Interaction between DRD2 C957T polymorphism and an acute psychosocial stressor on reward-related behavioral impulsivity

Melanie J. White[1*], Bruce R. Lawford[1,2], C. Phillip Morris[1], Ross McD. Young[1]

[1]Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, Brisbane, Queensland, Australia

[2]Department of Psychiatry, Royal Brisbane and Women's Hospital, Herston, Brisbane, Queensland, Australia

* To whom correspondence should be addressed.

Mailing address: Melanie J. White, Centre for Accident Research & Road Safety – Queensland (CARRS-Q), School of Psychology & Counselling, Institute of Health and Biomedical Innovation, Queensland University of Technology, Victoria Park Road, Kelvin Grove, Queensland, Australia, 4059

Tel. +61 7 3138 4884

Fax. +61 7 3138 4734

Email: m2.white@qut.edu.au

Suggested running head: DRD2 C957T-acute stress interaction on behavioral impulsivity

Abstract

The dopamine D2 receptor (DRD2) C957T polymorphism CC genotype is associated with decreased striatal binding of DRD2 and executive function and working memory impairments in healthy adults. We investigated the relationships between C957T and acute stress with behavioral phenotypes of impulsivity in 72 young adults randomly allocated to either an acute psychosocial stress or relaxation induction condition. Homozygotes for 957C showed increased reward responsiveness after stress induction. They were also quicker when making immediate choices on the delay discounting task when stressed, compared with homozygotes who were not stressed. No effects were found for response inhibition, a dimension of impulsivity not related to extrinsic rewards. These data suggest that C957T is associated with a reward-related impulsivity endophenotype in response to acute psychosocial stress. Future studies should examine whether the greater sensitivity of 957C homozygotes to the effects of stress is mediated through dopamine release.

Keywords

Association; Dopamine; D2 receptor; Genetics; Impulsivity; Reward; c.957C>T Polymorphism, Acute Stress.

Interaction between DRD2 C957T polymorphism and an acute psychosocial stressor on reward-related behavioral impulsivity

Introduction

Impulsivity is a multidimensional construct underlying several psychological disorders (American Psychiatric Association 1994) and risky behaviors (Zuckerman and Kuhlman 2000). Dimensions of impulsivity have variously been defined as hypersensitivity to reward or novelty, delay discounting (greater preference for, or valuing of, immediate rewards than delayed rewards), and reduced response control or disinhibition (see Enticott and Ogloff 2006; Evenden 1999 for reviews). The neurotransmitter dopamine is integral to two leading theories of impulsive personality, Gray's reinforcement sensitivity theory (Gray and McNaughton 2000) and Cloninger's psychobiological model of personality (Cloninger et al. 1993), which conceptualize impulsive individuals as being hypersensitive to reward and as novelty seekers respectively. Brain dopamine activity plays an important role in behavioral activation, reward mechanisms, and goal-directed behavior (Noble 2003). Further, disorders of reward processing are observed in human disorders that implicate the mesolimbic dopamine system, such as Attention Deficit Hyperactivity Disorder (ADHD), addiction and schizophrenia (Bobb et al. 2006; Noble 2003).

Personality theories of impulsivity consistently emphasize the role of genetics, reflecting evidence from twin studies of high heritability on self-report measures, including sensation seeking (55%, Hur and Bouchard 1997), novelty seeking (40%, Heath et al. 1994) and "rash/unplanned" impulsivity (15-40%, Eysenck 1983). Subsequently, genes associated with brain dopaminergic activity have been commonly studied candidates. The D2 dopamine receptor gene (DRD2) region is one of the most extensively investigated gene regions associated with dopamine receptor function, particularly the *Taq*IA polymorphism (Bowirrat and Oscar-Berman

2005). The *Taq*IA had been historically described as residing in the DRD2 gene but has more recently been referred to as being within the *ANKK1* (Neville et al. 2004). While the *Taq*IA has recent support for an association with impulsivity (White et al. 2008), the functional significance of this polymorphism remains undetermined. The C allele of a common synonymous polymorphism in the DRD2 gene (c.957C>T, commonly referred to as C957T), which is in linkage disequilibrium with *Taq*IA, is associated with *in vitro* DRD2 mRNA stability and protein translation (Duan et al. 2003). *In vivo* this polymorphism explained 18% of the variance in striatal DRD2 binding potential in healthy participants using [^{11}C]raclopride and positron emission tomography (PET) (Hirvonen et al., 2004) with erratum (Hirvonen et al. 2005). Inconsistent with the direction of the *in vitro* findings, this study found that 957C homozygotes (i.e., CC genotype) had the lowest striatal binding, heterozygotes (CT genotype) had intermediate binding while 957T homozygotes (TT genotype) had the highest binding (Hirvonen et al. 2005). It is worth noting that these *in vivo* findings were originally incorrectly reported in the opposite direction (Hirvonen et al. 2004), resulting in some inconsistent interpretation in the literature published in the interim period. Both the Duan et al. (2003) and Hirvonen et al. (2005) studies confirm the functional significance of the C957T to striatal DRD2, however the resulting behavioral phenotypes have not yet been elucidated.

Recently, significantly reduced D2/D3 receptor availability in the ventral striatum was found for rats categorized as having high preclinical trait impulsivity (Dalley et al. 2007). The rats were grouped into low and high trait impulsivity according to their levels of anticipatory responses made before the presentation of a food-predictive, brief light stimulus in a five-choice serial reaction time. Conversely, a series of behavioral experiments found transgenic mice with reversibly increased levels of DRD2 striatal binding showed selective working memory deficits

on a radial-arm maze task (Kellendonk et al. 2006). Working memory impairment persisted in the transgenic mice even after normalization of DRD2 striatal binding by "switching off" the gene (by administration of 2 weeks of doxycycline treatment), indicating the effect of a key developmental period. However, human imaging studies using healthy populations have predominantly found that decreased striatal DRD2 density is associated with a range of executive function impairments, including aspects of response inhibition, working memory and planning. These types of studies have also showed that striatal dopamine activity is involved in reward processing, working memory and attentional processes (see Cropley et al. 2006 for a recent review). Taken together, such findings suggest polymorphisms associated with DRD2 striatal binding, such as C957T, may relate differentially to aspects of impulsive behavior.

