threshold of $P < 0.05$ were determined. They were applied in all further image-analyses ensuring a corrected α -level of 5% (respective *F*- or *t*-statistics of the peak voxel are provided in parentheses).

RESULTS

Behavioral Data

Participants attained a mean score of 8.72 (SD = 3.74) and 8.36 (SD = 4.64) on the SR and the SP scale, respectively. In accordance with previous findings, both scales were not significantly correlated ($r = -0.08$; $P = 0.57$). Additionally, mean SR- ($t_{(51)} = -0.078$; $P = 0.94$) and SP scores ($t_{(51)} = 1.374$; $P = 0.18$) did not differ between *DAT* genotype groups. Significant gender differences could neither be verified for SR ($t_{(51)} = 1.137$; $P = 0.26$) nor for SP ($t_{(51)} = -1.139$; $P = 0.26$).

During the MID-task, participants succeeded on an average of 64% (SD = 2.2) of the trials. Thus, the proportion of hits and misses is comparable with the value we aimed for in accordance with [Knutson et al., 2001; Hahn et al., 2009].

fMRI Data

In line with previous findings, a significant bilateral response was observed in the VS during reward anticipation ($t_{(52)} = 9.61$; $P < 0.05$, corrected). Furthermore, regions within the VS showed a significant positive association of SR scores and activation during reward anticipation ($t_{(51)} = 2.80$; $P < 0.05$, corrected). To estimate the overall amount of variance explained by SR scores, subject-specific ROI-means were obtained by calculating the mean of all significantly correlated voxels in the VS for each participant. These values were then correlated with SR scores, which leads to a correlation of $r = 0.39$ ($P = 0.002$). None of the activations showed a significant correlation with SP scores. Furthermore, we did not observe any significant negative correlations with SR or SP scores. Additionally, no significant effect of genotype could be observed. Interestingly, a direct main effect of *DAT* genotype variation on VS activation was only present, when *DAT* variation data is entered into the GLM while excluding all other regressors ($t_{(52)} = 2.46$; $P < 0.05$, corrected). By statistically controlling for the effects of reward sensitivity no significant influence of *DAT* on VS activation was present.

Interaction of *DAT* Variation and Reward Sensitivity Scores

Extending earlier evidence [Beaver et al., 2006; Hahn et al., 2009], we revealed a significant interaction of the investigated *DAT* genotype and SR scores affecting reward-related VS-activation ($F_{(1,50)} = 11.24$; $P < 0.05$, corrected), indicating that the correlation between reward-related activity in the VS and reward sensitivity is affected by differences in DA neurotransmission (Fig. 1, top panel). Investigating this interaction more closely, the correlation between VS-activation and SR scores as described above turned out to be present only in the *DAT* 10-repeat allele homozygotes ($r = 0.58$; $P = 0.002$), while 9-repeat allele carriers showed no such association ($r = -0.29$; $P = 0.13$;

Figure 1.

Interaction of *DAT* 3'VNTR and trait sensitivity to reward (SR) scores affecting reward-related VS-activation ($P < 0.05$, corrected; top panel). Scatter plots (bottom) display the association between z-standardized trait SR scores and ventral striatal activation (averaged over activation-clusters showing a significant

interaction of the 3'VNTR and SR scores). The correlation between ventral striatal activation and SR scores is present only in the *DAT* 10-repeat allele homozygotes (bottom right panel), while no such association is found in 9-repeat allele carriers (bottom left panel).

Fig. 1, bottom panel; for the results of an exploratory whole-brain analysis, see Supplemental Results online).

DISCUSSION

In the present study, we were able to reliably elicit activation in the VS during reward anticipation. Additionally, this activation was substantially correlated with trait SR. These results replicate findings of previous studies focusing on the impact of individual differences in reward processing [Beaver et al., 2006; Hahn et al., 2009]. However, when statistically controlling for the effects of trait reward sensitivity, no significant main effect of *DAT* genotype variation on VS activation during reward anticipation can be

found. As other studies in the domain of "imaging genetics" which found such an effect [Dreher et al., 2008a; Forbes et al., 2007] did not control for individual differences in personality traits such as SR, differences in brain activation due to e.g., *DAT* genotype may be partly explained by the lack of such regressors. This underlines the necessity to investigate multiple factors such as genotype, brain activation, psychometric data, and their interaction.

