BDNF VAL66MET IS A STRONG PREDICTOR OF DECISION MAKING AND ATTENTION PERFORMANCE ON THE CONVIRT VIRTUAL REALITY COGNITIVE BATTERY

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Abstract

The val66met polymorphism of the brain-derived neurotrophic factor gene has been associated with changes in components of executive functioning such as decision making; however, this relationship remains unclear. Val66met-related changes in attention and visual processing speed may explain potential changes in decision making. Furthermore, chronic stress disrupts executive functions and alters autonomic activity. Because the relationship between val66met and cognition has not been investigated in the context of chronic stress or stress-related autonomic changes, in this study 55 healthy university students completed self-report measures of chronic stress and mental health. Participants then completed a virtual reality cognitive test battery (CONVIRT) measuring decision making, attention, and visual processing reaction times. To measure autonomic activity, saliva alpha amylase and heart rate variability were assessed at baseline and after CONVIRT testing. Saliva samples were used to identify val66met genotype. Regression analyses demonstrated that val66met was the strongest predictor of decision making and attention, but not visual processing, where Val/met participants had faster reaction times than Val/val participants. Val/met participants also had higher perceived chronic stress and heightened increases in sympathetic activity, but not parasympathetic activity. Neither stress nor autonomic activity moderated the effect of val66met on decision making or attention. This study is the first to investigate the role of val66met in decision making, attention, and visual processing while taking into account chronic stress and autonomic activity. This multifactorial approach revealed that carriers of the Val/met genotype may have better decision making and attention than Val/val carriers.

- Val/met carriers demonstrated faster decision-making and attention
- No association between val66met and visual processing speed
- High stress and sympathetic dominance associated with slower visual processing
- Val/met carriers demonstrated heightened increases in sympathetic activity

Keywords: CONVIRT; VR; brain-derived neurotrophic factor; chronic stress; autonomic activity

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INTRODUCTION

Executive functions are a collection of top-down cognitive processes, the most important of which are cognitive flexibility, working memory, and inhibition. These functions work together to complete complex, higher order processes such as planning, problem solving, and decision making (Diamond, 2013). Deficits in executive functions are present in numerous psychiatric and neurodevelopmental disorders (Bosaipo et al., 2017; Craig et al., 2016; Heinrichs and Zakzanis, 1998) and can be elicited in healthy people under stressful situations (G. S. Shields et al., 2016) or when chronically stressed (Kuhnell et al., 2020; Landolt et al., 2017). Given that cognitive impairments within populations vary considerably, many have suggested possible genetic causes for this variation.

The common val66met single nucleotide polymorphism of the brain-derived neurotropic factor (BDNF) gene is one such gene variant that has been implicated in changes of executive functioning (Gabrys et al., 2017; LeMoult et al., 2015; Tramontina et al., 2009). BDNF is a neurotrophin vital for neuroplasticity, neurogenesis, and neuronal survival (Notaras et al., 2015). The polymorphism occurs when the nucleotide adenine replaces guanine at codon 66 resulting in synthesis of methionine (met) instead of valine (val)(val; Notaras et al., 2015; Notaras and van den Buuse, 2018). The presence of val66met causes reduced activity-dependent synaptic release of BDNF, with reductions subject to the number of alleles affected. As a result, heterozygotic carriers of val66met (Val/met) have an approximately 18% reduction of activity-dependent BDNF release and homozygotic val66met carriers (Met/met) have approximately 29% reductions (Chen et al., 2006; Egan et al., 2003).

Decision making is a complex, multifaceted process that involves responding quickly and accurately to stimuli under uncertainty in order to choose between two or more options. Despite some evidence that the core executive functions may be altered in carriers of val66met, the current literature on the association between val66met and decision making is limited and there appears to be no consensus on its role. Although there is evidence that the met allele is associated with altered decision making strategies and poorer decision making quality overall (da Rocha et al., 2011; Tulviste et al., 2019), these studies focused on higherorder decision making. Current research examining the relationship between val66met and choice reaction time indicates there is no effect of the genotype on decision making speed and accuracy (Canivet et al., 2017; Schofield et al., 2009). However, the data from one of these studies (Canivet et al., 2017) emanates from older adults ($\geq$ 55 years). As BDNF levels and expression decrease with age (Hattiangady et al., 2005; Romanczyk et al., 2002), there may be differences in decision making between genotypes in younger adults that is no longer present in older populations. Therefore, more evidence is needed to determine whether val66met may affect decision making in younger adults. Further, there are no studies that investigate if chronic stress moderates the relationship between val66met and choice reaction time.

A potential explanation for changes in decision making only seen in higher order decision making tasks may be related to changes in attention. While most research has found no association between val66met and attention, studies that have found an association have been predominantly in clinical populations (Toh et al., 2018). Moreover, if there is an association between val66met and attention in either healthy or clinical populations, the direction of these relationships is not yet clear. Given the current trajectory of evidence, it is likely that there is no direct relationship between val66met and attention. This relationship has not been examined in the context of chronic stress, however, and chronic stress may moderate the relationship between val66met and attention.

