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Note

Effects of L-Tyrosine on working memory and inhibitory control are determined by *DRD2* genotypes: A randomized controlled trial

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ABSTRACT

L-Tyrosine (TYR), the precursor of dopamine (DA), has been shown to enhance facets of cognitive control in situations with high cognitive demands. However some previous outcomes were mixed: some studies reported significant improvements, while other did not. Given that TYR increases DA level in the brain, we investigated, in a double-blind, randomized, placebo-controlled design, whether the C957T genotypes of a functional synonymous polymorphism in the human dopamine D2 receptor (*DRD2*) gene (rs6277) contribute to individual differences in the reactivity to TYR administration and whether this factor predicts the magnitude of TYR-induced performance differences on inhibiting behavioral responses in a stop-signal task and working memory (WM) updating in a N-back task. Our findings show that T/T homozygotes (i.e., individuals potentially associated with lower striatal DA level) showed larger beneficial effects of TYR supplementation than C/C homozygotes (i.e., individuals potentially associated with higher striatal DA level), suggesting that genetically determined differences in DA function may explain inter-individual differences in response to TYR supplementation. These findings reinforce the idea that genetic predisposition modulates the effect of TYR in its role as cognitive enhancer.

1. Introduction

L-Tyrosine (TYR), precursor of dopamine (DA), enhances DA release in the human brain (Growdon, Melamed, Logue, Hefti, & Wurtman, 1982). Once the optimal DA level is reached, TYR is no longer converted into DA because tyrosine hydroxylase, the enzyme responsible for the conversion, is inhibited (Weiner, Lee, Barnes, & Dreyer, 1977). Several lines of evidence suggest that mechanisms regulating tyrosine turnover

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modulate cognitive control (Beste, Getzmann, Gajewski, Golka, & Falkenstein, 2014; Stock, von Heinegg, Köhling, & Beste, 2014). However, previous studies on the effect of TYR focused mainly on deficits in TYR to DA conversion (Pietz et al., 1995; Van Spronsen, Van Rijn, Bekhof, Koch, & Smit, 2001) and on the depletion of TYR (Fernstrom & Fernstrom, 2007; Harmer, McTavish, Clark, Goodwin, & Cowen, 2001). The outcomes were mixed: following TYR intake, some patients reported significant improvements, while other did not (see Jongkees, Hommel, Kühn, & Colzato, 2015 for a recent review). In healthy individuals, TYR has also been used to counteract the negative effects of conditions that deplete the brain's dopaminergic resources, such as extreme stress. The outcomes were again mixed: the supply of TYR was found to reduce stress-induced impairments of working memory (WM) and attention, but more so in individuals who were particularly sensitive to the stressors (Deijen & Orlebeke, 1994; Mahoney, Castellani, Kramer, Young, & Lieberman, 2007; Shurtleff, Thomas, Schrot, Kowalski, & Harford, 1994). Only recently, the focus has moved to the possible enhancing effects of TYR on challenging cognitive performance in the absence of physical stress. Indeed, even without exposure to stress, the supplementation of TYR has been revealed to have a short-term beneficial effect on challenging task performance driven by DA, such as multitasking (Thomas, Lockwood, Sing, & Deuster, 1999), the updating and monitoring of WM (Colzato, Jongkees, Sellaro, & Hommel, 2013), inhibitory control (Colzato, Jongkees, Sellaro, van den Wildenberg, & Hommel, 2014) and cognitive flexibility (Steenbergen, Sellaro, Hommel, & Colzato, 2015). Hence, high cognitive challenges lead to a stress-like state inducing DA depletion and associated cognitive impairments, which are then reversed by TYR (Jongkees et al., 2015).

We suggest that mixed findings with respect to TYR supplementation in previous studies were due to the failure to consider individual differences in DA function. Given that TYR increases DA level in the brain (Growdon et al., 1982), we took into account individual differences (i.e., genetic differences) in DA function to explain the effectivity of TYR (Jongkees, Hommel, & Colzato, 2014). Our gene candidate is C957T (rs6277) single nucleotide polymorphism (SNP) of the D2 receptor gene (*DRD2*) given that this is the only gene that has been demonstrated to (a) regulate dopaminergic functioning (Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009); (b) be implicated in the two cognitive control functions (Colzato, van den Wildenberg, van der Does, & Hommel, 2010; Li et al., 2013; Stock, Arning, Epplen, & Beste, 2014) that have been found to benefit from TYR administration (response inhibition and WM updating; Colzato, Jongkees, et al., 2013; Colzato et al., 2014).