Preliminary evidence for a potential role of the C957T polymorphism in impulsivity is provided by recent reported associations of the CC genotype with executive function in humans, particularly switching behavior and aspects of working memory. In 83 healthy Spanish Caucasian adults, CC genotypes, compared with CT/TT genotypes, demonstrated poorer executive function on measures from the Wisconsin Card Sorting Test, including achieving fewer categories, and making more perseverative errors and with greater perseveration (Rodriguez-Jimenez et al. 2006). Another recent study of 188 healthy adults showed that those with the CC genotype demonstrated poorer performance (compared with CT and TT genotypes) on a word serial position test of working memory (Xu et al. 2007).

However, in behavioral genetics, simple Mendelian genetic influences are rare, with most traits reflecting the interplay of genes and environment (Caspi and Moffitt 2006). Environmental factors may also explain conflicting findings reported for associations between alternate models of C957T inheritance for nicotine-related effects in clinical samples (Jacobsen et al. 2006;

Lerman et al. 2006). Acute environmental stress is a correlate of impulsive behavior (Bogdan and Pizzagalli 2006; Conner et al. 2005; Roberti 2003) and has been found to increase dopamine neurotransmission in the ventral striatum of humans (Pruessner et al. 2004). No previous study has examined the combined influence of the C957T polymorphism and environmental stress on impulsivity.

The primary aim of this study was to test two hypotheses examining a polymorphism associated with altered striatal dopamine functioning and impulsivity: whether the C957T is related to individual differences in impulsive behavior and whether exposure to acute environmental stress moderates this relationship. To reduce the influence of potential confounds associated with psychopathology, we studied a community sample of young adults screened for psychiatric illness. On balance, the results of previous research investigating the C957T in similarly healthy populations suggested the selection of the recessive model of inheritance for the effect of C957T on impulsive behavior. That is, the CC genotype was selected as the 'risky' genotype focus based on previous research associations with cognitive aspects of impulsivity in similarly healthy adults (Rodriguez-Jimenez et al. 2006; Xu et al. 2007) and supported by *in vivo* data showing reduced striatal DRD2 binding in this group (Hirvonen et al. 2005). This selection is also consistent with previous associations between a range of executive control impairments including reward-related impulsivity and lower DRD2 binding (Cropley et al. 2006; Dalley et al. 2007). Given these associations and the potential of acute stress to increase striatal dopamine release (Pruessner et al. 2004), it was hypothesized that acute stress would increase behavioral impulsivity in 957C homozygotes, but not affect those without this genotype (i.e., CT and TT genotypes). Consistent with the multidimensional nature of impulsivity (Dougherty et al. 2005)

the laboratory paradigm incorporated three independent measures of impulsivity, assessing reward-cued approach, delay discounting and response disinhibition, respectively.

Materials and methods

Participants

Seventy-three participants (44 female and 29 male) aged between 17 and 25 (M = 19.29 years, SD = 1.89) were recruited from Brisbane Technical and Further Education college campuses through advertising. The procedure was also piloted on one male and one female and their data are not included here. Potential participants were screened at initial contact via self-report for exclusion criteria: outside the age range of 17 to 25 years, history of head injury or psychiatric disorder, current gum or mucosal tissue disease and insufficient English language to complete the questionnaires. All participants provided signed informed consent as approved by the Queensland University of Technology Human Research Ethics Committee and were compensated.

Of the 73 participants, 51 (69.9%) were Australian-born and 51 (69.9%) were of Caucasian/European ethnicity, with 6 (8.2%) reporting Polynesian ethnicity, 5 (6.8%) Asian ethnicity, 1 (1.4%) Aboriginal and/or Torres Strait Islander ethnicity and 9 (12.3%) reporting 'Other' ethnicity. On the highest level of education attained, 60 (82.2%) participants reported completing high school. Despite prior screening criteria, 2 (2.7%) participants reported a history of head injury in the demographic questionnaire and 8 (11.0%) reported a history/prior diagnosis of a psychiatric disorder. However, all participants were assessed as having normal cognitive function by the Trail Making Test (TMT) (Spreen and Strauss 1998) and an absence of

psychiatric symptoms according to the General Health Questionnaire-28 (GHQ-28) (Goldberg and Williams 1988). On this basis, their data were retained and used in subsequent analyses. Seven (9.6%) participants reported a forensic history, typically involving minor offences.

Materials and Procedure

Participants were tested individually in 2-hour sessions conducted in small rooms at the participant's place of study. We manipulated acute stress by randomly allocating participants to either a pre-testing stress induction group (preparation period for a video-taped speech) or a pre-testing relaxation group (listening to relaxing music), with each induction period lasting five minutes. This experimental manipulation consistently increases subjective feelings of stress and accompanying neuroendocrine and cardiovascular responses (Elsenbruch et al. 2006; Feldman et al. 2004; Kirschbaum et al. 1993). The experiment was conducted individually in order to maximize the effect of the psychosocial stressor and minimize social support confounds (Thorsteinsson and James 1999). Specifically, those in the acute stress condition were told they were to spend the next five minutes preparing a speech on their least favorite body part which may be videotaped at the end of the testing session. These instructions are similar to those used successfully in previous research (though these did not use a video camera) on the effect of alcohol on psychological stress (Steele and Josephs 1988) and the effect of psychosocial stress on decision-making performance (Preston et al. 2007). A video camera was positioned on a tripod and visibly connected to the power supply in full view of the participant.

A behavioral measure of reward sensitivity, the Card Arranging Reward Responsiveness Objective Test (CARROT; Powell et al. 1996) was administered before and after the stress or relaxation induction. This is a simple card-sorting task that measures over four trials the extent to

which participants increase their speed of performance when financially rewarded (see Table I for further details). The CARROT has sound validity as a behavioral measure of Gray's reward sensitivity, with scores correlating with self-reported reward sensitivity in an Australian university sample (Kambouropoulos and Staiger 2004) and with clinically rated motivation in brain injured patients (Al Adawi et al. 1998).

