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ORIGINAL ARTICLE

The *SLC6A3* 3′-UTR VNTR and intron 8 VNTR polymorphisms association in the time estimation

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Abstract

Objective The present study investigated the association of 3′-UTR VNTR and intron 8 VNTR polymorphisms with a time estimation task performance.

Materials and methods One hundred and eight men in a Brazilian Northeast population (18–32 years old) participated in the experiment. The 3′-UTR VNTR and intron 8 VNTR polymorphisms were associated alone and combined to absolute error (AE) and relative error (RE) in a time estimation task (target duration: 1 s, 4 s, 7 s and 9 s).

Results We found an association of the behavioral variable with intron 8 VNTR for the time intervals of 1 s and 9 s ($p < 0.001$) and polymorphisms combinatorial effect for 1 s ($p \leq 0.05$).

Conclusion The intron 8 VNTR polymorphism and the combinatorial effect can modulate the time estimate in the domain of supra seconds, and thus our study indicates a role of the dopamine transporter in the neurobiological areas related to the time intervals judgment.

Keywords Time perception · Dopamine · Time estimation · *SLC6A3* 3′-UTR VNTR · *SLC6A3* intron 8 VNTR

Introduction

Dopaminergic signaling is believed to be related to temporal processing underlying many physiological and behavioral actions (e.g., hormone level, walking, speaking, executive functions and cognition) that range in scale from millisecond to hours (Matthews and Meck 2014; Fontes et al. 2016). Pharmacological studies in humans and animal model studies, as well as neuroimaging studies suggest that dopaminergic neural pathways have a significant role in the internal clock speed during the time intervals interpretation (Cheng et al. 2006; Lake and Meck 2013; Coull et al. 2012). Molecular and genetic studies further support the role of dopaminergic pathways in neurobiological domains embedded in the timing (e.g., memory, learning, decision making and stimulus perception) (Meck et al. 2012; Balci et al. 2013; Bartholomew et al. 2015; Wiener et al. 2014; Marinho et al. 2018a), in particular the *SLC6A3* gene, located at 5p15.3 and encoding the dopamine transporter (DAT), expressed at presynaptic terminals of dopaminergic neurons (Vasconcelos et al. 2015; Tong et al. 2015).

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The DAT transcriptional regulation is modulated by the 3′-UTR VNTR (rs28363170) polymorphism, located in Exon 15, which contains different allelic forms with series of 40 bp ranging from 3 repetitions (3R) to 13 repetitions (13R); with the 9R and 10R alleles being the most common in the population (Šerý et al. 2015). Compared to the 9R allele, the 10R allele was associated with significantly increased DAT expression, and consequently increased dopamine reuptake in synaptic clefts (Faraone et al. 2014; Maksimov et al. 2015). In this context, previous studies demonstrated that the 9R allele in the 3′-UTR as a regulator of *SLC6A3* expression. The association of the 9R allele with higher DAT binding site density could result from a number of possible pathways: interaction of the 3′-UTR with regulatory proteins or microRNAs, shunting of mRNA to distinct compartments in the neuron, regulation of mRNA stability, turnover, increases in translational efficiency, or even remote interaction with regulatory elements of other genes that may affect *SLC6A3* expression, stability and trafficking. A parsimonious interpretation of the functional consequences of elevated DAT is more efficient in clearing extracellular dopamine, yielding lower extracellular levels and reduced dopamine signaling (Faraone et al. 2014; Ettinger et al. 2016; Marinho et al. 2018b).

Another polymorphism acting at the dopaminergic levels, the intron 8 VNTR (rs3836790) has alleles with series of 30 bp ranging from 3 repetitions (3R) to 7 (7R). However, only the 6R and 5R alleles have roles in the DAT transcriptional regulation, the 6R allele is associated with greater dopaminergic reuptake in the synaptic clefts than in the 5R allele (Franke et al. 2008; Šerý et al. 2015). Additional mechanisms were implicated to DAT binding, supported by Faraone et al. (2014) study. For example, DAT is constitutively recycled through the endosome. Although the majority of DAT is sequestered intracellularly in the recycling endosome, only membrane-associated DAT is functionally available for the reuptake of dopamine. Thus, some studies suggest that DAT recycling is effect by *SLC6A3* 3′-UTR VNTR with specific haplotypes formed with the intron 8 VNTR (Rommelse et al. 2008; Faraone and Mick 2010; Hawi et al. 2010; Šerý et al. 2015). Haplotype analyses by Shumay et al. (2010) suggested that higher DAT levels were associated with intron 8 alleles. Thus, it may be the combinations allelic formed by the 3′-UTR VNTR/intron 8 VNTR is worthy of further study as the variant increases DAT density. Thus, these polymorphisms are related to the expression of gene products, which could modify the behavioral phenotypes related to stimuli perception and executive functions (Green et al. 2008; Šerý et al. 2015).