An alternate explanation for the conflicting reports on the relationship between val66met and decision making is the influence of bottom-up processes. Given that visual processing speed may be intrinsically

linked to efficient decision making, especially on choice reaction time measures involving visual stimuli (Delorme et al., 2004), it is possible that genetic variation in visual processing may mediate changes in decision making. Evidence of the relationship between val66met and visual processing has been limited and highly variable, with meta-analytic evidence suggesting no clear association (Mandelman and Grigorenko, 2012). However, this analysis did not include potential confounding variables such as age, gender, or stress and included both healthy and clinical populations without any analysis between the two subgroups. Further, studies of visual processing often rely on classic neuropsychological testing that require a participant to identify or reconstruct visual targets, often requiring other complex cognitive processes. Consequently, measures of visual processing speed such as ultra-rapid object detection using saccadic eye movement have been proposed as a promising alternative (Kirchner and Thorpe, 2006). Currently, there are no published val66met studies that have used saccadic eye movement as a measure of visual processing speed.

Given that the relationship between val66met and cognitive performance is more consistently seen in clinical populations than healthy populations, it is likely that val66met moderates other causes of cognitive impairment. There is some preliminary evidence that val66met moderates the relationship between stress and executive functions (Gabrys et al., 2017; Gatt et al., 2009; Yu et al., 2012), though this relationship has not been extensively explored. Changes in autonomic activity related to stress such as increased alpha amylase level and heart rate variability (HRV) reactivity have generally been associated with poorer cognitive performance (Holzman and Bridgett, 2017; Kuhnell et al., 2020; Maldonado et al., 2019). One study found no association between val66met and salivary alpha amylase (sAA) at rest or after stress (Tsuru et al., 2014), yet this relationship has not been widely explored. The relationship between val66met and HRV has been the subject of some previous studies. Previous studies (Tsuru et al., 2014; Yang et al., 2010) have demonstrated altered HRV between genotypes as Met/met participants had reduced parasympathetic activity, resulting in sympathetic dominance, compared to Val/val and Val/met participants. Further, Chang et al. (2014) demonstrated that val66met was associated with altered HRV in a gender-specific manner. They found that met carriers had reduced parasympathetic activity, but only male met carriers demonstrated parasympathetic dominance. This suggests that the relationship between val66met and HRV may be moderated by gender, however the direction of this relationship is not yet clear.

Given the discrepancies in the val66met and cognition literature and the lack of exploration into the potential moderating effect of stress and autonomic activity in this relationship, the present study investigated the role of val66met in decision making, attention, and visual processing after considering the influence of known predictors (i.e., chronic stress and autonomic activity). This study used CONVIRT, a cognitive test battery utilising virtual reality (VR) and eye-tracking technology to assess performance on both bottom up attention (i.e., visual processing speed) and top down cognition (i.e. attention decision making) measures. Further, this study assessed autonomic reactivity using a non-linear method of analysing electrocardiographic (ECG) data, the Poincaré plot, to measure HRV.

It was hypothesised that there would be no direct effect of genotype on decision making, attention or visual processing speed. However, it was anticipated that met carriers who report higher chronic stress and display heightened autonomic activity would demonstrate poorer cognitive performance than Val/val participants in all three domains. That is, chronic stress and autonomic arousal would moderate the association between genotype and performance on the CONVIRT battery. Relating to autonomic activity specifically, it was predicted that met carriers would demonstrate increased sympathetic and reduced parasympathetic activity compared to Val/val participants, but there would be no association between genotype and sAA levels.

Method

2.1 Participants

Participants were healthy young adults (*N* = 55, females = 31), aged 18 to 28 years (*M* = 20.6, *SD* = 2.1). Participants were invited to participate face-to-face on the grounds of a La Trobe University, Melbourne, Australia using a recruitment script. Candidates were included if they were currently full-time students (criterion for a related study) and could read English. Exclusion criteria included a self-reported history of loss of consciousness, psychiatric, cardiac, or neurological illness, dementia, head injury, insomnia or other sleep disorders, and the use of medications that may affect concentration. Candidates were also excluded if they reported feeling physically fragile or acutely unwell (cold, flu), or regularly used drugs or alcohol as indicated by a score of 8 or above on the Alcohol Use Disorders Identification Test(Saunders et al., 1993). Participants provided written informed consent in line with institutional ethics (HEC19036) and were compensated for their time with a $100 shopping voucher.

Materials

Alcohol consumption screening

The 10-item AUDIT (Saunders et al., 1993) was used to screen for regular alcohol use. The questionnaire assesses alcohol consumption, drinking behaviour and consequences related to drinking. An AUDIT score ≥8 indicates risky/hazardous levels of alcohol use. The AUDIT has high internal consistency (α = .81; A. L. Shields and Caruso, 2003) and strong test-retest reliability (Selin, 2003). No participants were excluded based on this criterion.

Chronic stress

The 10-item Perceived Stress Scale (PSS) (Cohen et al., 1983) measures perceived chronic stress on a 5-point Likert scale (0 – never, to 4 – very often). An example item is “In the last month, how often have you felt nervous and stressed?” (Cohen et al., 1983, p. 394). High scores denote high levels of perceived stress. In this study, the PSS had high internal consistency (α = .83).