Measures of state anxiety (State Trait Anxiety Inventory – State form, STAI-S; Speilberger 1983) and feelings of relaxation or stress via a Visual Analogue Scale (VAS) were also administered before and after the induction, with the pretest measures contained within an initial questionnaire pack. Each VAS was 100mm in length, with "very relaxed" anchoring the left side and "very stressed" anchoring the right side. The pack comprised a demographic form, a psychiatric health screening measure (GHQ-28) (Goldberg and Williams 1988) and other questionnaires analyzed cross-sectionally as part of a larger study and not reported here. After completion of the questionnaires participants then provided mouth swab samples and completed the TMT as a screening measure of cognitive function (Spreen and Strauss 1998). Two computerized impulsivity tasks were administered after the induction and second CARROT administration. These were a forced-choice delay discounting task (smaller, sooner-obtained rewards vs. larger, longer delayed rewards) named the Two Choice Impulsivity Paradigm (TCIP; Dougherty et al. 2005) followed by a stop signal task assessing the ability to withhold a prepotent response, the GoStop task (Dougherty et al. 2005). Due to the nature of the scoring of these tasks and the potential influence of carry-over practice effects, they could only be administered post-induction. Table I provides further detail on these behavioural measures of impulsivity. These measures were selected to reflect three impulsivity dimensions of reward-cued approach, delay discounting and response disinhibition, as supported by factor analytic

studies (e.g., Dom et al. 2007). Neuroimaging research linking these dimensions to differential brain activation patterns indicate reward-related processing and delay discounting is linked to greater activation in the ventral striatum of humans (Hariri et al. 2006) and differential striatal DRD2 binding in animals (Dalley et al. 2007). By contrast, response inhibition is associated with activation in orbitofrontal circuits (Horn et al. 2003).

Following the post-induction behavioral tests, participants who had undergone the stress induction were asked to select one envelope from a larger collection to discover whether their video-taped speech would proceed. All envelopes advised the speech would not take place and that their session was finished. This mild deception was essential to avoid participants from talking with new recruits and invalidating the stress induction task. Participants were then paid and were offered the opportunity to undergo a further period of 5 minutes relaxation before leaving the test room.

DRD2 C957T Genotyping

Buccal mucosa cells were collected via mouth swab samples using *Cytosoft* nylon bristle cytology brushes (Medical Packing Corporation, California, USA). Mouth swabs were used to obtain samples for DNA analysis to avoid a selective exclusion of participants with blood and injection phobias. These buccal mucosa cells were spun and DNA was extracted from leucocytes using standard techniques and subsequently used as a template for determination of genotypes (Grandy et al. 1993). Genotyping was performed by kinetic real-time polymerase chain reaction (PCR) using the Applied Biosystems 7000 sequence detection system (Applied Biosystems, Foster City, California, USA) . Sequence specific primers were designed for the C allele (5'-ATGGTCTCCACAGCACTCTC-3'), the T allele (5'-ATGGTCTCCACAGCACTCTT-3') and a

common reverse primer (5'-CATTGGGCATGGTCTGGATC-3'). A total of 5–10 ng of genomic DNA was amplified in 1×SYBR green PCR master mix (Applied Biosystems) containing 0.4 μM of allele specific forward primer and 0.4 μM of common reverse primer in a 25 μL volume. Amplification conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A cycle time (C_t) value was obtained by setting the threshold during geometric phase of amplification and scored relative to the ΔC_t generated between the matched and mismatched primer pairs. The C957T was one of four polymorphisms tested as part of a larger research program, with the others not reported here. These included the *Taq*IA polymorphism of the ANKK1 gene for which we have elsewhere reported an association (White et al. 2008) and two serotonin receptor genes not yet published.

Statistical analysis

This experimental design was mixed including one repeated measures variable of time of testing for the CARROT (pre- and post-induction), a between groups variable of genotype for the DRD2 C957T and a between groups variable of induction group (stress vs. rest induction condition). The between groups C957T genotype variable compared CC genotypes with CT and TT genotypes (i.e., using a recessive model) based on the previous observation of an association between the CC genotype and executive function impairments (Rodriguez-Jimenez et al. 2006; Xu et al. 2007). A within-groups variable of SOA was present for the GoStop task. A square root transformation corrected a significant positive skew on TCIP mean choice latency for immediate rewards. Separate analyses were conducted for each dependent variable of each task. All relevant assumptions were met.

Results

Genotyping

C957T genotyping identified 25 (34.7%) participants as CC genotype, 32 (44.4%) as CT genotype and 15 (20.8%) as TT genotype. These frequencies are in Hardy-Weinberg equilibrium, $\chi^2(1, N = 72) = 0.63$, $P > 0.05$. Subsequent analyses were performed combining the CT and TT genotypes, comparing presence or absence of the CC genotype. One sample could not be genotyped, thus excluding it from further analyses. The gender and ethnicity distribution for these two genotypes are indicated in Table II. As an initial check, separate 2 x 2 analyses of variance were conducted to explore whether gender and ethnicity interacted with genotype on the baseline measure of the first impulsivity measure, the CARROT (although ethnicity cell sizes necessitate collapsed group comparisons between Caucasian and non-Caucasian only). As shown in Tables III and IV, gender and ethnicity did not show any main effects (P = .318 and .918 respectively) or interactions with genotype (P = .446 and .532 respectively) on baseline reward responsiveness.

Experimental manipulation checks

Manipulation checks via paired t-tests on pre- and post-induction STAI-S scores supported the validity of the induction process. Those exposed to the stress induction reported significantly more anxiety after exposure (M = 40.67, SD = 12.17) than at baseline (M = 36.78, SD = 10.75), $t(1, 35) = -2.50$, $P = 0.017$. Those exposed to the relaxation induction significantly reduced their anxiety scores from baseline (M = 37.35, SD = 11.72) to post-induction (M = 31.24, SD = 8.15), $t(1, 36) = 4.59$, $P < 0.001$. Further tests revealed no baseline differences between the two

induction groups, $t(1, 71) = 0.22$, $P = 0.828$, and no effect of C957T genotype on anxiety scores at baseline or over time, $P > 0.05$. These test results were replicated using the VAS measure of stress, further supporting the validity of the stress manipulation.