In a nutshell, the goal of this study was to investigate whether individual differences in the C957T polymorphism at *DRD2* gene contribute to individual differences in the reactivity to TYR to predict performance on (a) inhibiting behavioral responses in a stop-signal task and (b) WM updating in a *N*-back task. In particular, we expect T/T homozygotes (i.e., individuals potentially associated with lower striatal DA level; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009) to show larger beneficial effects of TYR supplementation than C/C homozygotes (i.e., individuals potentially associated with higher striatal DA level; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009), suggesting that TYR might eliminate the genetic impact of C957T polymorphism at *DRD2* gene on response inhibition and WM updating.

2. Materials and methods

2.1. Participants

166 healthy Caucasian bachelor students (level of education in the Dutch system VWO) of the Leiden University with no cardiac, hepatic, renal, neurological or psychiatric disorders participated in the experiment, see Table 1. All participants were selected individually using the Mini International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998). The M.I.N.I. is a well-established brief diagnostic tool in clinical and pharmacological research that screens for several psychiatric disorders and drug use (Colzato & Hommel, 2008; Colzato, Kool, & Hommel, 2008; Sheehan et al., 1998). We made sure that the participants met the following criteria: (1) no clinically significant medical and psychiatric disease and (2) no use of drugs and/or medication. Written informed consent was obtained from all subjects; the protocol and the remuneration arrangements of 15 euro in cash payment were approved by the local ethical committee (Leiden University, Institute for Psychological Research). A double blind, placebo-controlled design was used. Randomly, 85 participants were assigned to the TYR group, whereas 81 were assigned to the placebo group. Following previous published protocols (Colzato, Jongkees, et al., 2013; Colzato et al., 2014), participants in the TYR group were exposed to an oral dose (powder) of 2.0 gr of TYR (supplied by Bulkpowders Ltd.), whereas those in the placebo group were exposed to an oral dose of 2.0 gr of microcrystalline cellulose (Sigma–Aldrich Co. LLC), a neutral placebo. Both TYR and placebo were dissolved in 400 ml of orange juice. All female participants were using contraception pill and were tested when they actually used the pill given that fluctuations in hormone levels associated with the menstruation cycle can influence DA function and thereby confound results related to DA (Colzato & Hommel, 2014).

2.2. Genotyping

Rs6277 is a synonymous SNP (Pro319Pro) within the coding sequence of *DRD2*. Genotyping was performed by PCR-RFLP techniques. Primers were designed with Primer Express 2.0 software (Applied Biosystems). In particular, the T allele has been linked to reduced messenger ribonucleic acid (mRNA) stability, affecting the density of D2 receptors (Duan et al., 2003). Indeed, instead of being "silent", the T allele altered the predicted mRNA folding leading to a decrease in mRNA stability and translation that dramatically changed dopamine-induced up-regulation of *DRD2* expression (Duan et al., 2003). This allele has also been associated with reduced extrastriatal D2 receptor availability (Hirvonen, Lumme, et al., 2009) and lower striatal DA levels (Hirvonen, Laakso, et al., 2009). However, a recent meta-analysis of human in vivo molecular imaging studies states that there

Table 1 – Sample and genotype-specific demographics as a function of group (TYR *vs* Placebo).

Group		N	Sex		Age	BMI
			Male	Female		
TYR	Total	85				
	C/C homozygotes	22	3	19	20.1	22.0
	C/T heterozygotes	44	7	37	20.9	21.8
	T/T homozygotes	19	5	14	22.4	21.8
Placebo	Total	81				
	C/C homozygotes	26	5	21	20.5	22.0
	C/T heterozygotes	35	5	30	19.8	21.5
	T/T homozygotes	20	4	16	19.9	21.9
ALL	_	166	29	137	20.6	21.8

were no replications "in which two or more comparable, independent, studies of striatal D2 receptors" tested for effects of this variant (Gluskin & Mickey, 2016). In the current study no other *DRD2* variants have been tested in relation to TYR enhancement of cognitive function.

All other details of the methodology and primer sequences are available upon request.

