Differential Associations of Dopamine-Related Polymorphisms with Discrete Components of Reaction Time Variability: Relevance for Attention Deficit/Hyperactivity Disorder

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Abstract

Background: Reaction time variability (RTV) is considered a valid endophenotype of attention deficit/hyperactivity disorder (ADHD). It is also often used to examine the efficacy of drug treatment or individual patients’ treatment responses and has been furthermore suggested to significantly reduce the potential number of false-positive diagnoses. Among the most commonly investigated candidate genes for ADHD are DRD2, SLC6A3 (DAT), COMT and MAOA. Genetic associations have, however, proven inconclusive or inconsistent.

Methods: Due to the complexity of dopaminergic neurotransmission in the two distinct prosencephalic dopamine pathways, we examined whether the effects of dopamine-related candidate polymorphisms in the genes DRD2, SLC6A3, COMT and MAOA may be differentially associated with discrete subcomponents of RTV, rather than global RTV. A total of 260 healthy volunteers were genotyped for the aforementioned polymorphisms and performed a reaction time paradigm able to distinguish between sensory and motor reaction time. Results: We found that functional polymorphisms in the genes encoding for dopamine-catabolizing enzymes (i.e. COMT and MAOA) are associated with motor RTV but not with sensory RTV, whereas vice versa the gene DRD2 influences sensory but not motor RTV. No significant associations for the gene SLC6A3 (DAT) were found.

Conclusions: Our results give new insight into the inconsistent state of the literature regarding genetic associations of RTV and clearly show that the examination of subcomponents thereof explains far more variance compared to global RTV. This could be of great relevance to the use of RTV in basic research, clinical diagnostics and pharmacological studies examining the efficacy of novel drug treatments.

Introduction

Increased intraindividual variability of reaction time (RT variability, RTV) has consistently been found in patients diagnosed with attention deficit/hyperactivity disorder (ADHD) [1] and is also considered a useful endophenotype of the disorder [2].
Reviews of genetic associations of RT [3] show that the most commonly investigated dopamine-related genes are DRD4, SLC6A3 (DAT), COMT, MAOA and DBH, whereby only the gene DRD4 appears to have the most consistent influences, wherefore we chose not to examine the associations of this gene further within the scope of this study.

Other researches additionally report relevant associations between the gene DRD2/ANKK1 and ADHD [4, 5], while others report negative associations both regarding the association with ADHD [6] as well as RTV [7].

Since dopaminergic neurotransmission in the forebrain relies on two functionally discrete systems – namely the nigrostriatal system involved in the modulation of striatal gating and the mesolimbic system involved in stimulus processing and salience attribution [for an overview, see 8] – we assumed that the inconsistency in findings regarding the aforementioned genetic associations may be due to a lack of separation between these two distinct aspects of dopamine functioning. We therefore used an RT paradigm capable of distinguishing between sensory and motor subcomponents of RT (sRT and mRT) and their respective variabilities and examined the associations of these individual RT components with functional polymorphisms of the genes DRD2/ANKK1, SLC6A3 (DAT), COMT (catechol-O-methyltransferase) and MAOA (monoamine oxidase isozyme A). These polymorphisms were the Val158 Met polymorphism (rs4860) of the gene coding for COMT, the upstream variable number tandem repeat (uVNTR) of the gene encoding for MAOA, the 3′-untranslated region variable number tandem repeat (3′-UTR-VNTR) of the gene encoding for the dopamine active transporter (SLC6A3) and the Taq1A polymorphism (rs1800497) of the gene ANKK1, formerly believed to lie within the gene encoding for the postsynaptic D2 receptor (DRD2).

The Val allele of rs4860 has been shown to result in a higher active and more thermostable form of COMT [9] compared to the Met allele. Similarly, the number of repeats of the MAOA uVNTR influences the expression and therefore the abundance of MAOA [10, 11]. Both genes are highly expressed, i.a. in the basal ganglia – with higher expression values for COMT compared to MAOA [12]. Both of the aforementioned polymorphisms will impact striatal dopamine and noradrenaline metabolism. Since dopamine levels in the striatum are 13–30 times higher than those of noradrenaline [13], which is also degraded by COMT, the striatal effects of the rs4860 are primarily on dopaminergic degradation. Furthermore, as dopamine has been shown to be equally degraded by both MAO isozymes [14], and both isozymes have nearly identical Michaelis-Menten constants and Vmax values for dopamine [15], the MAOA uVNTR will also influence striatal dopamine availability. It should be mentioned that COMT is often reported to be of higher influence in the frontal cortex; this is, however, due to lower levels of dopamine reuptake and MAO availability and not because of low COMT levels in the striatum – this as well as the importance of both COMT and MAOs in the striatum are clearly shown by the rates of 3,4-dihydroxyphenylacetic acid production (DOPAC, the MAO metabolite of dopamine degradation) and the ratios of 3-methoxytyramine (the COMT metabolite of dopamine degradation) to DOPAC (3-methoxytyramine/ DOPAC ratios) in the striatum compared to the frontal cortex [16].