Apart from this, the fact that the genotype groups do not differ in regard to their SR- and SP scores does appear counterintuitive. However, studies investigating direct genetic effects on complex behaviors and traits regularly report very small effect sizes. Thus, several hundred subjects can easily be necessary to show such effects. In this study, an intermediate biological marker (i.e., brain

activity) between genes and traits was investigated. This “endophenotype” approach can reveal genetic influences too weak to be detected when investigating direct associations between genes and behavior in small samples [Gottesman and Gould, 2003]. In summary, we thus attribute the absence of a genotype effect on SR- and SP scores to our sample size which—while medium to large for an fMRI study—is very small in comparison with the sample sizes of studies solely investigating the effects of genotype on behavioral or trait level.

In an effort to shed light on the genetic basis of individual differences in reward anticipation, we reveal an interaction effect of *DAT* variation and reward sensitivity impacting VS activation. Disentangling this interaction, the association between VS activation and reward sensitivity was only present in individuals homozygous for the *DAT* 10-repeat allele, but not in 9-repeat allele carriers. With this, we provide direct evidence linking neural correlates of reward sensitivity as defined by Gray’s reinforcement sensitivity theory [Gray, 1987] to a genetic polymorphism which has been repeatedly suggested to alter DA function by affecting *DAT* gene expression [VanNess et al., 2005].

However, inconsistent findings regarding the qualitative and quantitative influence of *DAT* 10- and 9-repeat alleles on dopaminergic neurotransmission demand cautious interpretations. The functionality of the *DAT* VNTR polymorphism has been repeatedly studied in vitro [Fuke et al., 2001; Greenwood and Kelsoe, 2003; Mill et al., 2002, 2005; Miller and Madras, 2002]. While it is difficult to compare these studies due to different transfection systems, cell lines and protocols [Haddley et al., 2008] findings were divergent or inconclusive regarding the decrease or increase of *DAT* gene expression for 9- or 10-repeat alleles, respectively. However, several possible molecular/cellular mechanisms by which the 30-UTR genotype may influence *DAT* expression have been suggested, such as nuclear export, polyadenylation, subcellular targeting, and rates of transcription and degradation of mRNA [Conne et al., 2000]. Additionally, single photon emission computed tomography (SPECT) has been repeatedly used to quantify the impact of the *DAT* VNTR genotype on in vivo differences in striatal *DAT* density [Martinez et al., 2001; Heinz et al., 2000; van Dyck et al., 2005; van de Giessen et al., 2009]. Heinz et al. [2001] ($n = 25$; mixed sample of abstinent alcoholics and healthy subjects) reported a 22% reduction of *DAT* protein in the putamen of 9/10 compared with 10/10 genotype carriers, whereas Martinez et al. [2001] ($n = 31$; healthy controls) found no differences in striatal radiotracer ($[^{123}\text{I}]\beta\text{-CIT}$) binding. More recently, two SPECT studies addressed the issue by investigating larger samples of healthy subjects [$n = 81$ and $n = 96$; van de Giessen et al., 2009; van Dyck et al., 2005]. Both consistently showed that carriers of at least one 9-repeat allele when compared with 10/10 genotype carriers exhibit increased striatal *DAT* availability. In summary, inconsistencies of these in vitro and in vivo findings

have to be carefully considered when suggesting interpretations based on *DAT* VNTR genotype dependent *DAT* density.

As an approach for interpreting the present findings, we speculate that dopamine-dependent processes of neural plasticity might underlie the moderator effect of *DAT* variation on the association between VS activation and trait reward sensitivity. On one hand, *DAT* calibrates duration and intensity of DA neurotransmission in VS [Horn, 1990; Jones et al., 1998]. On the other hand, DA has been shown to control reward learning in the VS [Schultz, 2002] as well as the regulation of corticostriatal synaptic plasticity [Berridge, 2007]. Here, genotype-dependent *DAT* functioning may affect these DA-dependent plastic processes and thereby the association between VS activation and trait reward sensitivity. Following this approach, we discuss two interpretations of how the respective *DAT* genotype could impact DA availability and DA mediated plasticity.

High Dopamine Availability

The most essential mechanism of plasticity for learning is long-term potentiation (LTP). Within the VS, LTP requires convergence of cortical glutamatergic and dopaminergic inputs from the ventral tegmental area [Kerr and Wickens, 2001; Haluk and Floresco 2009; Cepeda et al., 1998; Hernandez-Lopez et al., 1997; Wickens et al., 1996]. DA-signals from VTA have to depolarize VS neurons via D1/D5 dopamine receptors so cortical glutamate signals can activate NMDA-receptors to open Ca^{2+} channels inducing LTP. This coincidence of glutamate and DA signals as well as activation of the DA-dependent cAMP-DARPP32 pathway may depend on DA availability and synaptic DA clearance via *DAT* [Svenningsson et al., 2004; Calabresi et al., 2007]. Accordingly, if relatively increased DA availability was assumed for *DAT* 10/10 carriers, this might foster plasticity in VS contributing to the positive correlation of reward sensitivity and VS activation. In turn, decreased DA availability, as assumed for 9-allele carriers in this case, may reduce DA-dependent VS plasticity and thus trait-VS association.

