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SHORT COMMUNICATION

DRD4 polymorphism associated with greater positive affect in response to negative and neutral social stimuli

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Abstract: Despite the robustness of *DRD4* polymorphism associations with brain-based behavioral characteristics in candidate gene research, investigations have minimally explored associations between these polymorphisms and emotional responses. In particular, the prevalent single nucleotide polymorphism (SNP) -521C/T (rs1800955) in the promoter region of *DRD4* remains unexplored relative to emotions. Here, two independent samples were evaluated using different emotion elicitation tasks involving social stimuli: Study 1 (N = 120) evoked positive and negative emotional responses to validated film clips; Study 2 (N = 122) utilized Cyberball to simulate social rejection and acceptance. Across studies, C/C individuals self-reported higher mean positive affect scores using Likert scales versus T carrier individuals, selectively when presented with neutral or negative (but not positive) social stimuli. The consistent findings across these two studies supports a functional consequence of this *DRD4* SNP on emotion processing during changing social contexts. Continued investigation will help clarify if a C/C genotype enhances positive emotions under negative circumstances, or if the presence of the T allele reduces positive emotions, and how rs1800955 behavioral associations might generalize across different demographics. Future studies could also reveal if this SNP interacts with other changing environmental conditions to affect emotional responses, such as social limitations during the COVID-19 pandemic.

KEYWORDS
affect, dopamine, emotions, mental health, ostracism, polymorphism, receptors, single nucleotide

1 | INTRODUCTION

Dopamine signaling in the brain contributes to a multitude of behavioral processes, including motor movement, learning processes, and emotional responses. Study of functional genetic polymorphisms in genes coding for dopamine receptors, particularly the G protein-coupled dopamine receptor D4 (*DRD4*), have demonstrated replicable polymorphism-behavior associations (Abrahams et al., 2019; Gizer et al., 2009; Thomson et al., 2013) and significant associations with brain activity as measured by functional magnetic resonance imaging (Agam et al., 2014; Camara et al., 2010). Indeed, associations between *DRD4* polymorphisms and behavior have been supported by

literature reviews and meta-analyses (Gizer et al., 2009; Munafò et al., 2008). However, studies of other candidate gene polymorphisms have faced greater replicability challenges, leading to candidate gene studies as a whole to fall out of favor in the scientific realm (Border et al., 2019).

While much *DRD4* polymorphism research has centered around impulsivity, addiction, and other externalized behaviors, some researchers have also explored how *DRD4* polymorphisms relate to emotional responses. These have thus far exclusively focused on the *DRD4* exon III variable number tandem repeat (VNTR). However, associations between another DRD4 polymorphism, the single nucleotide polymorphism (SNP) -521C/T (rs1800955) in the promoter region (Okuyama et al., 1999), and emotional responses have not been evaluated despite the role of emotions in externalizing behaviors and broadly defined psychological health. Nonetheless, both alleles of this SNP have been differentially associated with externalizing behavioral measures including smoking (C allele) (Pérez-Rubio et al., 2017), novelty seeking (T and C alleles, respectively) (Abrahams et al., 2019; Munafò et al., 2008), error processing (T allele) (Agam et al., 2014), and attention-deficit hyperactivity disorder (T allele) (Gizer et al., 2009). In particular, behaviors associated with reward and novelty seeking are heavily influenced by emotional responses, suggesting rs1800955 might also be associated with positive emotional responses (Abrahams et al., 2019; Camara et al., 2010; Pérez-Rubio et al., 2017). Here, we used two separate studies to assess how the *DRD4* SNP -521C/T polymorphism was associated with *positive* emotional responses to positively and negatively valenced social stimuli. Positive emotions are an established factor in long-term psychological and physical health, and evolved to facilitate social connection and reward (Coifman et al., 2021; Fredrickson, 1998; Pressman et al., 2018). Given this polymorphism had not been previously evaluated with respect to emotion responses, we did not have any a priori hypotheses about how these genotypes might be associated with positive affective responses to negatively and positively valenced social stimuli.

2 | MATERIALS AND METHODS

Undergraduates of the Psychological Sciences department subject pool at a large midwestern public university in the United States were recruited as independent samples for Study 1 ($N = 120$) or Study 2 ($N = 122$). Study 1 was 79% of Western Eurasian ancestry, 62% female, 94% non-Hispanic/Latino, and Study 2 was 81% of Western Eurasian ancestry, 62% female, and 95% non-Hispanic/Latino. The present results are secondary data analyses of data collected between 2012 and 2015 for a parent project (Gilman et al., 2015, see Supporting Information Material), and for which sample sizes were determined based on power analyses, though Ns were limited in a few instances by practical considerations, such as the quality of extracted and amplified DNA. Full demographic data broken down into genotype categories is in Table 1. Written informed consent was obtained from all participants prior to each study, and all procedures were approved by the Kent State University Institutional Review Board and in accord with the Declaration of Helsinki.