Since COMT, MAOs and SLC6A3 are more involved in synaptic dopamine availability, we believe, due to the aforementioned argumentation regarding the expression of these genes and the differential functional effects of their respective proteins, that the related polymorphisms would more likely influence striatal gating and thus motor control, whereby those genotypes leading to higher dopamine concentrations would probably result in more stable motor control (i.e., reduced RTV), but should not significantly influence average motor speed or sRT. These alleles would be the Met allele of the Val158 Met polymorphism in COMT and the low-activity alleles of the MAOA uVNTR. Regarding the DAT 3′-UTR-VNTR, we expected the 9-repeat allele to be associated with higher motor control and reduced RTV, since imaging studies show increased deactivation of the striatum in carriers of this allele in an executive processing paradigm [17]. The number of repeats of the DAT 3′-UTR-VNTR [18] is believed to influence the expression of the SLC6A3 gene, although these results are inconsistent [17].

The A1 allele of the DRD2/ANKK1 Taq1A polymorphism leads to reduced D2 receptor availability [19]. Due to the functional aspects of the dopamine pathways and the described polymorphisms it was therefore hypothesized that the DRD2 Taq1A would be more likely to be associated with the sensory aspect of RT, since it is postsynaptic and therefore more related to the efficacy of dopamine transmission, especially in regard to the speed of salience attribution to a given stimulus. Therefore, persons carrying the A1 allele were expected to show longer durations between stimulus presentation and the initialization of movement as well as higher variability thereof.
Methods and Materials

Sample
The sample consisted of 260 students (211 female, 49 male) of Western European descent at Justus Liebig University, Giessen, Germany. The mean age was 23.15 years (median = 22 years) with a standard deviation of 4.9 years. They were asked to fill in a number of personality inventories (results not shown here) for the Department of Individual Differences ‘Giessen Gene Brain Behaviour Project’, give a sample of buccal epithelial cells for genotyping and perform an RT paradigm. This study was approved by the local ethics committee of the Psychological Faculty at Justus Liebig University (Locale Ethik-Kommission des Fachbereichs 06 der Justus-Liebig-Universität Giessen, LEK-FB06). All participants provided their written informed consent to participate in this study.

Participants were screened via self-report for factors that could influence experimental procedures or results (e.g. current use of prescription or illegal drugs, acute psychiatric or other clinical diagnosis, past psychiatric or neurological diagnosis within the past year) prior to invitation to participate in the design.

Genotyping
Extraction of DNA from buccal epithelia and genotyping using PCR amplification were performed according to standard protocols previously published by us [20].

Due to molecular biological reasoning, females heterozygous for the MAOA uVNTR polymorphism were excluded from related analyses. Regarding the classification of high-versus low-activity alleles, we chose to follow the classification of the 5-repeat allele as highly active [11].

In case of the SLC6A3 (DAT) 3′-UTR-VNTR polymorphism, which usually consists of 9- or 10-repeat alleles, those participants with other numbers of repeats were omitted, since these alleles are extremely rare [18].

All genotyping was performed within our own laboratory, and all genotyping results were double checked by the technician, an experienced researcher (P.G. or Y.K.), as well as by the group’s administrative secretary upon entering into our genotyping database. Additionally, goodness-of-fit to the Hardy-Weinberg principle was examined (see first paragraph of Results section).

RT Paradigm
Participants performed a choice RT paradigm using a Leeds psychomotor test (ZAK GmbH, Germany). Participants are asked to place the index finger of their preferred hand on the central home button, which is surrounded by 6 equidistant response buttons, each located in front of a red LED. These LEDs light up at random, and participants are asked to extinguish the respective light by pressing the corresponding target button.

This allows for the calculation of two individual components of the whole RT. These are the time between the lighting up of an LED and the participant’s lifting the finger off the home button (reaction time or sRT) and the time between the lifting off from the home button and the touching of the (correct) target button (mRT). After each trial participants place their index finger back on the home button, which is acknowledged by lighting up of a green LED next to the home button. Participants completed a total of 120 trials.

RT data were examined for outliers (defined as 2 standard deviations outside of the group and/or individual averages); no participant had to be excluded on these grounds.