Mental health

The 12-item Short Form Health survey (SF-12) (J. E. Ware et al., 1996) assesses selfreported physical and mental health. The Mental Health composite score (MCS) was used to measure mental health functioning. The MCS is effective in identifying the presence and severity of clinical psychiatric disorders as well as related disruptions to the central nervous system and gastrointestinal functioning (J. E. Ware et al., 1996). Higher scores on the MCS denote better mental health. In this study, the MCS had high internal consistency (α = .80).

Heart rate variability

HRV is a measure of heartbeat fluctuations that are primarily controlled by autonomic activity. Dysregulated HRV is commonly associated with alteration in cognitive performance (Collins et al., 2012; Forte et al., 2019). Continuous heart rate was measured using a five-lead ambulatory digital recorder (AR12plus, Medilog, Schiller AG, Switzerland) recording ECG signals at a sampling rate of 1000 Hz. Recordings were subsequently examined using Darwin V2 software (Medilog, Schiller AG, Switzerland) to determine R-R peak

intervals during the baseline and CONVIRT test phases and non-sinus intervals were excluded. These baseline and CONVIRT testing periods were both approximately 10 min. in duration and analysed using a Poincaré plot, a non-linear method of measuring HRV where each data point refers to the relative change between one R-R interval and the successive R-R interval (Roy and Ghatak, 2013). Non-linear methods of analysing ECG data were developed to identify nonlinear patterns in HRV that occur due to the complexity of the autonomic nervous system (Roy and Ghatak, 2013; Shaffer and Ginsberg, 2017). HRV in val66met studies has currently only been analysed by linear, time- and frequency-domain methods and therefore it would be advantageous to examine the non-linear patterns to help elucidate the relationship between val66met and HRV.

The Poincaré plot provides three indices of HRV: SD1, SD2, and SD12. SD1 refers to the standard deviation of instantaneous beat-to-beat interval variability and is a measure of parasympathetic activity. SD2 refers to the standard deviation of the continuous long-term RR interval variability and is a measure of sympathetic activity. SD12 is the ratio of SD1/SD2 and indicates autonomic balance. A SD1/SD2 change variable was calculated by subtracting the baseline phase measure from the CONVIRT phase measure. **Salivary alpha-amylase** sAA is a reliable and non-invasive measure of autonomic arousal and stress reactivity (Vineetha et al., 2014). Participants were asked to provide saliva samples at baseline and immediately after CONVIRT testing. Unstimulated saliva samples were collected using cotton swabs (Salivette®, Sarstedt, Chur, Switzerland). The participant was instructed to move the swab around their mouth in a circular motion for one minute. The Salivettes® were placed in a centrifuge tube and immediately stored in a freezer (−20° C). sAA activity was assayed using a Salimetrics ®96 Well Kinetic Enzyme Assay Kit (No.1-1902; State College, PA, USA). Optical density measurements were performed at 450 nm with a SynergyTM HT Multi-Detection Micro-Plate Reader (Bio-Tek Instruments Inc., Winooski, VT). Concentrations were calculated using KC4 v3.4 Software (Bio-Tek Instruments). The assay has a lower detection limit of 0.1 nmol/L with intra- and inter-assay coefficients of variations <8%. A sAA change variable was calculated by subtracting the baseline phase measure from the CONVIRT phase measure.

Genotyping

A portion of the baseline saliva sample from each participant was used to identify genotype and was transported on dry ice to the Australian Genome Research Facility (AGRF) for DNA extraction and genotyping. The samples were analysed using Agena MassArray for the single nucleotide polymorphism rs6265 (BDNF val66met).

CONVIRT battery

The CONVIRT test battery is a recently validated measure of cognitive performance using VR and eye-tracking technology (Horan et al., 2020). The test battery involves three computerised tests: the saccadic reaction time test (SAC-VR), a measure of visual processing speed, the detection test (DET-VR), a measure of attention, and the identification test (IDNVR), a measure of decision-making. The tests have high test-retest reliability (SAC-VR-VR, r = .79; DET-VR, r = .90; IDN-VR, r = .88; Horan et al., 2020). The CONVIRT system operates through a laptop while the participant wears a FOVE 0 VR Head Mounted Display (HMD) with embedded eye tracking hardware. Participants experience the first-person perspective of a jockey riding a horse in a horserace and are asked to respond to target shapes appearing in their virtual environment by pressing a button on a custom wireless riding crop, or focussing their gaze on the object as quickly as possible. Although the CONVIRT was developed for jockeys it has shown high convergent validity with other well validated neuropsychological tests, namely the attention and decision making subtests of

the CogState battery, in healthy young adults who are not jockeys (Horan et al., 2020). Prior to each test, instructions are presented in the visual display, and participants undertake a short practice trial.