2.3. *Stop-signal task*

The version of the stop-signal task used in this study was adapted from Colzato et al. (2010), Colzato, Jongkees, et al. (2013), Colzato, van den Wildenberg, and Hommel (2013). Arrows were presented pseudo-randomly for a maximum of 1500 ms, with the constraint that they signaled left- and right-hand responses equally often. Arrow presentation was response-terminated. The green arrow changed to red on 30% of the trials, upon which the choice response had to be aborted (stop trials). A staircase-tracking procedure dynamically adjusted the delay between the onset of the go signal and the onset of the stop signal to control inhibition probability (Levitt, 1971). The algorithm ensured that motor actions were successfully inhibited in about half of the stop trials, which yields accurate estimates of stop-signal reaction-time (SSRT) and compensates for differences in GO RT between participants (Band, van der Molen, & Logan, 2003). Shorter SSRTs are a sign of better ability of stopping on time, i.e., better response inhibition. Hence, given that the beneficial effect of TYR seems to be limited to cognitively challenging conditions (Colzato, Jongkees, et al., 2013; Colzato et al., 2014; Thomas et al., 1999), we would expect lower SSRTs, but no effect on RTs to go-signals after TYR administration, compared to placebo, in T/T homozygotes (i.e., individuals potentially associated with lower striatal DA level).

2.4. N-*back task*

The three conditions of the letter-based version of the *N*-back task were adapted from Colzato, Jongkees, et al. (2013). Participants responded to targets (presented in 33% of the trials) and to nontargets by pressing the z and m keys. The response button assignment was randomized across participants. All participants performed the 1-back condition first and then the 2-back and 3-back conditions. *N*-back task performance was analyzed using the signal detection theory (Swets, Tanner, & Birdsall, 1961) to derive d prime (d′), a net score that takes into consideration an individual's hit and false alarm rates, which has been proposed to provide a more suitable index of WM performance in the *N*-back task (Haatveit et al., 2010). For each individual participant, d′ scores were calculated separately for the three n-back conditions, and perfect scores were corrected for, as described earlier (Colzato, Jongkees, et al., 2013). A high d′ indicates better target sensitivity, reflecting task performance that maximizes hits while minimizing FAs.

In the 2-back and 3-back conditions, the task requires the on-line monitoring and updating of WM content, which is known to be cognitively demanding (Kane, Conway, Miura, & Colflesh, 2007). In contrast, performance in the easiest 1-back condition depend on immediate perceptual priming (as the two matching items appear in direct succession), which makes this condition a suitable (i.e., WM-undemanding) control condition. Given that the enhancing effect of TYR seems to be restricted to cognitively challenging conditions (Colzato, Jongkees, et al., 2013; Colzato et al., 2014; Thomas et al., 1999), we expected that the beneficial effect of TYR (i.e., higher d′ scores) in T/T homozygotes (i.e., individuals potentially associated with lower striatal DA level) will be restricted to the cognitively demanding 2- and 3-back conditions.

2.5. *Procedure and design*

All participants were tested individually. Upon arrival, from each participant DNA was collected via buccal swaps. Then, they were asked to practice for 20 min the *N*-back and the stop-signal task. One hour following the administration of TYR (corresponding to the beginning of the 1 h-peak of the plasma concentration; Glaeser, Melamed, Growdon, & Wurtman, 1979) or placebo, participants were asked to perform a color vision test unrelated to the purposes of the present study (data not reported here) and immediately after the *N*-back (30 min) and the stop-signal task (30 min) measuring WM updating and response inhibition, respectively. Because of technical problems, two participants (T/T homozygotes) did not perform the *N*-back task and two participants (one T/T homozygotes and one C/T heterozygotes) did not perform the stop-signal task. Also, for two participants

(one T/T homozygotes and one C/T heterozygotes), data of the 1-back condition were not saved.