Statistical Analyses
Hardy-Weinberg statistics were calculated using an online Hardy-Weinberg equilibrium calculator [21].

All other statistical procedures were performed using the Statistical Package for the Social Sciences (SPSS, version 15.0). Individual sRTs and mRTs were arithmetically averaged, and the standard deviations of these means were then divided by the mean in order to receive each participant’s individual coefficient of variation (ICV). The ICVs serve as an optimal measure for a person’s RTV [22].

For each person we therefore used 4 dependent variables: the arithmetic means of the sRT (sRT_M) and mRT (mRT_M) as well as the sensory and motor ICVs (s_ICV and m_ICV, respectively). It has to be stressed, however, that maximally 2 of the respective dependent variables were entered into any given analysis (i.e. sRT_M and s_ICV or mRT_M and m_ICV).

Initially, effects of sex and age were examined regarding their effect on any of the dependent variables via t tests and correlation analyses, respectively. In any case where such main effects were found (see Results section), the variance of the confounding variable on the dependent variable was removed by entering only the confounding variable as a covariate into a generalized linear model (GLM) analysis and saving of the standardized residuals of the respective dependent variable. Further analyses of genetic associations were then only performed for the said standardized residuals (see Results section).

The genetic associations regarding the dependent variables (or their standardized residuals) were performed using the GLM.

In cases of directed hypotheses reached due to our functional argumentation (see Introduction section) – i.e. ‘carriers of the A1 allele of the Taq1A have a higher sRT_M and s_ICV than A2/A2 homozygotes’ and ‘carriers of genetic indicators of higher dopamine availability regarding the COMT, MAOA and SLC6A3 polymorphisms have a reduced m_ICV compared to participants with genetic indicators of lower dopamine availability (see Introduction section) – one-tailed testing was performed. In these cases, any deviations (independently of significance) in the direction not proposed by our alternative hypothesis leads to nonrejection of the null hypothesis, wherefore one-tailed testing is called for [23]. For all other hypotheses two-tailed testing was performed.

Results

Genotype Distributions
Note that for the single polymorphisms the number of participants does not always add up to 260. This is mainly due to the argumentation regarding the repeat polymorphisms of the SLC6A3 and MAOA genes in the Methods section.

Table 1 shows that all genotypes are in Hardy-Weinberg equilibrium with the exception of the Taq1A. In this case, however, the marginal deviation from the Hardy-Weinberg equilibrium is negligible, as a distribution with one TT homozygote more and one TC heterozygote less would lead to an insignificant χ² statistic. Additionally, in

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cases of low minor allele frequencies, χ²-based Hardy-Weinberg calculators have been shown to underestimate the true p values [24, 25].

**Effects of Age and Sex**

Since RTs may be influenced by age and could be sexually dimorphic, we performed correlations between all dependent variables and participants’ age as well as independent-sample t tests for sex.

We found no significant correlations for age, wherefore this factor was not entered into any subsequent analyses.

Regarding sex, however, we found a significant sexual dimorphism for the average mRTs and the m_ICV, but not for sensory aspects of RT. Regarding the motor components, women showed significantly slower (T 61.997 = –3.149; p < 0.001) but more stable reactions (T 59.172 = 4.617; p = 0.009) than men. Subsequent analyses were therefore performed on the standardized residuals of mRT_M and m_ICV, with variance due to the influence of sex removed as described in the section on statistical analyses.

**Genetic Associations of Aspects of mRTs**

We found no significant effect of the DAT 3'-UTR-VNTR on aspects of mRT and its variability.

Regarding the other two polymorphisms hypothesized to influence motor RTV, descriptive statistics showed that both groups with relatively high dopamine turnover resulting in lower dopamine abundance (i.e. the Val/Val heterozygotes of the COMT Val158Met polymorphism and the high-activity groups of the MAOA uVNTR) showed significantly higher standardized residuals of m_ICV values compared to carriers of the Met allele (COMT Val158Met) or low-activity groups of the MAOA uVNTR (table 2).

Due to the argumentation above regarding the correct use of ANCOVAs, the standardized residuals from a multivariate covariance analysis of the effects of sex on the mRT_M and the m_ICV were examined regarding their associations with the Met allele of the rs4680 (COMT Val158Met) and the low-activity alleles of the MAOA uVNTR.

There was a significant effect of the Met allele of the rs4680 on aspects of motor reaction time (F 2,244 = 5.142; p = 0.003). Univariate analyses showed that this effect was dominated by the differences in m_ICV (F1 = 9.95; p = 0.001) while, as expected, differences in mRT_M were not significant (F1 = 0.081; p = 0.777, two-tailed).