Visual processing speed

The SAC-VR test begins with the participant fixating their gaze on a grey sphere within the virtual environment. Participants observe two green eye symbols in their environment and these symbols indicate the gaze of their left and right eye. Once the participant has fixated their gaze on the grey sphere a blue sphere appears in a random position at a random time within two seconds. The participant must move their gaze as quickly as possible to the blue sphere and when the green eye symbols converge on the blue sphere, auditory and animated explosive effects signify trial completion. The grey sphere then reappears, and the participant is required to fixate on this sphere again to initiate the next trial. The SAC-VR test is finished once 35 trials have been completed. The SAC-VR measures the time it takes for the participant's gaze to reach 50% of the distance to the blue sphere, as this portion of the saccade does not involve deceleration and accuracy corrections which are driven by other complex neural processes (Orban de Xivry and Lefèvre, 2007). In this way, the SAC-VR measures saccadic latency and a portion of acceleration towards the target.

Attention

The DET-VR measures simple reaction time. During each trial, the participant is presented with an orange triangle in a random position. After seeing the triangle, the participant must press the button on the riding crop as quickly as possible. The triangle disappears for a varied duration of 1 to 2.37 seconds, after which the next shape is presented. The test is completed after 120 seconds and 35 trials have been completed.

Simple reaction time is measured based on the time elapsed (in milliseconds) between each triangle appearing and the riding crop button being pressed. False positives occur when the button is pressed but no triangle is present. DET-VR tests with false positives constituting more than 10% of all button presses were excluded. No tests were excluded based on this criterion.

Decision-making

Lastly, participants undergo the IDN-VR, which is a measure of choice reaction time. Participants are presented with a random shape (orange sphere, blue sphere, or blue triangle) in a random position within their peripheral vision. The participant must press the riding crop button as quickly as possible when they see an orange sphere, but not when a blue sphere or blue triangle is present. The test is completed after 120 seconds, with 31 shapes presented in total.

Choice reaction time is measured by the time elapsed (in milliseconds) between each orange sphere appearing and the riding crop button being pressed. False positives occur when the button is s pressed prior to the presentation of the correct stimuli, or in response to the distractor stimuli. IDN-VR tests with false positives constituting more than 20% of all button presses are excluded. No tests were excluded based on this criterion.

Procedure

Participants were screened using the AUDIT and provided basic demographic information (i.e., age, gender, height, and weight), prior to attending the lab to take part in the study. Participants were excluded if they received an AUDIT score of 8 or above, however, no participants were excluded based on this criterion. This study is a part of a larger research project assessing the impact of alcohol consumption upon cognition and measures of autonomic reactivity; however, these data are not presented here. Nonetheless, screening for high levels of alcohol use controls for potential substance-related cognitive impairment. Upon arrival,

participants provided informed consent. Two researchers were present during testing, wearing white lab coats. Two participants were tested in separate rooms simultaneously, approximately four participants were tested over one day.

Baseline

Participants provided a saliva sample for sAA and genotype analysis and were instructed to fit the ECG recorder and electrodes, which were worn for the duration of testing. Researchers checked the recorder to ensure the electrodes were fitted correctly and operational. Participants then completed the PSS and MCS questionnaires.

CONVIRT testing

Participants were fitted with the CONVIRT HMD and were familiarised with the riding crop and virtual environment. Participants then completed the CONVIRT test battery, with each test presented in the same order for all participants (i.e., SAC-VR, DET-VR, IDNVR). Each test included a familiarisation phase before the test commenced where the participants were provided instructions and a practice trial. Once the CONVIRT testing was complete, participants provided another saliva sample for sAA analysis.

Statistical Analysis

IBM SPSS statistics computer software package (version 26) was used for all statistical analyses. An independent samples *t*-test was used to assess if participants with Val/val or Val/met genotype were equivalent on measures of age and BMI. A Chi square analysis was used to assess if the differing genotypes were equal in gender distribution. A paired *t* -test assessed if the autonomic measures (i.e., sAA, SD1, SD2, and SD12) differed across baseline and CONVIRT testing phases. A 2 (phase; baseline, CONVIRT) x 2 (genotype; Val/val, Val/met) ANOVA assessed if changes in these markers between phases differed by genotype. Associations between participant characteristics (genotype, sex, age, and BMI), self-report measures of stress (PSS and mental health) autonomic measures (change in sAA and change in SD12) and CONVIRT measures (DET-VR, IDN-VR, and SAC-VR) were assessed with Pearson and Spearman correlations as appropriate. Three hierarchal regressions were conducted to assess the association of genotype, age, and sex (step 1), PSS and mental health scores (step 2) and autonomic arousal (sAA and SD12; step 3) with the three measures of cognition (DET-VR, IDN-VR, SAC-VR).

Results

Data Management

Two participants were removed from the dataset, one who carried the Met/met genotype and one who was missing sAA data. Data were checked for outliers and violations of normality, multicollinearity, and homoscedasticity with only normality violations requiring correction. The reaction time measures (i.e., DET-VR, IDN-VR, SAC-VR) and sAA data at both timepoints were positively skewed ($p > .001$) and corrected with natural logarithmic transformations. Assessing demographic equivalence across genotypes.

The descriptive statistics are provided for each genotype and the entire sample for gender, age and BMI data (Table 1).The genotype groups did not differ in gender distribution ($X^2(1, N = 55) = 0.81$, $p = .368$), mean age ($t(53) = -0.56$, $p = .834$), or mean BMI ($t(53) = 0.08$, $p = .671$).