2.6. Statistical analysis

First, separate univariate ANOVAs were performed to rule out possible differences between genotype groups in terms of age and body max index (BMI). A Chi-square test was performed to verify whether the genotype groups were comparable in terms of gender distribution. Second, for all participants, individual SSRTs for stop-signal trials and mean RTs to go-signals were calculated to index response inhibition and response execution, respectively. Third, in order to test whether TYR eliminates the genetic effect on inhibitory control without affecting response execution, SSRTs and mean RTs to go-signals were analyzed separately by means of univariate ANOVAs with C957T polymorphism at *DRD2* (C/C homozygotes *vs* C/T heterozygotes *vs* T/T homozygotes) and group (TYR *vs* Placebo) as between-subjects factors. Finally, in order to test whether TYR eliminates the genetic effect on WM updating, d′ scores were submitted to a repeated measures ANOVA with C957T polymorphism at *DRD2* (C/C homozygotes *vs* C/T heterozygotes *vs* T/T homozygotes) and group (TYR *vs* Placebo) as between-subjects factors and WM load (1 *vs* 2 *vs* 3-back condition) as within-subjects factor. Further, means of other *N*-back task parameters (RTs on target and nontarget trials, hits, correct rejections, false alarms and misses in percent for 1-back, 2-back and 3-back) are listed in Table 2. A significance level of $p < .05$ was adopted for all statistical tests. In case of significant interaction, post hoc analyses were conducted using Fisher HSD test.

3. Results

3.1. Participants

Sample information and genotype-specific demographics are shown in Table 1.

Genotype distribution for C957T polymorphism at *DRD2* in our Dutch healthy population was 48 C/C homozygous subjects (28.9%), 79 C/T heterozygous subjects (47.6%) and 39 T/T homozygous subjects (23.5%). All resulting genotype frequencies from our cohort of participants did not deviate from Hardy–Weinberg equilibrium ($p > .5$). No significant differences were found among genotype groups with respect to age, $F(2, 163) = 1.016$, $p = .36$, $\eta 2p = .012$, BMI, $F(2, 163) < 1$, $p = .76$, $\eta 2p = .003$, or sex, $\chi^2 = 1.16$, $p = .56$.

3.2. Stop-signal task

Means of the stop-signal task parameters are listed in Table 2.

Analyses of SSRTs revealed a significant main effect of group, $F(1, 158) = 4.343$, $p = .039$, $\eta 2p = .027$. Replicating

Table 2 – Parameters of the stop-signal and the *N*-back tasks.

	C/C Hom.				C/T Het				T/T Hom			
	Placebo		Tyrosine		Placebo		Tyrosine		Placebo		Tyrosine	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Stop-signal												
Mean stop-signal RT (SSRT)	240	20	237	45	239	26	243	35	253	33	212	67
Mean RTs on go trials	440	74	421	61	408	54	424	62	403	50	423	58
N-back task												
Hits												
1-back	91	5.4	91	5.3	92	7.1	91	7.3	91	8.9	94	5.0
2-back	88	11.6	85	18.2	90	6.9	87	9.5	81	10.7	94	4.4
3-back	76	14.5	70	15.5	75	14.9	69	13.7	73	13.9	80	14.0
Misses												
1-back	9	5.4	9	5.3	8	7.1	9	7.3	9	8.9	6	5.0
2-back	12	11.6	15	18.2	10	6.9	13	9.5	19	10.7	6	4.4
3-back	24	14.5	30	15.5	25	14.9	31	13.7	27	13.9	20	14.0
Correct rejections												
1-back	97	3.8	97	2.1	96	3.0	97	3.0	96	5.8	98	1.5
2-back	93	5.8	92	1.2	92	6.2	93	5.3	91	4.9	96	3.7
3-back	86	8.3	87	8.0	85	9.7	83	10.0	86	9.2	90	9.0
False alarms												
1-back	3	3.8	3	2.1	4	3.0	3	3.0	4	5.8	2	1.5
2-back	7	5.8	8	1.2	8	6.2	7	5.3	9	4.9	4	3.7
3-back	14	8.3	13	8.0	15	9.7	17	10.0	14	9.2	10	9.0
Reaction times 1-back												
Target	437	43.7	432	66.5	441	47.6	461	40.2	471	55.2	414	51.2
Non-target	465	55.5	453	70.0	481	58.0	478	44.3	475	45.5	434	46.8
Reaction times 2-back												
Target	487	76.7	456	66.0	490	68.5	492	63.8	523	92.3	452	72.2
Non-target	525	76.7	486	61.0	536	70.3	539	54.6	534	68.5	507	70.2
Reaction times 3 back												
Target	530	89.2	531	71.1	521	74.9	546	72.4	562	63.6	495	90.2
Non-target	579	87.1	534	49.1	566	80.8	562	73.6	561	65.1	538	82.5

previous findings (Colzato et al., 2014), participants who received TYR showed faster SSRTs (i.e., better inhibitory control), as compared to participants who received placebo ($M = 231$, SEM = 4.4 *vs* $M = 244$, SEM = 4.5, Cohen's $d = .31$). The main effect of C957T polymorphism at *DRD2* was not significant, $F(2,158) < 1$, $p = .45$, $\eta 2p = .008$.