Low Dopamine Availability

ADHD has been shown to be associated with increased trait reward sensitivity [Mitchell, 2009], hypodopaminergic state within the VS and, interestingly, hyporesponsiveness in the VS during reward anticipation (see clinical implications) compared with healthy controls [Scheres et al., 2007; Strohle et al., 2008]. The healthy subjects showing high reward sensitivity and low DA-availability (as assumed for 10/10 repeat allele carriers in this case) investigated in this study exhibit increased VS activation. This difference in VS activity compared with ADHD might represent one process of (network) plasticity preventing healthy subjects from exhibiting extreme behavioral phenotypes regarding

reward-seeking. Conversely, relatively high DA-availability might decouple VS activation from trait reward sensitivity as compensatory modulation of VS activation might not be required to regulate behavioral phenotypes in healthy individuals (or medicated patients with ADHD).

Though the notion of DA- and DAT -dependent neuroplasticity as a moderator of the relation between VS activity and trait reward sensitivity appears plausible, functioning of DA neurotransmission is highly complex. It is generally influenced by a multitude of DA system-related compounds such as receptors, metabolic enzymes, and synaptic proteins as well as other neurotransmitter and modulator systems. Furthermore, gene expression and protein conformation/function of each compound is influenced by a larger number of genetic polymorphisms, (epi)genetic mechanisms, and genetic epistasis. One mechanistic example might be the adaptive post-synaptic up- and downregulation, respectively, of both D1- and D2-like receptor gene expression, which has been shown by manipulating dopamine availability though *DAT* overexpression and *DAT* gene knock-out in striatal neurons of mice. This may represent a mechanism by which altered synaptic DA availability due to *DAT* genotype may be adaptively counterbalanced. Thus, our considerations regarding the effect of DAT bioavailability have to be viewed in the light of additional potential genetic influences and complex relationships between dopamine function, striatal processing, and trait reward sensitivity. Overall, further investigations concerning the impact of DA availability, particularly at early ages in relation to the (neuro)development of trait reward sensitivity over longer periods of time appear necessary.

Our results may also have important implications for research in the clinical domain. Individual differences in dopaminergic neurotransmission have been proposed to play a significant role in the neurobiology of severe psychiatric disorders, such as drug addiction and ADHD. For instance, Franklin et al. [2009] showed that nicotine-dependent but otherwise healthy *DAT* 9-repeat allele carriers responded more strongly to smoking cues compared with 10-repeat allele homozygotes. In the light of the present study's results, it appears to be particularly interesting that they observed a correlation between activation in key regions of the reward circuitry and a behavioral measure of craving only for 10/10-carriers, while it was not present in the 9-repeat allele carriers. Our results thus underline the need for a broader view of this data, potentially hinting at alternative interpretations considering the concept of neural plasticity. Concerning ADHD, recent fMRI studies indicate diminished anticipatory reward processing within the VS of patients with ADHD. Pharmacological evidence (Knutson and Gibbs 2007) suggests that these findings are related to hypo-dopaminergic responses during reward anticipation, whereas in vivo neuroimaging of *DAT* density in ADHD remain discrepant (see for a review). A part of these inconsistencies might be due to unaccounted *DAT* interaction effects with, for example,

trait-level factors (compare Fig. 1) that are putatively heterogeneous in different intermediate phenotypes of a disorder (endophenotypes). Therefore, our results call for an extension of the classical endophenotype approaches by including genetic, trait, and neural measures and test for their joint effects in healthy subjects as well as psychiatric patients. In particular, those studies showing an association between psychometric scores and neural activation need to carefully consider the possible impact of altered neurotransmission and neuroplasticity. While such associations might be reliable, they may be selectively missing in subgroups of the samples as shown here. Investigating interactions of all three pivotal players (behavior/trait, neural activity, and genes) can thus be viewed as a means to complement previous studies relating behavior, traits, and psychopathologies to neural activation. Generally, studying the impact of variations in DA neurotransmission on the relation between reward-related brain activity and reward sensitivity by building on the current approach might aid in uncovering the complex mechanisms underlying disorders associated with DA neurotransmission, such as drug addiction and ADHD.

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