Reward sensitivity: CARROT scores

A 2x2x2 split-plot ANOVA was conducted to examine differences in pre- and post-induction reward responsiveness (CARROT1 and CARROT2 scores) by DRD2 C957T polymorphism CC genotype (presence vs. absence) and induction condition (stress vs. rest). Table V presents the results and shows a significant 3-way interaction between genotype, stress induction group and time of testing on reward sensitivity (CARROT scores). Further comparisons were conducted for each genotype group (presence or absence of the CC genotype) using a Bonferroni adjusted alpha level of 0.025. A significant simple effect of time (pre- vs. post-induction scores) was found for those CC genotypes exposed to the stress induction only, $F(1,23) = 8.13$, $P = 0.009$ ($\eta_p^2 = 0.261$), with 957C homozygotes demonstrating greater reward responsiveness after stress induction, as shown in Figure 1. In contrast, 957C homozygotes reduced their reward sensitivity after the rest induction, though not significantly, $F(1,23) = 1.24$, $P = 0.277$ ($\eta_p^2 = 0.051$). For the combined CT and TT genotype group, only a multivariate main effect of time was significant, $F(1,45) = 9.25$, $P = 0.004$ ($\eta_p^2 = 0.171$), with this group demonstrating greater reward responsiveness after exposure to either the acute stress or rest induction, as shown in Figure 1. While the a priori hypothesis focused on the recessive model, as a further check the data was reanalyzed using the alternative dominant model of inheritance (TT vs. CT/CC genotypes) which was not significant at all levels of analysis, $P > 0.05$.

Delay discounting: TCIP measures

Separate 2x2 between-groups ANOVAs were conducted to examine effects of the C957T CC genotype (presence vs. absence) and induction condition (stress vs. rest) on the two delay discounting (TCIP) measures, proportion of smaller sooner 'immediate' reward choices made and square root transformed mean response latencies for 'immediate' reward selections. Table V shows a significant interaction between genotype and induction condition on transformed mean latencies for immediate choice responses. Further comparisons using a Bonferroni adjusted alpha level of 0.025 revealed a significant simple main effect of induction condition for those with the CC genotype, $F(1,66) = 5.52$, $P = 0.022$ ($\eta_p^2 = 0.077$). 957C homozygotes exposed to the acute stress induction were quicker in making their immediate choices than those 957C homozygotes who underwent the rest induction (as shown in Figure 2). For participants with CT and TT genotypes, mean latencies for immediate choices (transformed) were not significantly different between induction conditions, $F(1,66) = 0.16$, $P = 0.687$ ($\eta_p^2 = 0.002$). As shown in Table V, there were no significant effects for genotype or induction on mean proportions of immediate choices made in this task. Again, the analysis was repeated on the alternative dominant model of inheritance (TT vs. CT/CC genotypes). This dominant model was again not significant at all levels of the analysis, $P > 0.05$.

Inhibitory control: GoStop measures

Finally, we investigated a third dimension of impulsivity, response disinhibition, also referred to as rash impulsiveness, using the GoStop, a computerized stop inhibition task. We examined the interaction and main effects of C957T genotype and stress exposure via separate mixed-design ANOVAs for the two indices of rash impulsiveness, poorer stop inhibition (lower percentage of

stop trial responses successfully inhibited) and faster mean response latencies when responding to the stop signal. Mean latency data at 50 ms SOA contained 21% missing data (15 participants successfully inhibited the prepotent response 100% of the time) and was thus excluded from the analysis. Table V shows there were no significant effects involving genotype or induction condition. There were expected significant main effects of SOA for each measure, with poorer stop inhibition and faster response latencies as SOA increased. There were also no significant effects involving genotype or induction condition when reanalyzing the data using the dominant model of inheritance for C957T, $P > 0.05$.

Discussion

The results support the hypothesis that the DRD2 C957T polymorphism interacts with environmental stress on impulsivity, such that acute stress will lead to an increase in impulsive behavior for individuals with the CC genotype, but not for those without this genotype. This hypothesis was supported by results for two of the three dimensions tested. Homozygotes for 957C showed speeded reward-directed approach behavior (CARROT) and quicker delay discounting (TCIP) after exposure to the acute stressor, compared with those 957C homozygotes exposed to the rest induction. In contrast, acute stress exposure did not affect the performance of other young people with the CT or TT genotypes. There were no effects of C957T genotype or acute stress on response inhibition, a dimension of impulsivity not conceptually related to extrinsic reward.

As yet, there has been no other research examining the impact of acute stress on impulsivity (or any behavior) as a function of C957T genotype with which to compare these results. However, the results are consistent with previous research associations of acute stress

and impulsivity more generally (Conner et al. 2005; Roberti 2003) and the finding that acute stress increases striatal dopaminergic neurotransmission in humans (Pruessner et al. 2004), given that such activity has been associated with cognitive task performance and reward-cued behavior (Cropley et al. 2006). Importantly, the differential pattern of results revealed acute stress-induced C957T effects on two independent behavioral paradigms of impulsivity, which share processing of reward-related information. Only those with the CC genotype showed these stress-induced effects on impulsive behavior, which corresponds to early reports of an association of the CC genotype with executive function impairments (Rodriguez-Jimenez et al. 2006; Xu et al. 2007), potentially because of reduced striatal DRD2 density (Hirvonen et al. 2005). Our findings add to this emerging evidence base supporting a recessive model of inheritance for the C957T association with impulsive risk in healthy populations. The alternate dominant model failed to reach significance on any variable. In the clinical literature, schizophrenia has also been associated with the CC genotype (Hoenicka et al. 2006; Lawford et al. 2005), and with the C allele (Hanninen et al. 2006). Executive function deficits are part of the phenotype in schizophrenia (Goldman-Rakic 1994). The CC genotype has also been associated with psychopathic traits in alcohol dependent patients (Ponce et al. 2008).