Meck et al. (2012) studied the role of genetics in temporal processing using peak interval tasks in rats knocked out for *SLC6A3*. The authors found less precision in the knockout rats' tasks, when compared to the control rats, due to the non-expression of DAT—an essential product at the cognitive level and decision making in time intervals task judgments. Based on previous studies of genetic influence on time perception in animal models, Bartholomew et al. (2015), through genome-wide association studies (GWAS), reinforced the importance of dopaminergic levels and internal clock speed in contributing to the variability in timing tasks. These findings favor a quantitative time model, suggesting that dopamine levels could alter the firing rate of neurons responsible for a temporal information accumulator (Shea-Brown et al. 2006).

In a study reported here, we investigated the association between the polymorphisms that alter the expression and availability of neurotransmitters, including the *SLC6A3* 3′-UTR VNTR, and the variations in internal time representation in supra seconds range. The results show that the chemical modulation in the central nervous system (CNS) in timing activities is not related to the active polymorphism in DAT regulation (Sysoeva et al. 2010). However, other polymorphisms that regulate DAT have not been investigated, including the *SLC6A3* intron 8 VNTR polymorphism and the combinatorial effect of 3′-UTR VNTR and intron 8 VNTR in the time estimation task. Thus, for a possible association of 3′-UTR VNTR, we hypothesized the adjuvant action of intron 8 VNTR, since both modulate neurotransmission in cortical as well as subcortical areas that act as modular parts of neural clock mechanisms underlying subjective perception (Gupta 2014). The DAT performance in time perception is supported by modulations in cognition in individuals who have the 10R allele of 3′-UTR VNTR polymorphisms combined to the 6R allele of the intron 8 VNTR during cognitive task performance (Franke et al. 2010).

We hypothesize that the performance of *SLC6A3* 3′-UTR and/or intron 8 VNTRs, as well as their combinatory effects, can promote a differential sensitivity to the stimuli and, consequently, alter the internal representation of time, which may affect judgments of time intervals leading to underestimation or overestimation. Thus, we have investigated the association of the *SLC6A3* 3′-UTR and intron 8 VNTRs polymorphisms, as well as their combinatorial effects, on time perception through time-interval estimation tasks.

Materials and methods

Participants

We recruited 108 healthy male individuals, students of the Federal University of Piauí (UFPI) with mean age ± standard deviation [SD] = 22 ± 1.5 years (age range 18–32 years), belonging to a population from Brazilian Northeastern. Only right-handed individuals were selected based on the Edinburgh Inventory (Oldfield 1971), and were not using any

substance that could influence brain activities (e.g., tobacco, coffee, alcoholic beverages, caffeine-containing foods or medications) 12 h before or during the study period. The Ethics Committee of the UFPI approved all procedures, and participants provided written, informed consent (#: 1.087.450).

Genotyping

Anticoagulated venous blood samples were collected with EDTA. We isolated the peripheral blood leukocyte DNA from the Wizard® Genomic DNA Purification (Promega) kit, according to the manufacturer's specifications.

The analysis of *SLC6A3* 3′-UTR VNTR of 40 bp was performed using the polymerase chain reaction (PCR) using the *primers forward* 5′-TGT GGT GTA GGG AAC GGC GTG AG-3′ and *reverse* 5′-CCT CCT GGA GGT CAC GCG TCA AGG-3′. For a total reaction volume of 25 µL, the following conditions were used: 1.0 µL DNA, 2.5 µL of buffer 10 × (50 mM KCl, 20 mM Tris–HCl/pH 8.4), 0.75 µL of $MgCl_2$, 1.0 µL of each primer, 5.0 µL of dNTPs, 0.3 µL of Taq DNA polymerase, and 13.45 µL of distilled H_2O was added to make up the final volume. Also, the *SLC6A3* intron 8 VNTR of 30 bp was performed utilizing PCR using *primers forward* 5′-CCC AGG GAC ATC TGC TAA TG-3′and *reverse* 5′-CAC AAA TGA GTG TTC GTG CAT G-3′. For a total reaction volume of 25 µL, the following conditions were used: 1.0 µL DNA, 2.5 µL buffer 10 × (50 mM de KCl, 20 mM Tris–HCl/pH 8.4), 0.9 µL de $MgCl_2$, 0.4 µL of each primer, 4.0 µL of dNTPs, 0.2 µL of Taq DNA polymerase and 15.6 µL of distilled H_2O was added to make up the final volume. Primers were commercially manufactured by Thermo Fisher Scientific Inc. (Waltham MA, USA) and all other reagents used were supplied by Ludwig Biotechnology Ltd. (Porto Alegre, RS, Brazil).