Table 1. Demographics of Study Sample Compared with Genotype

	Total Sample	Genotype	
Variable		Val/val	Val/met
Gender (Male: Female)	M = 24: F = 31	M = 11: F = 18	M = 13: F = 13
Age *M* (SD)	20.63 (2.14)	20.52 (0.39)	20.85 (0.44)
BMI *M* (SD)	24.01 (3.96)	24.10 (0.76)	24.00 (0.79)

Normative comparisons

A one-sample *t*-test was used to compare the participants' perceived chronic stress (PSS scores) and mental health (MCS) to a normative age-matched sample (Table 2). Participants reported lower perceived chronic stress and better mental health than normative samples.

Table 2. Age-matched Normative Comparisons of Means and Standard Deviations for Self-Report Measures

	Sample data		Normative data			
Scale	M	SD	M	SD	t	d
PSS	15.47	5.78	18.89	6.78	-3.60***	0.51
MCS	70.39	18.94	49.18	9.74	14.04***	1.97

*Note. *** = p < .001, PSS = Perceived Stress Scale, MCS = Mental health composite score. Normative data: PSS (Örücü and Demir, 2009), n = 508, age = 15-29; MCS (J. Ware et al., 1998), n = 636, age = 18-34.*

Effect of testing and genotype on autonomic activity.

A mixed 2 (phase; baseline, CONVIRT) x 2 (genotype; Val/val, val/met) ANOVA was used to assess if the physiological variables (sAA, SD1, SD2, and SD12) changed between baseline and CONVIRT testing phases (Table 3) and whether these changes differed between genotype. Increases were observed in the CONVIRT phase for all variables except SD1. The effect size for SD2 was very large (Cohen, 1992), suggesting that the CONVIRT testing induced a large sympathetic reaction, and this is consistent with the increases observed in sAA.

Table 3 Comparison of Physiological Indices at Baseline and CONVIRT Testing

	Baseline M (SD)	CONVIRT M (SD)	Cohen's d	p
sAA (U/ml)	85.88 (81.14)	98.29 (72.10)	0.546	.006
SD1	28.65 (17.94)	30.40 (15.08)	0.101	.601
SD2	76.09 (26.11)	100.41 (40.62)	1.271	< .001
SD12	0.38 (0.17)	0.31 (0.10)	0.626	.002

Note. For the sAA variables the untransformed means are reported, but the log transformed versions were used in parametric tests.

Of these physiological variables, only the change in SD2 across phases differed between genotype (Figure 1), $F(1, 53) = 4.228$, $p = .045$, $\eta_p^2 = .074$. Participants with the Val/val genotype had a smaller increase in SD2 (baseline $M = 72.74$, CONVIRT $M = 88.52$) compared to Val/met participants (baseline $M = 79.25$, CONVIRT $M = 109.30$), demonstrating that compared to Val/val participants, Val/met participants had heightened increases in sympathetic activity between baseline and CONVIRT testing.