In contrast, studies focusing on addiction have found inconsistent associations for the presence of the C allele or T allele with alcohol dependence (Hill et al. 2008; Ponce et al. 2008) and nicotine response (Jacobsen et al. 2006; Lerman et al. 2006). Only two reports have been published showing an association between alcohol dependence and the DRD2 C957T polymorphism (Hill et al. 2008; Ponce et al. 2008). Hill et al. found an association between the TT genotype and alcohol dependence (81 cases vs. 78 controls). In contrast, Ponce et al. (176 cases vs. 150 controls) found evidence for a C association (higher prevalence in CC genotypes

vs. CT/TT genotypes). The smaller Hill et al. study should be viewed with caution as they started with family analysis and used those families to select alcohol dependent participants for their population association study. This is therefore a highly selected group that may not be representative of the general population. Reports of an association of the T allele with nicotine-related variables are also unclear, particularly in terms of how they relate to dependence or to impulsivity. While they found poorer smoking cessation in response to a transdermal nicotine patch for T homozygotes (Lerman et al. 2006) and a nicotine-induced decrement on verbal working memory for carriers of the T allele (Jacobsen et al. 2006), these variables in themselves do not necessarily indicate an association with dependence. Alternatively, the CC and CT genotypes may be more 'risky' in terms of non-responsiveness to treatment for substance dependence and facilitatory working memory effects from nicotine which may contribute to dependence. These conflicting clinical research findings may also alternatively reflect the influence of environmental factors, or suggest differential effects on aspects of cognition and behaviour as a function of either reduced or increased striatal DRD2 density. A strength of the current study is the inclusion of laboratory manipulated acute psychosocial stress in the design and the measurement of multiple dimensions of impulsive behavior to further elucidate the phenotypic effects of the C957T in a healthy young adult population. Prospective research is clearly needed to further delineate the link between impulsivity and the developmental pathways to related clinical disorders.

A limitation of the current study is its modest sample size, requiring a cautious interpretation of our results, though this is typical of other studies of this type (e.g., Rodriguez-Jimenez et al. 2006). Further, the sample was heterogeneous for both gender and ethnicity, with the small cell sizes and resulting power issues precluding the inclusion of these variables into the

full design ANOVAs to fully investigate their effects. However, an analysis of their effects on baseline impulsivity did not find any significant main effects or interactions with genotype on baseline reward responsiveness (CARROT measure of impulsivity). While a larger sample size would have made it possible to test the effect of gender and ethnicity on the measures of interest, results conducted on this sample suggest that an effect would not be seen in a larger sample. The effect of a heterogeneous population is to reduce power. However, despite this significant effects of the C957T and acute stress induction were found.

The study suggests the C957T CC genotype is associated with a reward-related impulsivity endophenotype that is associated with acute psychosocial stress. This may reflect a greater sensitivity of these individuals to the effects of acute social stress, potentially through striatal dopamine release (Pruessner et al. 2004), consistent with reduced striatal DRD2 availability (Hirvonen et al. 2005). Recent PET scan findings suggest acute stress-induced striatal dopamine release, particularly in the ventral striatum, may be greater in healthy individuals at risk of developing psychosis (having elevated scores for physical anhedonia/negative schizotypy) (Soliman et al. 2008). The current findings suggest that those with the CC genotype may be more sensitive to stress-induced effects on impulsive behaviour. Because we did not measure dopamine activity, we can only speculate that this sensitivity may involve dopaminergic processes, given that 957C homozygotes are characterized by reduced dopamine receptor density in key brain reward areas (Hirvonen et al. 2005). Future research should incorporate assessment of dopamine release to examine the dual hypothesis that 1) the C957T polymorphism may actually affect stress-induced dopamine release and 2) this in turn affects reward-related impulsive behavior. Further, it is likely there are multiple environmental and genetic risk factors contributing to impulsive behavior. Future research using a larger sample

could also investigate potential gene-gene interactions on impulsivity, possibly between the DRD2 C957T and ANKK1 *Taq*IA, given their similar *in vivo* associations with reduced striatal DRD2 density (Hirvonen et al. 2005; Jonsson et al. 1999; Pohjalainen et al. 1998) and recent association between the *Taq*IA and other aspects of impulsivity in a healthy population (White et al. 2008).

The validity of the psychosocial stress induction paradigm was supported, with self-reported anxiety and feelings of stress significantly increased after stress induction and significantly decreased after rest induction. This adds to a substantial body of research support for the validity of speech tasks characterized by a preparation period and evaluative component in increasing subjective feelings of stress. Previous research has also consistently demonstrated this self-reported stress is accompanied by appropriate neuroendocrine and cardiovascular responses (Elsenbruch et al. 2006). The inclusion of similar physiological markers such as salivary cortisol levels would strengthen the validity of the manipulation used in this study. Importantly however, the current results have shown the C957T gene effect is strong enough to reveal behavioral differences in impulsivity even with a mild stressor. It would be useful for future research to examine whether chronic exposure to stressors has similar effects to the single acute stress exposure in this study.

This study was innovative in incorporating a multidimensional behavioral assessment of impulsivity that integrated psychosocial and molecular genetic influences in a community sample of young adults. It is the first study to investigate and provide evidence of a moderating role of acute stress on the relationship between C957T genotype and behavior, and the first to suggest an association of the CC genotype with a reward-related impulsivity endophenotype.

This may reflect a greater sensitivity of these individuals to the effects of acute social stress, which operates through enhanced dopamine release.

Acknowledgments We thank D. Dougherty for providing the software for the delay discounting and stop inhibition tasks. We also thank C.D. Swagell and A. Liao for assistance with genotyping. This study was financially supported by an Australian Postgraduate Award and Institute of Health and Biomedical Innovation Postgraduate Award.