PCR was carried out as follows: initial denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C for 45 s, 62 °C (3′-UTR) or 63 °C (intron 8) for 45 s, followed by 72 °C for 45 s, and a final extension at 72 °C for 7 min. The resultant PCR products for 10R and 9R alleles (479 bp and 439 bp) of *SLC6A3* 3′-UTR VNTR, and 6R and 5R alleles (254 bp and 284 bp) of intron 8 VNTR were separated on an 8.0% polyacrylamide gels and visualized using silver nitrate staining. PCR quality control was performed by randomly selecting 20% of the samples for re-genotyping by an independent technician. The correlation observed for the genotyping assays was 100%.

Time estimation task

A 42′ inch monitor was placed on a table in front of the participants at a distance of 50 cm and was turned on only during the task. The time estimate was analyzed using a program that records the displayed time interval (e.g., 1 s, 4 s, 7 s or 9 s) (Wittmann 2013; Jozefowiez et al. 2014). The task was performed in two phases. In the first, the display shows the command "enter" to start, then, the program produces a yellow circle in the monitor center that remains randomly in 1 s, 4 s, 7 s or 9 s. In the second phase, the software platform Matlab displays an empty field on the monitor to enter the estimated time interval, and then the subject presses the "enter" key to complete the task (Fig. 1). Each participant performed 2 task blocks with 40 tracks per block.

Data analysis

The behavioral variable was transformed into measures representing the absolute error value (AE) and the estimated proportion of target duration (Brown 1985). The AE value is a measure of the difference between the target-time and judged time-intervals, making it useful to assess the timing precision. The AE was calculated by putting in absolute value the difference between the time estimation (Ed) and the target duration (Td) [AE=|Ed − Td] (Mioni et al. 2014; Brown 1985). The target duration estimation ratio was obtained by dividing each participant's time performance by the time duration of the interval presented for that trial [Target duration estimation = Ed/Td]. This analysis is similar to the relative error (RE), and a coefficient below 1.0 indicates a trial of the time estimate less than the real time, while the coefficient above 1.0 represents the time trial longer than the real duration, an underestimation or overestimation of time, respectively (Brown 1985).

We associate the behavioral performance to the genotypes of *SLC6A3*, 3′-UTR VNTR and intron 8 VNTR based on DAT functionality, to influence the time estimation task:

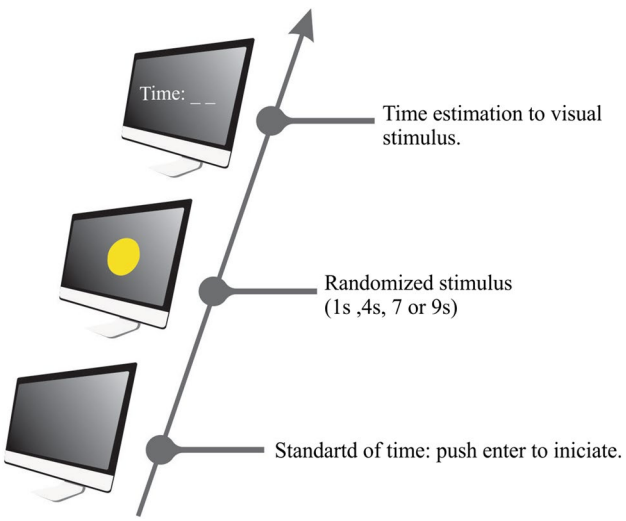

Fig. 1 Experimental procedure of the time estimation task

1. 3′-UTR VNTR: 10R/10R (high protein-coding of DAT) *vs*. 10R/9R or 9R/9R (low protein-coding of DAT) (Rommelse et al. 2008).
2. Intron 8 VNTR: 6R/6R (high dopaminergic reuptake) *vs*. 6R/5R ou 5R/5R (low dopaminergic reuptake) (Tong et al. 2015).
3. Combinatorial effect of 3′-UTR and the intron 8 VNTRs on behavioral parameters, based on the DAT regulation level: Grouping 10R/10R and 6R/6R (G1); Grouping 10R/10R and 6R/5R or 5R/5R (G2); Grouping 10R/9R or 9R/9R and 6R/6R (G3); Grouping 10R/9R or 9R/9R and 6R/5R or 5R/5R (G4).