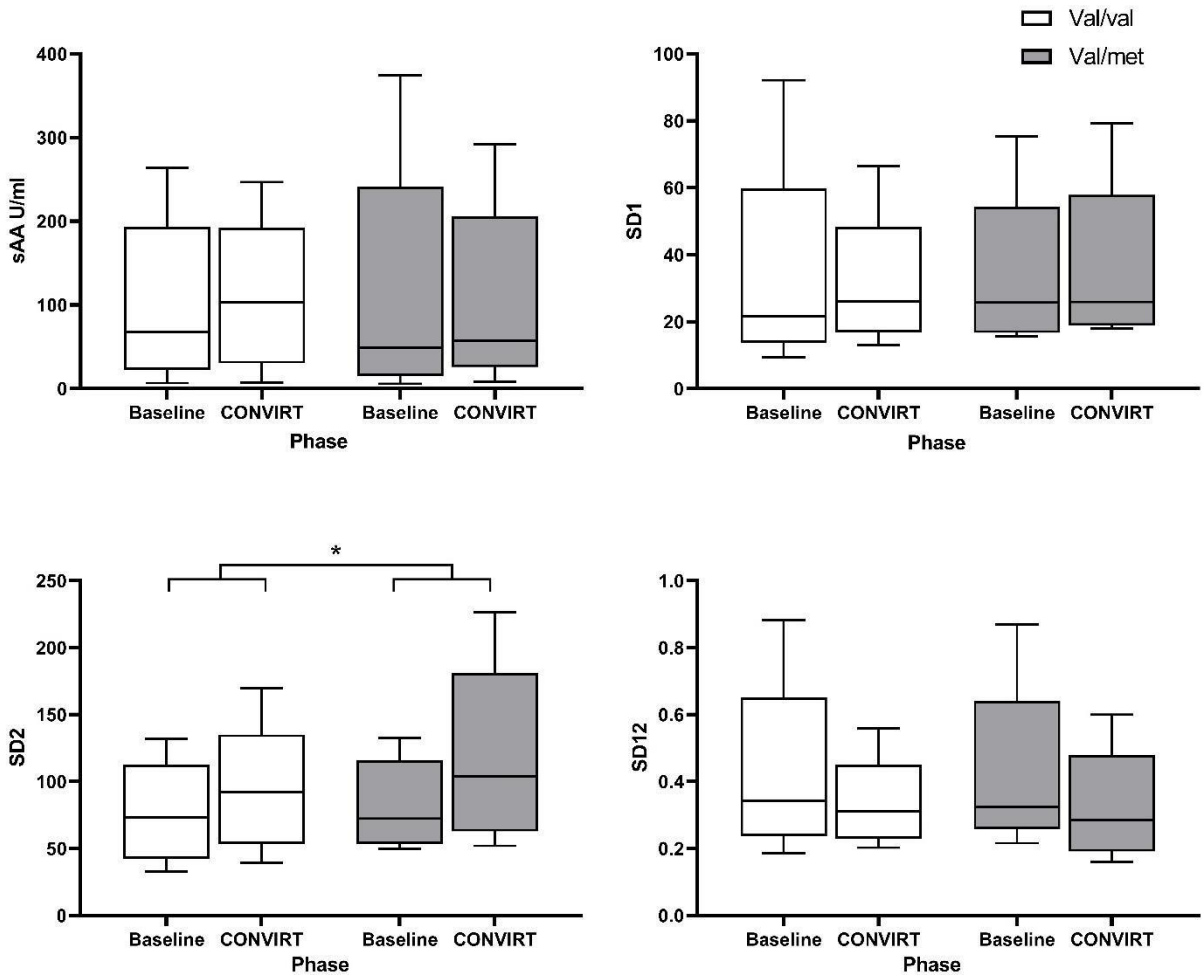

Figure 1. Changes in autonomic activity between baseline and CONVIRT testing between genotypes. sAA = salivary alpha-amylase, error bars show standard error of the mean. The box plot shows the 5th and 95th percentiles, the interquartile range, and the median. *$p < .05$.

Associations between key variables

The associations between participant characteristics, self-report measures of stress, physiological markers of arousal, and CONVIRT measures were assessed (Table 4). Of interest, met carriers were associated with higher chronic stress (PSS) and better attention (DET-VR) and decision-making (IDN-VR). Genotype was not associated with autonomic activity.

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Table 4. Correlations Comparing Genotype, Gender, Age, Self-Report Measures of Stress and Wellbeing, Physiological Measures of Arousal, Simple and Choice Reaction Time, and Saccadic Eye Movement.

Variable	1 Genotype	2	3	4	5	6	7	8	9	10
2. Gender	-.103[a]									
3. Age	.076	-.100								
4. BMI	-.011	-.237	.309*							
5. PSS	.284*	.207	-.132	.058						
6. MCS	-.257	-.126	-.023	-.218	-.678**					
7. sAA $^{\Delta}$	-.053	.208	-.027	-.253	-.200	.190				
8. SD12$^{\Delta}$	-.039	-.101	.039	.070	-.005	-.092	.038			
9. IDN-VR	-.275*	.044	-.079	.080	.059	.088	-.100	-.048		
10. DET-VR	-.292*	.110	.080	.233	.056	-.007	.041	.079	.628**	
11. SAC-VR	.066	.286*	.012	-.081	.443**	-.301*	.146	-.282*	.077	.191

*Note. * p < .05, ** p < .01, [a] = Spearman's correlation, BMI = Body mass index, PSS = Perceived Stress Scale, MCS = Mental health composite score, sAA $^{\Delta}$ = change in salivary alpha amylase between baseline and CONVIRT testing SD12$^{\Delta}$ = change in SD12 between baseline and CONVIRT testing, IDN-VR = decision making, DET-VR = attention, SAC-VR- visual processing speed.*

Assessing the relationship of the genotype, psychological and autonomic variables with measures of cognition. The regression models with the outcome variables of attention (DET-VR) and decision-making (IDN-VR) produced very similar results. In both models after accounting for all other variables in the model, genotype was the sole predictor of attention and decisionmaking (Table 5). Specifically, articipants with the Val/met genotype performed better on these tasks than those with a Val/val genotype.

Table 5. Hierarchical Regression Assessing Association of Genotype, Gender, Age, PSS, Mental Health, sAA and SD12 Activity with Measures of Attention (DET-VR) and Decision-Making (IDN-VR).

	Attention (DET-VR)					Decision-making (IDN-VR)				
Predictor	Final Step Summary			Step Summary		Final Step Summary			Step Summary	
	Beta	*r*	*sr*	$R^2\Delta$	*p*	*Beta*	*r*	*sr*	$R^2\Delta$	*p*
Step 1				.10	.13				.08	.24
Genotype	-.34*	-.29	-.31			-.30*	-.28	-.28		
Gender	.04	.11	.04			-.01	.04	-.01		
Age	.14	.08	.13			-.02	-.08	-.02		
Step 2				.02	.54				.04	.34
Chronic stress	.21	.06	.14			.26	.06	.18		
Mental Health	.05	-.01	.04			.20	.09	.14		
Step 3				.01	.83				.01	.75
sAA $^{\Delta}$	.05	.04	.04			-.10	-.10	-.09		
SD12 $^{\Delta}$	.07	.08	.07			-.04	-.05	-.04		

*Note. * p< .05, ** p < .01; $R^2\Delta = R^2$ change; Genotype 1 = Val/val, 2 = Val/met; Gender 1 = male, 2 = female; Total attention model: $R^2 = 0.13$, $F(7, 47) = 1.03$, $p = .427$; Total decisionmaking model: $R^2 = 0.13$, $F(7, 47) = 1.00$, $p = .443$.*

For the hierarchical regression with saccadic reaction time (SAC-VR) as the outcome, a different trend emerged. Genotype was not associated with SAC-VR, but participants with higher chronic stress (PSS) or low HRV reactivity (SD12), had slower/worse SAC-VR scores (Table 6).