References

Al Adawi S, Powell J., Greenwood RJ (1998) Motivational deficits after brain injury: A neuropsychological approach using new assessment techniques. Neuropsychology 12:115-124

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington, D.C.

Bobb AJ, Castellanos FX, Addington AM, Rapoport JL (2006) Molecular genetic studies of ADHD: 1991 to 2004. Am J Med Gen 141:551-565

Bogdan R., Pizzagalli DA (2006) Acute stress reduces reward responsiveness: Implications for depression. Biol Psychiatry 60:1147-1154

Bowirrat A, Oscar-Berman M (2005) Relationship between dopaminergic neurotransmission, alcoholism, and reward deficiency syndrome. Am J Med Gen 132:29-37

Caspi A., Moffitt TE (2006) Gene-environment interactions in psychiatry: Joining forces with neuroscience. Nature Reviews Neuroscience 7:583-590

Cloninger CR, Svrakic DM, Przybeck TR (1993) A psychobiological model of temperament and character. Arch Gen Psychiatry 50:975-990

Conner KR, Phillips MR, Meldrum S, Knox KL, Zhang Y, Yang G (2005) Low-planned suicides in China. Psychol Med 35:1197-1204

Cropley VL, Fujita M, Innis RB, Nathan PJ (2006) Molecular imaging of the dopaminergic system and its association with human cognitive function. Biol Psychiatry 59:898-907

Dalley JW, Fryer TD, Brichard L, Robinson ESJ, Theobald DEH, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron J-C, Everitt

BJ, Robbins TW (2007) Nucleus Accumbens D2/3 Receptors Predict Trait Impulsivity and Cocaine Reinforcement. Science 315:1267-1270

Dom G, De Wilde B, Hulstijn W, Sabbe B (2007) Dimensions of impulsive behaviour in abstinent alcoholics. Pers Indiv Diff 42:465-476

Dougherty DM, Mathias CW, Marsh DM, Jagar AA (2005) Laboratory behavioral measures of impulsivity. Behav Res Methods 37:82-90

Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV (2003) Synonymous mutations in the human dopamine receptor (DRD2) affect mRNA stability and synthesis of the receptor. Hum Mol Genet 12: 205-216

Elsenbruch S, Lucas A, Holtmann G, Haag S, Gerken G, Riemenschneider N, Langhorst J, Kavelaars A, Heijnen CJ, Schedlowski M (2006) Public speaking stress-induced neuroendocrine responses and circulating immune cell redistribution in irritable bowel syndrome. Am J Gastroenterol 101:2300-2307

Enticott PG, Ogloff JRP (2006) Elucidation of impulsivity. Aust Psychol 41:3-14

Evenden J (1999) Varieties of impulsivity. Psychopharmacology 146:348-361

Eysenck HJ (1983) A biometrical-genetical analysis of impulsive and sensation seeking behaviour. In: Zuckerman M (ed) Biological bases of sensation seeking, impulsivity and anxiety. Erlbaum, Hillside, NJ, pp. 1-27

Feldman PJ, Cohen S, Hamrick N, Lepore SJ (2004) Psychological stress, appraisal, emotion and cardiovascular response in a public speaking task. Psychol Health 19:353-368

Goldberg D, Williams P (1988) A User's Guide to the General Health Questionnaire. NFER-NELSON Publishing, Windsor, Berkshire

Goldman-Rakic PS (1994) Working memory dysfunction in schizophrenia. J Neuropsychiatry Clin Neurosci 6:348-357

Grandy DK, Zhang Y, Civelli O (1993) PCR detection of the Taq A RFLP at the DRD2 locus. Hum Mol Genet 2:2197

Gray JA, McNaughton N (2000) The neuropsychology of anxiety: An enquiry into the functions of the septo-hippocampal system, 2nd edn. Oxford University Press, New York

Hanninen K, Katila H, Kampman O, Anttila S, Illi A, Rontu R, Mattila KM, Hietala J, Hurme M, Leinonen E, Lehtimaki T (2006) Association between the C957T polymorphism of the dopamine D2 receptor gene and schizophrenia. Neurosci Lett 407:195-198

Hariri AR, Brown SM, Williamson DE, Flory JD, de Wit H, Manuck SB (2006) Preference for immediate over delayed rewards is associated with magnitude of ventral striatal activity. J Neurosci 26:13213-13217

Heath AC, Cloninger CR, Martin NG (1994) Testing a model for the genetic structure of personality: A comparison of the personality systems of Cloninger and Eysenck. J Pers Soc Psychol 66:702-775

Hill SY, Hoffman EK, Zezza N, Thalamuthu A, Weeks DE, Matthews AG, et al. (2008) Dopaminergic mutations: Within-family association and linkage in multiplex alcohol dependence families. Am J Med Genet Part B 147:517-526

Hirvonen M, Laakso A, Nagren K Rinne JO, Pohjalainen T, Hietala J (2004) C957T polymorphism of the dopamine D2 receptor (DRD2) gene affects striatal DRD2 availability in vivo. Mol Psychiatry 9:1060-1061

Hirvonen M, Laakso A, Nagren K, Rinne JO, Pohjalainen T, Hietala J (2005) C957T polymorphism of the dopamine D2 receptor (DRD2) gene affects striatal DRD2 availability in vivo (vol 9, pg 1060, 2005). Mol Psychiatry 10:889-889

Hoenicka J, Aragues M, Rodriguez-Jimenez R, Ponce G, Martinez I, Rubio G, Jimenez-Arriero MA, Palomo T (2006) C957T DRD2 polymorphism is associated with schizophrenia in Spanish patients. Acta Psychiatr Scand 114:435-438

Horn NR, Dolan M, Elliott R, Deakin JFW, Woodruff PWR (2003) Response inhibition and impulsivity: an fMRI study. Neuropsychologia 41:1959-1966

Hur Y, Bouchard TJ Jr (1997) The genetic correlation between impulsivity and sensation seeking traits. Behav Genet 27:455-463

Jacobsen LK, Pugh KR, Mencl WE, Gelernter J (2006) C957T polymorphism of the dopamine D2 receptor gene modulates the effect of nicotine on working memory performance and cortical processing efficiency. Psychopharmacology 188:530-540