The other genotypes of the 3′-UTR and the intron 8 VNTRs considered without functionality in dopaminergic regulation were excluded (Lim et al. 2012; Vasconcelos et al. 2015).

Statistical analysis

The genotype frequencies were tested for the Hardy–Weinberg equilibrium using χ^2-test. Subsequently, for the AE and RE analysis, we performed a Two-Way Mixed ANOVA with a group factor (3′-UTR and the intron 8 VNTRs) and time factor: (1 s, 4 s, 7 s, 9 s). Furthermore, to test a significant association among the 3′-UTR and the intron 8 VNTRs in the AE and RE variables, regression models were analyzed.

Comparison among four groups (10R/10R and 6R/6R genotypes; 10R/10R and 6R/5R or 5R/5R genotypes; 10R/9R or 9R/9R and 6R/6R genotypes; 10R/9R or 9R/9R and 6R/5R or 5R/5R genotypes) was tested by Two-Way Mixed ANOVA for combinatorial effect on AE and RE analysis. The interactions were investigated by univariate test, followed by the post hoc test, if necessary.

The effect size was estimated as Partial squared Eta (η^2p). Statistical power and the 95% confidence interval (95% CI) were calculated for the dependent variables. The magnitude of the effect was interpreted using the recommendations suggested by Hopkins et al. (2009): 0.0 = trivial; 0.2 = small; 0.6 = moderate; 1.2 = large; 2.0 = very large; 4.0 = almost perfect. Thus, to detect if there was a real difference in the population, statistical power was interpreted with 0.8 to 0.9 = high power (Fayers and Machin 1995). The probability of 5% for type I error was adopted in all analyzes ($p \leq 0.05$), with alpha-Bonferroni correction for the interaction analysis, adjusting the value for $p \leq 0.0125$. All analyzes were conducted using SPSS for Windows version 20.0 (SPSS Inc., Chicago, Il, USA).

Results

Genetic variables

The total genotyping rate was 99.07% (107/108 samples). Subsequently, we excluded other alleles found that are rare and in very low frequencies in different ethnic populations, and with lacking functionality in the regulation of protein reuptake (Lim et al. 2012).

Genotyping was completed for 107 samples for *SLC6A3* gene polymorphisms, but 06 samples were excluded for 3′-UTR VNTR (94.39% of samples were analyzed) and 02 samples were excluded for intron 8 VNTR (98.13% of samples were analyzed). For the 3′-UTR VNTR, the allelic frequencies of 10R and 9R were 0.76 and 0.24, respectively. Besides, for intron 8 VNTR, allelic frequencies of 6R and 5R were 0.72 and 0.28, respectively. The population studied was in Hardy–Weinberg equilibrium for both polymorphisms ($p \geq 0.05$) (Table 1). The allele frequencies were performed by simple counting and based on the functionality of the polymorphisms and their frequency.

Behavioral variable

The Two-way mixed ANOVA for AE and RE did not present a statistically significant difference between the polymorphisms studied ($p \geq 0.05$). However, the task execution errors increased as time intervals increased (Figs. 2, 3).

Regression analysis indicated that 3′-UTR VNTR genotypes (10R/10R *vs*. 10R/9R or 9R/9R), did not have a

Table 1 Allele counts and frequencies

Polymorphisms	Frequencies	Hardy–Weinberg equilibrium
SLC6A3 3′-UTR VNTR	($n = 101$)	($p = 0.975$)
10R/10R	58 (57.43%)	
10R/9R	37 (36.63%)	
9R/9R	6 (5.94%)	
Alleles		
10R	153 (75.74%)	
9R	49 (24.26%)	
SLC6A3 intron 8 VNTR	($n = 105$)	($p = 0.101$)
6R/6R	57 (54.29%)	
6R/5R	36 (34.29%)	
5R/5R	12 (11.42%)	
Alleles		
6R	150 (71.43%)	
5R	60 (28.57%)	