Table 6. Hierarchical Regression of Change in Genotype, Gender, Age, PSS, Mental Health, sAA, and SD12 Activity with Measures of Visual Processing Speed (SAC).

	Visual processing speed				
Predictor	Final Step Summary			Step Summary	
	Beta	*r*	*sr*	$R^2\Delta$	*p*
Step 1				.09	.169
Genotype	-.08	.07	- .07		
Gender	.11	.29	.10		
Age	.11	.01	.10		
Step 2				.15	.012
Chronic stress	.46**	.44	.32		
Mental Health	-.07	-.30	-.05		
Step 3				.12	.016
sAA $^{\Delta}$	- .24	.15	.22		
SD12$^{\Delta}$	- .29*	-.28	-.29		

*Note. * p< .05, ** p < .01; R2Δ = R2 change; Genotype 1 = Val/val, 2 = Val/met; Gender 1 = male, 2 = female; Total visual processing speed model: R2 = 0.37, F(7, 47) = 3.87, p = .002.*

Moderation analysis

Given that previous research has identified that chronic stress and autonomic arousal are directly or interactively associated with cognitive performance, a series of moderated regressions were conducted to assess whether the associations of genotype with cognition (DET-VR, IDN-VR, and SAC-VR) were compromised by heterogenous subgroups. Neither chronic stress (PSS) (b = 0.00, t = 0.47, p = .642) nor HRV (change in SD12) (b = -.52, t = -1.18, p = .246) moderated the relationship between genotype and attention (DET-VR). Similarly, neither chronic stress (b = 0.00, t = 0.50, p = .621) nor HRV (b = -0.62, t = -1.18, p = .246) moderated the relationship between genotype and decision-making (IDN-VR). Lastly, neither chronic stress (b = -0.00, t = -0.64, p = .527) nor HRV (b = -0.13, t = -0.24, p = .810) moderated the relationship between genotype and visual processing speed (SAC-VR).

Discussion

Contrary to our hypotheses, carriers of the met allele demonstrated better decision making and attention than Val/val participants. The results of this study suggest that the relationship between val66met and executive functioning persists after controlling for gender, visual processing, chronic stress, and autonomic activity. As predicted, there was no association between val66met and increased sAA levels in response to the CONVIRT protocol which, consistent with previous research, elicited a moderate acute stress response (Horan et al., 2020). We predicted that met carriers would demonstrate sympathetic dominance as a result of reduced parasympathetic activity compared to Val/val participants, however the results demonstrated

heightened increases in sympathetic activity in met carriers, but no change in the parasympathetic/sympathetic ratio.

This study demonstrated that val66met may be associated with top-down cognitive processes such as decision making and attention. Conversely, faster performance in bottom up processes (visual processing speed) was associated with lower chronic stress and physiological arousal, with no differentiation between genotypes. As the CONVIRT battery measures visual processing speed with ultra-rapid object detection using saccadic eye movement, the results are less likely to be influenced by complex cognitive processes (Kirchner and Thorpe, 2006) and may potentially be a better measurement of bottom-up processes than typical neuropsychological tests of visual processing speed. This study is the first to use saccadic eye movement when investigating the role val66met in complex cognition and demonstrates that the genotype may only affect top-down cognition and not bottom-up processes.

A novel relationship was found between val66met and top-down cognition. We demonstrated that while controlling for known factors that alter top-down cognition, met carriers were associated with better decision making compared to Val/val carriers. Previous research using choice reaction time measures have found no association between val66met and decision making (Canivet et al., 2017; Schofield et al., 2009). As one of these studies(Canivet et al., 2017) was tested in older adults (mean age = 71.5), it is possible that the relationship between val66met and decision making is present in young adults but as BDNF levels and expression decrease with age (Hattiangady et al., 2005; Romanczyk et al., 2002), the relationship between val66met and decision making dissipates. Some studies have found changes in higher-order decision making related to the val66met genotype (da Rocha et al., 2011; Tulviste et al., 2019), however, unlike the present study these studies found poorer decision making and decision-making strategies in met carriers compared to Val/val carriers. A potential explanation for the discrepancies may be that, unlike all previous literature, the present study controlled for psychological and physiological variables that are associated with performance on these cognitive measures (Canivet et al., 2017; da Rocha et al., 2011; Schofield et al., 2009; Toh et al., 2018; Tulviste et al., 2019).