Jonsson EG, Nothen MM, Grunhage F, Farde L, Nakashima Y, Propping P, Sedvall GC (1999) Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. Mol Psychiatry 4:290-296

Kambouropoulos N, Staiger PK (2004) Personality and responses to appetitive and aversive stimuli: The joint influence of behavioural approach and behavioural inhibition systems. Pers Indiv Diff 37:1153-1165

Kellendonk C, Simpson EH, Polan HJ, Malleret G, Vronskaya S, Winiger V, Moore H, Kandel ER (2006) Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. Neuron 49:603-615

Kirschbaum C, Pirke KM, Hellhammer DH (1993) The Trier Social Stress Test - a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28:76-81

Lawford BR, Young RM, Swagell CD, Barnes M, Burton SC, Ward WK, Heslop KR, Shadforth S, van Daal A, Morris CP (2005) The C/C genotype of the C957T polymorphism of the dopamine D2 receptor is associated with schizophrenia. Schizophr Res 73:31-37

Lerman C, Jepson C, Wileyto EP, Epstein LH, Rukstalis M, Patterson F, Kaufmann V, Restine S, Hawk L, Niaura R, Berrettini W (2006) Role of functional genetic variation in the dopamine D2 receptor (DRD2) in response to bupropion and nicotine replacement therapy for tobacco dependence: Results of two randomized clinical trials. Neuropsychopharmacology 31:231-242

Neville MJ, Johnstone EC, Walton RT (2004) Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat 23:540-545

Noble EP (2003) D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. Am J Med Genet 116:103-125

Pohjalainen T, Rinne JO, Naguen K, Naguen K, Lehikoinen P, Antila K, Syvalahti EK, Hietela J (1998) The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in health volunteers. Mol Psychiatry 3:256-260

Ponce G, Hoenicka J, Jimenez-Arriero MA, Rodriguez-Jimenez R, Aragues M, Martin-Sune N, Huertas E, Palomo T (2008) DRD2 and ANKK1 genotype in alcohol-dependent patients with psychopathic traits: association and interaction study. Br J Psychiatry 193:121–125

Powell J, Al-Adawi S, Morgan J, Greenwood RJ (1996) Motivational deficits after brain injury: Effects of bromocriptine in 11 patients. J Neurol Neurosurg Psychiatry 60:416-421

Preston SD, Tansfield RBS, Buchanan TW, Bechara A (2007) Effects of anticipatory stress on decision making in a gambling task. Behav Neurosci 121:257-263

Pruessner JC, Champagne F, Meaney MJ, Dagher A (2004) Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: A positron emission tomography study using [^{11}C]raclopride. J Neurosci 24:2825-2831

Roberti JW (2003) Sensation seeking characteristics and neuroendocrine responses to an acute psychological challenge for undergraduates with career interests in forensic sciences. Internet J Forensic Science 1:1-22

Rodriguez-Jimenez R, Hoenicka J, Jimenez-Arriero MA, Ponce G, Bagney A, Aragues M, Palomo T (2006) Performance in the Wisconsin card sorting test and the C957T polymorphism of the DRD2 gene in healthy volunteers. Neuropsychobiology 54:166-170

Soliman A, O'Driscoll GA, Pruessner J, Holahan ALV, Boileau I, Gagnon D, Dagher A (2008) Stress-induced dopamine release in humans at risk of psychosis: a [^{11}C]raclopride PET study. Neuropsychopharmacology 33:2033-2041

Speilberger CD (1983) State-Trait Anxiety Inventory for Adults (Form Y): Sampler Set. Mind Garden, Redwood City, CA

Spreen O, Strauss E (1998) A compendium of neuropsychological tests: administration, norms, and commentary, 2nd edn. Oxford University Press, New York

Steele CM, Josephs RA (1988) Drinking your troubles away II: An attention-allocation model of alcohol's effect on psychological stress. J Abnorm Psychol 97:196-205

Thorsteinsson EB, James JE (1999) A meta-analysis of the effects of experimental manipulations of social support during laboratory stress. Psychol Health 14:869-886

White MJ, Morris P, Lawford BR, Young RM (2008) Behavioral phenotypes of impulsivity related to the ANKK1 gene are independent of an acute stressor. Behav Brain Funct 4:54

Xu HY, Kellendonk CB, Simpson EH, Keilp JG, Bruder GE, Polan HJ, Kandel ER, Gilliam TC (2007) DRD2 C957T polymorphism interacts with the COMT Val158Met polymorphism in human working memory ability. Schizophr Res 90:104-107

Zuckerman M, Kuhlman DM (2000) Personality and risk-taking: Common biosocial factors. J Pers 68:999-1029