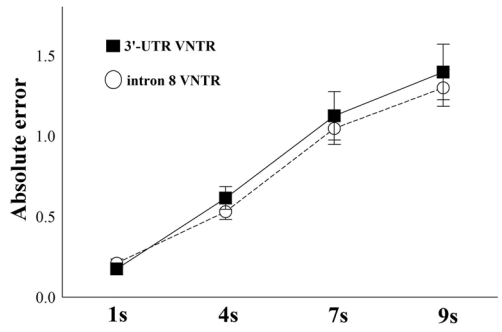

Fig. 2 Time estimation performance. Participants genotyped for 3′-UTR and intron 8 VNTRs increased the errors in the task as the time intervals increase

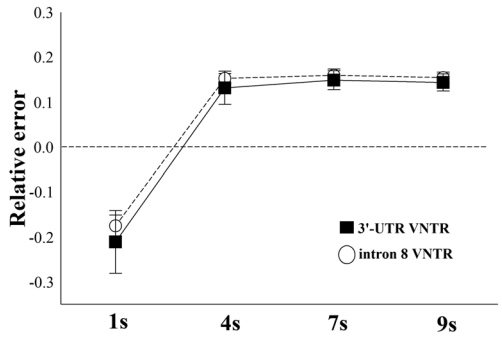

Fig. 3 Time estimation standard. Participants genotyped for 3′-UTR and intron 8 VNTRs underestimated the 1 s interval and overestimated the 4 s, 7 s, and 9 s intervals

significant effect in AE and RE for all target ranges in the time estimation task ($p \geq 0.05$) (Table 2).

The regression analyzes results for the 6R/6R *vs*. 6R/5R or 5R/5R of intron 8 VNTR polymorphism indicated significant effects only for the 1 s time intervals ($R^2 = 0.76$, $\beta = 0.87$, $p = 0.001$) and for 9 s ($R^2 = 0.81$, $\beta = 0.21$, $p = 0.002$) (Table 3). In consideration of ER revealing the magnitude of underestimation and overestimation in task performance, regression analysis showed association for the 1 s intervals ($R^2 = 0.88$, $\beta = 0.871$, $p = 0.001$) and 9 s ($R^2 = 0.86$, $\beta = 0.41$, $p = 0.002$), respectively (Table 3).

Subjects were classified into four combination groups of genotypes based on the 3′-UTR and the intron 8 VNTRs. A Two-way mixed ANOVA showed main effect for genotype in relation to AE, [$F(1948,3) = 753.8$; $p = 0.0001$; $\eta^2 p = 0.45$; power = 100%], with clusters of genotypes G1 increase AE in relation to G2 and G4 ($p = 0.0001$) (Fig. 4; Table 4). In addition, we found interaction between the genotype and time-target for RE, [$F(1948,3) = 343.1$; $p = 0.0002$; $\eta^2 p = 0.32$; power = 96%] (Fig. 5). When analyzing the interaction by means of univariate test, statistical difference was observed for time interval of 1 s, with [$F(7160,3) = 4.99$; $p = 0.0001$; $\eta^2 p = 0.12$; power = 96%]. For a reliable statistical comparison, clusters of genotypes with similar functional activity were combined. The post hoc inspection for RE revealed statistically significant differences during the time interval estimation interpretation. These findings indicate that clusters of genotypes G1 increase RE in relation to G2 and G4 ($p = 0.0001$) (Table 4).

Discussion

It is the first study to include a sample of healthy adults to examine the correlation of 3′-UTR and the intron 8 VNTRs, such as the combinatorial effect of *SLC6A3* gene polymorphisms with changes in time estimation task.

The analysis of AE and RE have revealed that task execution errors increased in different groups with the increase in the target time-intervals, consistent with the scalar expectancy theory of pulse-accumulator neural clocks. In addition, the internal representations transfer in memory suggests

Table 2 Regression model for the behavioral variables AE and RE based on the polymorphism 3′-UTR VNTR (10R/10R cluster compared to the 10R/9R or 9R/9R cluster)

Variables	*B*	SE	Beta	Wald	*p*	Odds ratio	CI 95% for odds ratio	
							Lower	High
AE1s	0.108	0.037	0.068	0.291	0.772	1.025	−0.063	0.085
AE4s	0.032	0.071	−0.011	−0.451	0.652	0.972	−0.169	0.106
AE7s	0.064	0.150	0.019	0.432	0.670	1.012	−0.231	0.359
AE9s	0.017	0.172	0.023	0.098	0.291	0.999	−0.320	0.353
RE1s	−0180	0.038	0.075	1.129	0.072	1.025	−0.063	0.085
RE4s	−0.085	0.017	−0.110	1.451	0.065	0.891	−0.042	0.026
RE7s	0.911	0.021	0.121	0.428	0.067	1.085	−0.033	0.051
RE9s	0.222	0.019	0.08	1.099	0.092	0.992	−0.036	0.039