Regarding the MAOA uVNTR, the multivariate analysis was only marginally significant (F 2, 142 = 2.148; p = 0.06). In the univariate analyses, however, differences in m_ICV were significant (F1 = 3.862; 0.025), while, again as expected, those in mRT_M were not (F1 = 0.007; p = 0.934, two-tailed).
Genetic Associations of Aspects of Sensory Reaction Time

The multivariate analysis of the effects of the A1 allele of the DRD2/ANKK1 Taq1A polymorphism was significant ($F_{2, 244} = 4.128; p = 0.001$). In univariate tests the effect was shown to be significant for both the s_ICV ($F_1 = 7.811; p = 0.003$) and the sRT_M ($F_1 = 3.319; p = 0.035$). After correction for the two multiple comparisons ($\alpha = 0.025$) however, the differences in the sRT_M only bordered on significance, although it has to be mentioned that due to the nature of our experiment and the drawn conclusions only comparisonwise and not experimentwise errors are relevant, wherefore correction for multiple testing is actually not necessary [26]. It could be shown, therefore, that carriers of the A1 allele showed significantly higher average sRTs and sensory ICVs compared to A2 homozygotes (table 3).

Specificity of These Associations

As assumed in our hypotheses, we found no significant effects of the rs4680, the MAOA uVNTR and the DAT 3′-UTR-VNTR on sRTs or effects of the DRD2/ANKK1 Taq1A on mRT, thereby showing that these effects are indeed specifically and differentially associated with the respective components of RT (online suppl. table 1; for all online supplementary material, see www.karger.com/doi/10.1159/000360367). Furthermore, even if we had used two-tailed testing, all of our results would have still been significant; with the exception of the association between the Taq1A and average sensory reaction time (sRT_M), which would have been marginally significant ($p = 0.07$, two-tailed).

Discussion

In accordance with our hypotheses we found differential associations of various genetic polymorphisms with an influence on dopaminergic neurotransmission on individual aspects of RTV. Using a Leeds psychomotor test, we were able to differentiate between the sensory and motor components of RT and its variability. While participants who carried 1 or 2 A1 alleles of the DRD2/ANKK1 Taq1A polymorphism, which are associated with reduced D2 receptor density in the postsynaptic membrane, showed both increased sRTs and sensory reaction variability, they did not differ regarding components of mRT.

On the other hand, those participants carrying allelic variations of the COMT Val158Met or MAOA uVNTR polymorphisms related to higher synaptic dopamine turnover and thereby a reduction in dopamine availability showed significantly higher variability in their mRTs. An ex-post-facto GLM analysis showed that these effects were significantly additive ($F_2 = 3.39; p = 0.018$, one-tailed) in that carriers of 2 high-activity genotypes had the highest standardized residuals of the m_ICV (mean = 0.35; SEM = 0.21) and those with 2 low-activity genotypes had the lowest values (mean = –3.3; SEM = 0.16), while those with 1 high- and 1 low-activity genotype were exactly in the middle (mean = 0.007; SEM = 0.11). As expected, the same polymorphisms did not influence the average mRT or any components of sRT.

Contrary to our initial predictions, we could not find any associations between any of the components of either mRT or sRT with the SLC6A3/DAT 3′-UTR-VNTR.

The relation of D2 receptor binding to movement disorders, i.a. akinesia which is defined as slowness in initiation and execution of willed movement [27], has already been shown by members of our group to be related to neuroleptics with high D2 affinity compared to atypical antipsychotics [28]. Akinesia has also been shown as a result of D2 receptor knockout in mice [29]. Additionally, many components of the regions involved in the proposed attentional network (i.a. the basal ganglia and the thalamus) impaired in ADHD [30] are known for their relatively high D2 receptor density [31] and respective DRD2 mRNA expression [32], wherefore these regions would be intra-

Table 3. GLM analyses of the effects of DRD2 Taq1A on the s_ICV and the sRT_M

<table>
<thead>
<tr>
<th>Variable</th>
<th>M and SEM group 1 (A1 carriers)</th>
<th>Sample size group 1</th>
<th>M and SEM group 2 (A2 homozygotes)</th>
<th>Sample size group 2</th>
<th>F (d.f. = 1)</th>
<th>p (one-tailed)</th>
<th>partial $\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sRT_M, ms</td>
<td>368.18 (4.87)</td>
<td>82</td>
<td>357.33 (3.43)</td>
<td>165</td>
<td>3.32</td>
<td>0.035</td>
<td>0.01</td>
</tr>
<tr>
<td>s_ICV</td>
<td>0.203 (0.08)</td>
<td>82</td>
<td>0.173 (0.08)</td>
<td>165</td>
<td>7.84</td>
<td>0.003</td>
<td>0.03</td>
</tr>
</tbody>
</table>