Tables

Table I Tests of behavioral impulsivity used in the current study

Test	Description	Dependent Variable
CARROT (Powell et al. 1996)	Participants complete four trials of sorting a pack of cards, each card with five digits, into three corresponding trays. The first trial (T1) involves sorting 60 cards while being timed, with this time used as the time limit for subsequent trials. In trial two (T2), the participant sorts a pack of 100 cards until told to stop. The third trial (T3) involves sorting 100 cards again with the time restriction of the previous trial, but with a small monetary reward offered for every five cards correctly sorted. A 20 cent coin is placed in front of the participant as the fifth card is sorted into the correct trays. The fourth trial (T4) is identical to T2 and controls for fatigue or practice effects on response speed. After T4, the participant is given the money earned during T3.	CARROT score of reward sensitivity, calculated by subtracting the mean of the number of cards sorted in T2 and T4 from the number of cards sorted in T3. CARROT = T3 – ((T2+T4)/2).
TCIP (Dougherty et al. 2005)	A forced-choice, reward-directed computerized task, modeled on delay discounting and delay of gratification tasks. Participants press a mouse button to select one of two shapes (a square and a circle), each associated with either a short delay (in this case, 5 seconds) followed by a small reward (in this case, 5 points) or a longer delay (15 seconds) followed by a larger reward (15 points). For this experiment, the parameters were set to include 10 training trials followed by 40 session trials using the "Reward Feedback" option. Pairing of shapes with immediate/delayed conditions was counterbalanced within each experimental induction group. Reward contingencies were not made explicit, with participants implicitly learning the relationship between the number of reward points displayed on the screen and each preceding geometric shape choice.	1. Proportion of more immediate reward choices (higher = more impulsive) 2. Reaction times when making these more immediate reward choices (faster = more impulsive).
GoStop (Dougherty et al. 2005)	Like other stop response inhibition procedures, participants are required to attend to a series of visual stimuli, respond when a target "go" signal appears, and withhold responding when a "stop" signal or non-target stimulus appears. In the GoStop, the stimuli are a series of five-digit numbers presented in black font one at a time on the screen. The "go" signal is a number that matches the previous number identically and is also presented in black. The "stop" signal is a matching number that changes color from black to red font sometime after the stimulus onset. In addition to No-Stop (only the "go" signal) and Stop trials, at least half of the trials are Novel trials, with randomly generated non-matching numbers presented in black. For this experiment, the parameters were the default option of two blocks, seven stop trials (default is 10), 28 non-stop trials (default is 40), and 56 novel trials (minimum of one Novel stimulus following every Stop and No-Stop Trial). Stop Interval settings (ms from stimulus onset, SOA) were set as default (four intervals of 50 ms, 150 ms, 250 ms, and 350 ms, quasi-randomized throughout the session). Stimuli were presented for 500 ms each followed by 600 ms blackout between stimuli presentations.	1. Percent inhibited responses (proportion of Stop trials where no response occurs) (lower = more impulsive). 2. Stop Latency (time in ms between the Stop Signal onset and response) (quicker = more impulsive).

Note: CARROT, Card Arranging Reward Responsiveness Objective Test; TCIP, Two Choice Impulsivity Paradigm.

Table II DRD2 C957T genotype group frequencies (% of total) by self-reported gender and ethnicity

Subgroup	CC	CT/TT
Gender		
Male	13 (18.1%)	16 (22.2%)
Female	12 (16.7%)	31 (43.1%)
Total	25 (34.7%)	47 (65.3%)
Ethnicity		
Aboriginal and/or Torres Strait	1 (1.4%)	0 (0%)
Islander		
Caucasian/European	11 (15.5%)	39 (54.9%)
Polynesian	5 (7.0%)	1 (1.4%)
Asian	4 (5.6%)	1 (1.4%)
Other	4 (5.6%)	5 (7.0%)
Total	25 (35.2%)	46 (64.8%)

Table III Analysis of variance for baseline CARROT by DRD2 C957T genotype (CC vs. CT/TT genotypes) and gender

Variable		*F*	*P*	η_p^2
CARROT	Genotype	.05	.829	.001
(pre-induction)	Gender	.32	.318	.015
	Genotype x Gender	.59	.446	.009

Note: CARROT, Card Arranging Reward Responsiveness Objective Test.

Table IV Analysis of variance for baseline CARROT by DRD2 C957T genotype (CC vs. CT/TT genotypes) and ethnicity (Caucasian vs. non-Caucasian)

Variable		*F*	*P*	η_p^2
CARROT	Genotype	.001	.979	<.001
(pre-induction)	Ethnicity	.01	.918	<.001
	Genotype x Ethnicity	.39	.532	.006

Note: CARROT, Card Arranging Reward Responsiveness Objective Test.

Table V Summary of analyses of variance for behavioral paradigms of impulsivity by DRD2 C957T genotype (CC vs. CT/TT genotypes) and induction condition (rest vs. stress)

Variable		*F*	*P*	η_p^2
CARROT (pre- and post-induction)	Genotype	0.04	0.852	0.001
	Induction	0.34	0.563	0.005
	Time	8.04	0.006	0.106
	Genotype x Induction	0.57	0.451	0.008
	Genotype x Time	0.38	0.542	0.005
	Induction x Time	5.44	0.023	0.074
	Genotype x Induction x Time	6.54	0.013	0.088
TCIP Proportion Immediate Choices	Genotype	0.16	0.688	0.002
	Induction	0.90	0.347	0.013
	Genotype x Induction	2.30	0.134	0.033
TCIP Mean Choice Latency (square root transformed scores)	Genotype	<0.001	0.996	<0.001
	Induction	2.78	0.100	0.040
	Genotype x Induction	4.59	0.036	0.065
GoStop Stop Inhibition	Genotype	0.43	0.515	0.006
	Induction	1.82	0.182	0.026
	SOA	88.69	<0.001	0.804
	Genotype x Induction	0.004	0.948	<0.001
	Genotype x SOA	0.75	0.528	0.033
	Induction x SOA	2.32	0.084	0.097
	Genotype x Induction x SOA	0.34	0.800	0.015
GoStop Mean Stop Response Latency	Genotype	0.07	0.796	0.001
	Induction	0.89	0.349	0.014
	SOA	291.37	<0.001	0.901
	Genotype x Induction	0.36	0.551	0.005
	Genotype x SOA	1.03	0.364	0.031
	Induction x SOA	0.15	0.865	0.005
	Genotype x Induction x SOA	0.30	0.745	0.009

Note: CARROT, Card Arranging Reward Responsiveness Objective Test; TCIP, Two Choice Impulsivity Paradigm. Follow-up results for significant interactions are reported in-text.

Figure Captions

Fig. 1 Reward responsiveness (Mean CARROT scores ± 2 *SEM*) pre- and post-induction by DRD2 C957T genotype for those in the rest induction condition (CC *n* = 13; CT, TT *n* = 23) and stress induction condition (CC *n* = 12; CT, TT *n* = 24).

Fig. 2 Delay discounting mean response latencies for impulsive choices (untransformed raw scores) and proportion of immediate choices post-induction by DRD2 C957T genotype for those in the rest induction condition (CC *n* = 12,13; CT, TT+ *n* = 23) and stress induction condition (CC *n* = 12; CT, TT *n* = 23,24). Error bars display ± 2 *SEM*.