Significant differences ($p \leq 0.05$) are represented by the asterisk (*)

AE absolute error, *RE* relative error, *B* regression coefficient, *SE* standard error, *df* degree of freedom, *OD* odds ratio

*$p \leq 0.05$

Table 3 Regression model for the AE and RE behavioral variables based on intron 8 VNTR (6R/6R grouping compared to 6R/5R or 5R/5R cluster)

Variables	*B*	SE	Beta	Wald	*p*	Odds ratio	CI 95% for odds ratio	
							Lower	High
AE1s	0.780	0.025	0.410	3.551	0.001*	1.274	0.039	0.136
AE4s	0.031	0.049	0.014	0.625	0.532	0.950	−0.062	0.126
AE7s	0.078	0.099	0.017	0.795	0.427	1.004	−0.115	0.273
AE9s	0.221	0.115	0.392	12.006	0.002*	1.001	−0.104	0.348
RE1s	0.870	0.025	0.781	6.551	0.001*	1.274	0.039	0.136
RE4s	0.080	0.012	0.140	1.625	0.532	0.851	−0.016	0.032
RE7s	0.110	0.014	0.170	0.795	0.079	0.768	−0.017	0.039
RE9s	0.410	0.013	0.231	5.058	0.002*	0.718	−0.012	0.039

Significant differences ($p \leq 0.05$) are represented by the asterisk (*)

AE absolute error, *RE* relative error, *B* regression coefficient, *SE* standard error, *df* degree of freedom, *OD* odds ratio

*$p \leq 0.05$

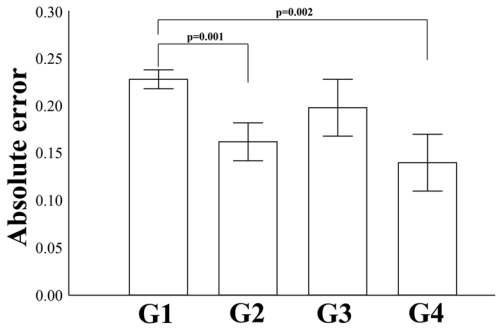

Fig. 4 The AE of the time estimation task of the four polymorphic types combined, shown as an average ± standard error. Significantly different results are highlighted indicated by a *p* value. A significant difference in AE between the G1 (Grouping 10R/10R and 6R/6R) with G2 (Grouping 10R/10R and 6R/5R or 5R/5R); A significant difference between the G1 (Grouping 10R/10R and 6R/6R) with G4 (Grouping 10R/9R or 9R/9R and 6R/5R or 5R/5R). (ANOVA followed by the Tukey post-hoc test)

emerging properties of interactions related to the changes in levels of expression and protein-coding of DAT (Wiener et al. 2011; Meck et al. 2012).

The observed results for the error increase, as the time interval progresses, as well as the underestimation for 1 s and overestimation for 4 s, 7 s and 9 s, the error increase demonstrate that the timing is dedicated to an "internal clock" model (Teixeira et al. 2013; Fontes et al. 2016). The time representation taken by this clock involves the pulses accumulation emitted by a pacemaker, suggest that the timing could be attributed to differences in the polymorphic expression of dopamine transporters in distinct circuits, serving different clock mechanisms for different target intervals (Rammsayer 1999). Moreover, 6R of intron 8 VNTR is less active than 5R allelic form of SLC6A3 gene (Hill et al. 2010), which could affect pulse accumulation emitted by a pacemaker or oscillator (Teixeira et al. 2013; Fontes et al.

Table 4 The combinatorial effect analysis of 3′-UTR and intron 8 VNTRs on the behavioral variable through Two-Way Mixed ANOVA, with analysis of the differences between polymorphic groupings

Behavioral results	*F*	*p* value	n^2p	Power	Post hoc	CI 95%	
						Lower	High
Absolute error[a]	753.8	0.0001*	0.45	100%	G2 < G1 in 0.06 s	−0.04	0.11
					G4 < G1 in 0.23 s	0.02	0.15
Relative error[b]	4.99	0.0001*	0.12	96%	G1 > G2 in 0.085 s	0.02	0.15
					G1 > G4 in 0.073 s	0.03	0.14