More importantly, group interacted significantly with the C957T polymorphism at *DRD2*, $F(2,158) = 4.39$, $p < .05$, $\eta 2p = .053$. As expected, Fisher HSD post-hoc tests showed that TYR administration, compared to placebo, had a significant beneficial effect (reduced SSRT) for T/T homozygotes (unfavorable genetic predisposition), $p < .005$, Cohen's $d = 1.05$, but not for C/T heterozygotes $p = .66$, Cohen's $d = .10$, and C/C homozygotes, $p = .80$, Cohen's $d = .07$, see Fig. 1. Additional post-hoc comparisons showed no significant differences between genotype groups for those participants who received placebo, $p_s \geq .21$, Cohen's $d_s \leq .36$. For those participants who received TYR, T/T homozygotes differed significantly from both C/C homozygotes ($p = .04$, Cohen's $d = .64$) and C/T heterozygotes ($p = .004$, Cohen's $d = .79$), who showed comparable performance ($p = .56$, Cohen's $d = .15$). In contrast to SSRTs, analyses of mean RTs to go-signals did not yield any significant main effect or interaction, $F_s \leq 1.22$, $p_s \geq .30$, $\eta 2p_s \leq .015$, indicating that TYR eliminates the genetic effect of C957T polymorphism at *DRD2* selectively in SSRTs, but not in RT to go-signals.

3.3. N-*back task*

ANOVA performed on d′ scores revealed a significant main effect of WM load, $F(2, 312) = 217.81$, $p < .001$, $\eta 2p = .58$. This indicates significantly higher d′ scores in the 1-back condition ($M = 3.50$, SEM = .1) than in the 2-back ($M = 2.92$, SEM = .1, $p < .001$, Cohen's $d = .65$) and in the 3-back condition ($M = 1.95$, SEM = .1, $p < .001$, Cohen's $d = 1.64$), which differed from each other too ($p < .001$, Cohen's $d = 1.17$). More importantly, group interacted significantly with the C957T polymorphism at *DRD2* and load, $F(4,312) = 2.47$, $p < .05$, $\eta 2p = .031$. As expected, Fisher HSD post-hoc tests revealed that for both the 2-back and the 3-back conditions TYR intake, compared to placebo, had a significant enhancing effect (increased target sensitivity) for T/T homozygotes (unfavorable genetic predisposition), $p \leq .01$, Cohen's $d_s = 1.82$ (2-back) and .67 (3-back), but not for C/T heterozygotes $p \geq .06$, Cohen's $d_s = .05$ (2-back) and .42 (3-back), and C/C homozygotes, $p \geq .55$, Cohen's $d_s = .05$ (2-back) and .17 (3-back), see Fig. 2. No significant differences between TYR and placebo intake were observed for the 1-back condition, $p_s \geq .28$, Cohen's $d_s \leq .40$. Additional post-hoc comparisons indicated no significant differences between genotype groups for those participants who received placebo, for both the 1-back and 3-back conditions, $p_s \geq .65$, Cohen's $d_s \leq .13$. For the 2-back condition, instead, significant differences were observed between C/C homozygotes and T/T homozygotes ($p = .02$, Cohen's $d = .62$) and between C/T heterozygotes and T/T homozygotes ($p = .03$, Cohen's $d = .71$), but not between C/C homozygotes and C/T heterozygotes ($p = .79$, Cohen's $d = .05$). In contrast, for participants who received TYR, significant differences between genotype groups were observed for both the 2-back and the 3-back conditions, but not for the 1-back condition ($p_s \geq .18$, Cohen's $d_s \leq .50$). Specifically, for both the 2-back and the 3-back conditions, T/T homozygotes differed significantly from C/C homozygotes ($p_s \leq .005$, Cohen's $d_s \geq .72$) and C/T heterozygotes ($p_s < .001$, Cohen's $d_s \geq 1.02$), whereas C/C homozygotes and C/T heterozygotes did not differ significantly from each other ($p_s \geq .24$, Cohen's $d_s \leq .32$).

Finally, neither group nor C957T polymorphism at *DRD2* were found to interact with load, $F_s \leq 2.43$, $p_s \geq .09$, $\eta 2p_s \leq .015$.