The relationship between val66met and decision making may also be attributed to the measurement of decision making using choice-reaction time. Choice-reaction time is strongly influenced by attentional processes whereas higher order decision making involves the use of strategies (Levine, 2009; Tulviste et al., 2019) and is influenced by emotional biases (Shukla et al., 2019). Given the strong correlation between the simple and choice-reaction time measures in this study, better decision making in met carriers could be a function of better attention. However, our findings conflict with some previous literature that have not found clear associations between attention and val66met in healthy populations but have seen changes predominantly in clinical populations, such as people with schizophrenia, mild traumatic brain injury, cardiovascular disease, and obsessive compulsive disorder (Toh et al., 2018). The participants in the present study, however, reported lower chronic stress and better mental health than age-matched normative samples, and this may explain differences in findings between studies. Further, our study excluded candidates if they had a history of mental or physical health conditions that would impede on cognitive functioning. Therefore, it is unlikely that better top-down cognition in met carriers is due to the presence of the met allele protecting against the influence of psychiatric conditions on cognition. Further, this sample contrasts with similar studies in healthy young adults who exhibited high stress (Kuhnell et al., 2020; Landolt et al., 2017). It is possible that, given our young adult sample was not highly stressed, the threshold for adverse autonomic reactivity to affect cognition may not have been met. Consequently, the relationship between val66met, chronic stress, and autonomic activity with top-down cognition may not be apparent in a healthy, high-stressed sample. Alternatively, the SAC-VR may present as a more sensitive measure of cognition than the IDN-VR or DET-VR and is better equipped to detect subtle differences in performance

between participants when chronic stress is low. Future research that incorporates prospective assessments of autonomic activity and cognition across high and low periods is required to determine if the cross-sectional associations we report are causal.

Although val66met was associated with chronic stress, with met carriers reporting more chronic stress than Val/val carriers, neither chronic stress or stress-related autonomic activation moderated the relationships between val66met and decision-making, attention, or visual processing. This has implications for research that further investigates the effect of chronic stress on cognition. Many studies that consider chronic stress will measure autonomic variables but largely do not include possible underlying genetic factors (Kuhnell et al., 2020; Landolt et al., 2017; Teixeira et al., 2015; Vineetha et al., 2014). This study demonstrates the importance of including genetic factors, as we demonstrate that val66met is the strongest predictor of some areas of executive functioning in this sample of healthy and moderately stressed students.

As for autonomic activity, this study is consistent with previous literature that reports no association between val66met and sAA (Tsuru et al., 2014). On the other hand, the HRV results are the inverse of previous literature. While previous studies(Tsuru et al., 2014; Yang et al., 2010) demonstrated sympathetic dominance as a result of reduced parasympathetic activity in met carriers compared to Val/val carriers, the present study found met carriers had increases in sympathetic activity but these changes were not strong enough to demonstrate sympathetic dominance.

Limitations of this study includes that it did not consider ethnicity of participants. There is substantial evidence that the val66met polymorphism elicits different effects between Caucasian and Asian populations (Lee and Song, 2014; Li et al., 2016; Tsai et al., 2011). However, we did control for several other important factors including gender, chronic stress, mental health, and physiological arousal. Future research should aim to include the factors that were considered in this study and determine whether these results are replicable between ethnicities.

Further, this study did not include participants that carried the Met/met genotype as genotyping was conducted after recruitment and only one Met/met carrier was identified. There is evidence that suggests that the Val/met and Met/met genotypes have differing effects on behaviour, cognition, and neurobiology (Hashimoto et al., 2008; Kim et al., 2013; Notaras et al., 2017; Schofield et al., 2009), likely due to the reduced activity-dependent secretion of BDNF in Met/met carriers compared to Val/met carriers (Chen et al., 2006). Although our findings are still appropriate for Val/met carriers, we were unable to fully examine the effects of all the val66met genotypes. While there are some concerns surrounding the use of the candidate gene approach (Sullivan, 2007), this approach is still valuable for the assessment of functional links between genetic variants and phenotypes (Moore, 2017). Further, the sample size was modest considering a candidate gene approach was adopted. Although this study controlled for a number of potential confounds and was adequately powered, it should be directly replicated in a larger sample nonetheless.

In conclusion, this study is the first to investigate the effect of val66met on decision making, attention, and visual processing while concurrently considering the roles of chronic stress and autonomic reactivity. Performance on cognitive measures have been shown to be associated with gender, age, chronic stress, physiological reactivity and in recent times, the val66met genotype. In this study, we can report that when these factors are considered concurrently, Val/met carriers have better attention and decision-making performance. In addition, higher chronic stress and increased HRV best predict faster visual processing speed. The relationships between val66met and top-down cognition were not moderated by chronic stress or autonomic reactivity, despite met carriers having higher levels of stress and increased sympathetic reactivity. Such knowledge is vital in understanding how best to intervene to improve and explain

decrements in cognition. Given the sample reported lower chronic stress and better mental health than normative populations, future research should consider replicating our design with a high-stress sample to determine if the val66met genotype is most strongly associated with measures of top-down cognition. Future research should also consider the role of the Met/met genotype and BDNF itself in decision-making and attention. Given the low prevalence of the Met/met genotype and the difficulty in measuring BDNF activity in human samples, animal model research may be an appropriate next step.

Data Availability Statement

The data that support the findings of this study are openly available in Open Science Framework (OFS) at https://osf.io/qs3pw/ (Wright, 2020).

Declarations of interest: none

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