G1: Grouping 10R/10R and 6R/6R; G2: Grouping 10R/10R and 6R/5R or 5R/5R; G3: Grouping 10R/9R or 9R/9R and 6R/ R; G4: Grouping 10R/9R or 9R/9R and 6R/5R or 5R/5R

Significant differences ($p \leq 0.05$) are represented by the asterisk (*)

$\eta^2 p$ Partial squared Eta, *95% CI* 95% confidence interval

[a]Main effect for Absolute error

[b]Analyze of the interaction to Relative error by a univariate test, statistical difference was observed for time interval of 1 s

*$p \leq 0.05$

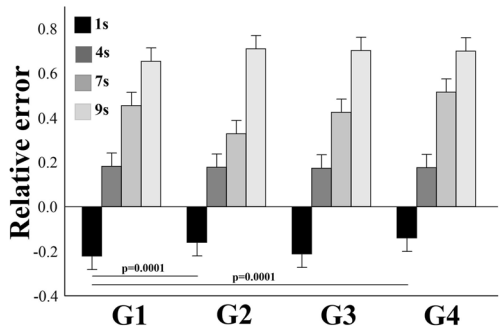

Fig. 5 The ER of the time estimation task of the four polymorphic types combined, shown as an average ± standard error. Significantly different results are highlighted indicated by a *p* value. The statistical difference is observed for time interval of 1 s. The findings demonstrate that G1 (Grouping 10R/10R and 6R/6R) increase RE in relation to G2 (Grouping 10R/10R and 6R/5R or 5R/5R) and G4 (Grouping 10R/9R or 9R/9R and 6R/5R or 5R/5R). (ANOVA followed by the Tukey post-hoc test)

2016), in a complex manner via a modular clock mechanism (Gupta 2014). Present findings are consistent with different neural oscillators for different target time-intervals, which could be engaged via dopaminergic signaling in the prefrontal cortex (Coull et al. 2012; Balci et al. 2010; Petter et al. 2016).

Thus, intron 8 VNTR polymorphism modulates subjective timekeeping for the 1 s and 9 s intervals and the combinatorial effect of the polymorphisms only in 1 s, which alters the internal clock speed and decreases the accuracy of temporal judgment. Moreover, the neural substrates underlying the three stages of the internal clock (clock phase, memory phase and decision) can be modulated by the joint action of both *SLC6A3* polymorphisms as a function of the essential protein coding in cognition, and the understanding of the genetic variables participation in the clock phase. Additionally, polymorphisms related to dopaminergic signaling could influence the sustained attention to the task visual stimulus. Since in the interval of 1 s it is suggested fewer pulses accumulation in the temporal information in comparison to the interval of 9 s. It may show a necessary conclusion that non-temporal factors influence the domain of supra seconds perception (Drew et al. 2003; Matthews and Meck 2014; Golombek et al. 2014). The Coull, Cheng and Meck (2011) and Barzman, Geise and Lin (2015) studies support our findings based on the applied neurobiology timing, since they demonstrated the prefrontal cortex, motor area and basal ganglia association in time estimation tasks with visual stimuli. However, each region may have a distinct functional role and differentially implicate depending on the task context. Thus, it is agreed that multi-regional performance in temporal perception, i.e., different mechanisms are responsible for the subjective duration interpretation at different time intervals, with dopaminergic system consistent performance (Meck 2009; Coull et al. 2012; Wiener et al. 2014). The current findings could be related to the dopaminergic pathways—nigrostriatal and mesocortical—related to the basal ganglia and prefrontal cortex, respectively, both of which are important regions for different cognitive timing functions (Buhusi and Meck 2005; Lewis and Mial 2006). In the current study, the results are directly or indirectly related to the transcriptional regulation of dopaminergic transport through 3′-UTR VNTR and intron 8 VNTR polymorphisms.

Past studies on the genetic influence of 3′-UTR VNTR on time interval interpretation activities did not demonstrate significant associations (Sysoeva et al. 2010; Balci et al. 2013; Wiener et al. 2014; Maksimov et al. 2015). However, our findings demonstrate that there is a combinatorial effect of 3′-UTR VNTR and intron 8 VNTR polymorphisms on the performance of time estimation task which could result from the changes in synaptic levels of dopamine, which suggests that changes in dopaminergic neurotransmission play an important role in cognitive timing.