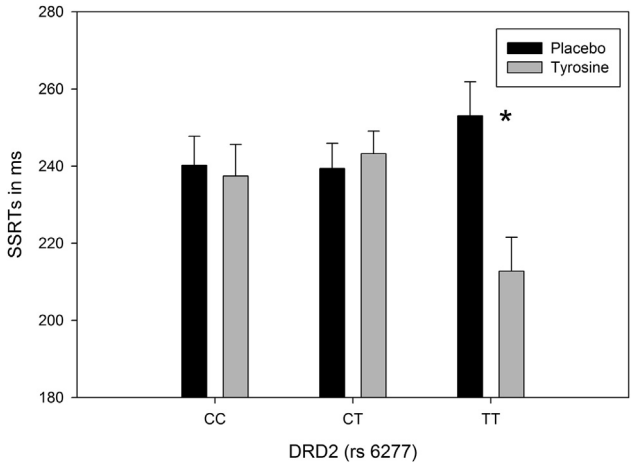

Fig. 1 – Mean SSRT (stopping latency) as a function of C957T polymorphism at *DRD2* gene (C/C homozygotes *vs* C/T heterozygotes *vs* T/T homozygotes) and group (TYR *vs* Placebo). Asterisks indicate significant (*$p < .05$) effects of TYR on mean SSRT. Vertical capped lines atop bars indicate standard error of the mean.

4. Discussion

In a double-blind, randomized, placebo-controlled design, we investigated the idea that mixed findings with respect to TYR supplementation in previous studies were due to the failure to consider individual differences in DA function. In particular, we examined whether individual differences in the C957T polymorphism at the *DRD2* gene contribute to individual differences in the reactivity to TYR to predict the ability of stopping on time and WM performance. Our findings show that T/T homozygotes (i.e., individuals potentially associated with lower striatal DA level; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009) showed larger beneficial effects of TYR supplementation than C/C homozygotes (i.e., individuals potentially associated with higher striatal DA level; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009), suggesting that TYR eliminates this genetic impact. As expected this beneficial effect was restricted to the cognitively challenging conditions (i.e., SSRTs in the stop-signal task and 2- and 3-back conditions in the *N*-back task) which is in line with other studies. It has been shown that TYR seems to show the most reliable beneficial effects on cognitive performance when healthy humans are exposed to either external stressors or cognitively challenging circumstances (Colzato, Jongkees, et al., 2013; Colzato et al., 2014; Thomas et al., 1999). Under these conditions, DA levels decrease and, consequently,

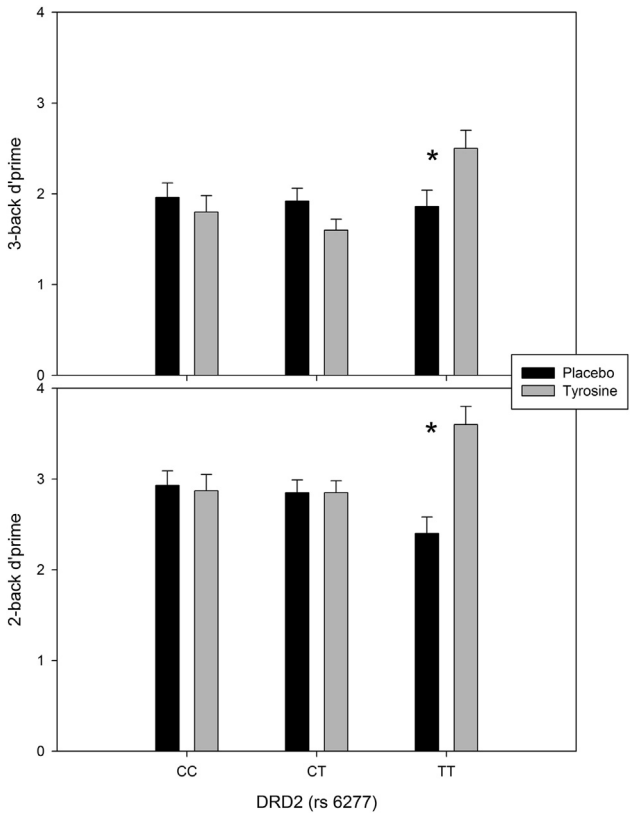

Fig. 2 – d′ as a function of load (2-back *vs* 3-back), of C957T polymorphism at *DRD2* gene (C/C homozygotes *vs* C/T heterozygotes *vs* T/T homozygotes) and group (TYR *vs* Placebo). Asterisk indicates significant (*$p < .05$) effect of TYR on the 2- and 3-back task. Vertical capped lines atop bars indicate standard error of the mean.