M = Arithmetic mean; SEM = standard error of mean; d.f. = degrees of freedom; p = conditional probability; s_ICV = sensory individual coefficient of variation; sRT_M = average sensory reaction time.
individually most affected by carrying 1 or 2 A1 alleles of the Taq1A. The reduced attentional capacities in A1 carriers of the Taq1A have also been shown within a study using the omission rates of calls in the continuous performance task as indicators of attention processes [33].

Regarding the observed effects of genetic variants related to reduced dopamine levels, studies in nonhuman primates have clearly shown a linear relationship between the level of dopamine depletion in the striatum and severity of motor symptoms [34]. This mechanism has been shown to be related to dysregulation of striatal gating as a consequence of dopamine denervation [35]. The fact that dopamine abundance is not relevant for reaction speed but only for its stability, is not surprising and was expected, since studies in rodents show that reduction in motor speed in a swim test as a result of dopamine depletion in the substantia nigra, pars compacta, or the caudate nucleus amounts to only a few milliseconds and is also attenuated with increasing trials [36, 37]. Similar findings are reported for patients with Parkinson’s disease [38]. We therefore did not expect the relatively moderate reduction of striatal dopamine abundance as an effect of high-performance genotypes in COMT Val158Met or MAOA uVNTR to significantly affect motor speed in healthy participants, especially considering the large number of trials used in our study.

The lack of associations with the 3′-UTR VNTR of the gene SLC6A3 (DAT) to either sRT or mRT is remarkable but not overly surprising, since results regarding the functional consequences of this polymorphism are highly ambiguous [39], and it has also often been reported not to be associated with overall RTV in the past [3].

We believe the fact of our sample being underpowered for the detection of effects of the size reported in table 3 (especially in the case of MAOA, where all heterozygotes were excluded from the analyses) makes our findings even more compelling [40]. Also, the unequal cell sizes in the case of the COMT Val158Met underscores this, as variation between individuals influences the indices of deviation more strongly in smaller (sub-)samples. Since the resulting heterogeneity in smaller (sub-)samples, however, makes rejection of the null hypothesis more difficult as outliers influence error variance more than they do the mean [41], again the verisimilitude of our findings is strengthened.

We therefore conclude that the inconsistency of findings regarding RTV in ADHD, both as an endophenotypic marker of ADHD and as a function of genetic polymorphisms, may be due to a lack of differentiation between sRT and mRT and their respective variability. We suggest future research should not solely investigate global RT and RTV, but rather distinguish the apparently functionally different aspects of sensory processing and motor control.

Additionally, since RTV has also been suggested to be able to greatly reduce the number of false-positive ADHD diagnoses [42, 43], we suggest that a further differentiation between the sensory and motor aspects of RTV may even more improve upon the use of this marker in ADHD diagnostics, follow-up examinations and finer differentiation between ADHD subtypes.

Finally, we believe that our results may have an implication for pharmacological ADHD treatment. A number of studies have shown that methylphenidate (MPH) significantly reduces RTV in ADHD patients [1]. Studies comparing the efficacy of other drugs with effects on dopamine abundance, i.a. reuptake inhibitors of noradrenaline (atomoxetine) or serotonin (citalopram), in comparison to MPH or placebo, however, report no effects of any pharmacological treatment other than MPH on RTV [44]. The authors argue that the primary effect of MPH is in the striatum, whereas atomoxetine, for example, works mainly on the frontal cortex. A differentiation between sensory and motor RTV may therefore show that these drugs do actually work, even though effects on global RTV are not measurable. Taking our findings into consideration may therefore lead to a more differentiated pharmacological treatment and possible alternatives to MPH.

Acknowledgments

The authors would like to thank Cornelia Meineke for her excellent technical assistance as well as Luise Blochberger, Andrea Hasselbach, Ann-Kathrin Kreuder, Julia Billek, Dorothea Borchert, Anne-Lisa Beerroh, Jasmin El Shazly and Marcel Riehle for their help in data acquisition. We would also like to thank the reviewers for their constructive comments and for pointing out the fact that a deviation of the genotype distribution in case of the Taq1A polymorphism by a single individual would lead to an insignificant χ² statistic.

Disclosure Statement

All authors declare that there are no existing conflicts of interest.

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Neuropsychobiology 2014;69:220–226
DOI: 10.1159/000360367