In the current study, precautions have been taken to exclude confounding variables. Only male participants were recruited for the study since previous studies have shown gender differences in functioning and dopaminergic levels in humans and animals (Rammsayer 1993; Buhusi et al. 2016). The experiments were conducted at the same time of day to eliminate or minimize a possible circadian influence (Spati 2015; Golombek et al. 2014). Bias reduction and data analysis were based on a model that has already been successfully used earlier in other studies (Hancock and Rausch 2010; Jozefowiez et al. 2014; Mioni et al. 2014). In this way, the results may indicate that the time representation parameters used for the specific purpose, i.e., AE and RE, demonstrate stable characteristics of the subjects regarding the timing at the supra seconds scales. Therefore, they provide a reliable basis for comparisons between carriers of different genotypes.

Furthermore, it may be important to consider that for intervals closer to 1 s implicit timing mechanisms through the neural integration of performance with subcortical structures (e.g., basal ganglia, cerebellum). However, the subjective interpretation of time relative to the 1 s interval becomes less close to the actual task duration (Coull et al. 2012). The possible explanation for the finding of underestimation in the range of 1 s is that the high recyclability of dopamine using the 6R/6R genotype in adjuvant action to 3′-UTR VNTR genotypes act at the time limit between automatic and cognitive synchronism, and may be shorter about the target actual duration. In this perspective, the 1 s interval coding can be considered automatic and, therefore, justify the underestimation in the time estimation task execution (Ivry and Spencer 2004; Jones et al. 2004; Finnerty et al. 2015).

We propose that the combinatorial effect of the *SLC6A3* gene polymorphisms modulates the information processing

underlying timing and cognitive functions of the brain. Due to the principle of reactivity to the external sensorial input, which is based on the model of sensorial capture associated with the internal reference model (IRM) (Matthews and Meck 2014). In this context, genetic polymorphisms modify the neural synchronism oscillations pattern in the discrimination precision of the stimuli and the memory-based comparison pattern. In fact, we have noticed that the combinatorial effect of polymorphisms indicates a more significant delay in comparing the target stimulus with an internal reference, which comprises a weighted linear combination of the first stimulus and the internal reference level of the response before the stimulus.

The present work has some limitations, among them the size of the sample. However, we may consider that our study, when compared to that of Bartholomew et al. (2015) is the second largest in analyzes of genetic variables associated with time perception tasks. Another limitation is non-association with tasks at the sub-second level because the software platform Matlab does not provide the possibility of stimuli with intervals below 1 s, as it could give a broader view of the timing's genetic performance. However, the findings suggest that the intron 8 VNTR can be considered a genetic marker of time perception and the combinatorial effect of polymorphisms can modulate the AE and RE interpretation for the intervals of supra seconds by greater fluctuation in dopaminergic transcription.

Conclusion

The present study is the first to include the role of the molecular bases related to the dopamine transporter by means of the intron 8 VNTR polymorphism, in addition to demonstrating the association of the 3′-UTR VNTR combined to the intron 8 VNTR during the time estimation in supra-second intervals. The transcriptional level of dopamine is regulated by the 3′-UTR VNTR and intron 8 VNTR polymorphisms, which modulate the chemistry of the neural bases, mainly in the circuits that involve the frontal cortex and basal ganglia action. The areas in question are modulating clocks that allow for the principle of accumulation and temporal information synchronization, which is associated with the determination of neurobiological domains pertinent to the time interval interpretation.

The findings of genetic influence on the perceptual capacity still require more investigations, mainly with larger sample size, more polymorphisms that act on behavioral phenotypes, use of other models of timing analysis associated to electrophysiological measurements of cortical and/or subcortical areas acting as modular clocks. Although it is the first work to show the intron 8 VNTR performance, in addition to the combinatorial effect of both *SLC6A3* polymorphisms in a time estimation task, our findings offer some support that leads to the understanding of the genetic influence on perceptual bases of stimuli. In this context, the study has implications not only for the neurobiological bases of time perception in healthy individuals but also points to the future understanding of possible relations for the behavior perception in neurological diseases. Furthermore, future studies may clarify the role of dopaminergic neurotransmission dysregulation in the mediation of behavioral and pathological phenotypes, since genetic and behavioral information compose means that direct the clarification of the mechanisms underlying the time perception in humans.

Author contributions Conceived and designed the experiments: VM, TO, GRP, and ST. Performed the experiments: VM, TO, AG, and VL. Analyzed the data: VM. Contributed reagents/materials/analysis tools: FM, KR, GRP, HFF, BV, PR, MC, DSG and VHB. Wrote the paper: VM. Headed the molecular genetic analysis: VM, TO, ST, and GRP.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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