performance on cognitive tasks declines. TYR supplementation acts as a cognitive enhancer by refilling DA levels in the brain and compensating for the depletion of cognitive resources (Jongkees et al., 2015). However, as demonstrated by the outcomes of this study, this improvement is modulated by genetically determined differences in striatal DA function. Indeed, by taking into account this genetic differences, we were able to show *why* some people may benefit more than others from TYR intake. Accordingly, we suggest that future studies involving TYR administration should take into account genetically determined differences in DA function to explain the effectivity of TYR. Indeed, only via the consideration of genetic profiles it is possible to predict the relative efficiency of TYR administration and to develop an individualized, tailored enhancement approach—with the aim to eliminate, or compensate for individual differences in performance as much as possible.

Notably, in contrast to dopaminergic agonist drugs, whose effects may be counterproductive in individuals with an already medium (optimal) or high DA levels (Cools, 2006), TYR administration does not show dose-dependent effects (Deijen & Orlebeke, 1994; Shurtleff et al., 1994). Indeed, the relation between TYR and cognitive performance does not follow the standard inverted U-shaped dose–effect curve (Arnsten, 1998; Williams & Goldman-Rakic, 1995). Indeed, as pointed out by Cools (2006), individuals may differ with respect to baseline levels of DA and may, consequently, exhibit differential sensitivity to the positive and negative effects of dopaminergic drugs. Once the optimal level is reached, higher levels of TYR will not lead to higher level of DA because the maximum production capacity of the enzyme tyrosine hydroxylase, which converts TYR into DA, has been reached (Daubner, Le, & Wang, 2011; Tam, Elsworth, Bradberry, & Roth, 1990).

4.1. *Conclusions*

Understanding exactly how TYR supplementation affects the interplay between the dopaminergic mesocortical maintenance system and the nigrostriatal flexibility system, from which cognitive control emerges (Cools & D'Esposito, 2011), requires the separation of supplementation effects on each of the two—a separation that is not possible based on behavioral outcomes only. Therefore, it may be useful to employ resting-state functional magnetic resonance imaging (3T-fMRI) to trace the impact of TYR on the mesocortical maintenance system and the nigrostriatal flexibility system. Indeed, optimal DA signaling seems to be crucial for the adaptive functioning of the striato-thalamo-cortical loops that enable task-relevant information to be dynamically updated in WM (Hazy, Frank, & O'Reilly, 2007; Miller & Cohen, 2001) and action plans to be executed and/or inhibited (Frank, Samanta, Moustafa, & Sherman, 2007). Interestingly, the communication within these loops can be assessed by temporal correlation of blood oxygen level–dependent signals across brain regions (called "functional connectivity") measured during a task-free resting state (Di Martino et al., 2008; Gordon, Devaney, Bean, & Vaidya, 2015). Given that previous studies have demonstrated that striato-frontal connectivity is affected by pharmacological dopaminergic manipulations (Cole et al., 2013; Kelly et al., 2009), we expect TYR administration to impact such connectivity in similar ways. Specifically, we expect T/T homozygotes (i.e., individuals potentially associated with lower striatal DA level; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009) to show larger beneficial effects of tyrosine supplementation (as indicated by increased striato-frontal connectivity) than C/C homozygotes. Further, future studies need to consider multilocus genetic risk scoring approaches that may allow for a more comprehensive account of genetic variability in DA function in future work (e.g., Nikolova, Ferrell, Manuck, & Hariri, 2011; Pearson-Fuhrhop et al., 2014).

One main limitation of the current study is that it employs a very small sample size with regards to studies that examine association with genetic variation. As a result, it will be important to replicate these preliminary results with an independent sample, that is larger in size and balanced for gender. A second major limitation is that we employed a between subjects design, which does not allow for a baseline comparison of task performance between participants who received tyrosine and those who received placebo.

Although more research is needed, the present study provides the first evidence that the genetic makeup contributes to explain individual differences in the reactivity to TYR administration. Our findings support the idea that individual

differences in DA base line level modulate the effect of TYR in its role as cognitive enhancer.

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