Each participant provided 2 ml of passive drool saliva that was stored at −20°C until processing. As previously described (Gilman et al., 2015), genomic DNA was extracted (with prepIT-L2P from DNA Genotek, Inc., Ottawa, Canada), purified (with Genomic DNA Clean & Concentrator kit, Zymo Research, Irvine, CA), and diluted to a standard working concentration of 5 ng/μl. A modified touchdown PCR protocol was used (Gilman et al., 2015), with 0.5 μmol/L of forward (5′-CGG GGG CTG AGC ACC AGA GGC TGC T-3′) and reverse (5′-GCA TCG ACG CCA GCG CCA TCC TAC C-3′) primers (Integrated DNA Technologies, Inc., Coralville, IA) (Okuyama et al., 1999). Following completion of the PCR, a restriction fragment length polymorphism analysis was run on each sample by combining 10 μl of PCR product with 4 U/reaction of *Fsp*I in 1X CutSmart buffer (New England BioLabs, Ipswich, MA) for 1 h at 37°C. Undigested PCR products were run alongside digested PCR products in a 2% agarose gel. C allele products were not digested, and resulted in 285 bp amplimers, whereas T allele products were digested by *Fsp*I, splitting them into fragments that migrated at 176 and 109 bp.

Study 1 involved participants viewing a sequence of validated emotionally evocative film clips following a neutral baseline clip. In order, these were as follows: (1) Big Cat Diary (baseline); (2) the Road to Guantanamo (negative); (3) Alive (positive); (4) the Champ (negative); (5) Between Two Ferns (positive); extensive details regarding these clips and their validation are published elsewhere (Gilman et al., 2017).

Study 2 involved participants engaging in a computer manipulation called Cyberball, validated to simulate social rejection and acceptance (Gilman et al., 2015). Briefly, this involved participants being told that they would play a video game with two other participants. In actuality, they were only playing with the computer program that, after a baseline (neutral) game involving equitable social participation, engages in a game that actively excludes the participant (rejection game), followed by a game that preferentially includes the participant (acceptance game).

After each film clip (Study 1) or Cyberball game (Study 2), participants were asked to self-report their emotional

TABLE 1 Demographics for Studies 1 and 2

Study 1 demographics	**C/C** ***N* = 28**	**C/T or T/T** ***N* = 92**	
Age	20.39 (5.08)	20.98 (6.78)	$t(118) = -0.42$, $p = 0.67$
Gender	16 Female	58 Female	$\chi^2 = 0.32$, $p = 0.57$
	12 Male	34 Male	
Biogeographic ancestry			
Western Eurasian	23	72	
Sub-Saharan	2	12	$\chi^2 = 4.36$, $p = 0.23$
East or Southern Asian	2	1	
Other	1	7	
Ethnicity	1 Hispanic/Latino	6 Hispanic/Latino	$\chi^2 = 0.34$, $p = 0.56$
Study 2 demographics	**C/C** **N = 35**	**C/T or T/T** **N = 87**	
Age	19.45 (2.38)	20.67 (5.00)	$t(120) = -1.38$, $p = 0.17$
Gender	20 Female	56 Female	$\chi^2 = 0.56$, $p = 0.46$
	15 Male	31 Male	
Biogeographic ancestry			
Western Eurasian	32	67	
Sub-Saharan	0	11	$\chi^2 = 7.40$, $p = 0.06$
East or Southern Asian	1	3	
Other	1	0	
Ethnicity	2 Hispanic/Latino	4 Hispanic/Latino	$\chi^2 = 0.05$, $p = 0.82$

experiences with Likert scales ranging from 1–7 with positive (affection, amusement, enjoyment, happiness, interest, relief) emotion words. Word scores were aggregated into mean positive affective scores. Additional procedural details of Study 1 and Study 2 have been described extensively elsewhere (Gilman et al., 2015). Though negative emotion words were also self-reported and aggregated into mean negative affective scores, these were not the focus of our precise research question. Moreover, no significant genotype effects were observed for self-reported negative emotions (see Supporting Information).

Relative to the preceding film clips in the sequence for Study 1, The Road to Guantanamo ($t(119) = 10.3$, $p < 0.001$) and The Champ ($t(119) = 5.59$, $p < 0.001$) significantly decreased positive affect as intended, and Alive ($t(119) = -6.06$, $p < 0.001$) and Between Two Ferns ($t(119) = -18.3$, $p < 0.001$) significantly increased positive affect. Similarly, the rejection game of Cyberball significantly reduced positive affect compared to the baseline game ($t(121) = 11.2$, $p < 0.001$), and the acceptance game significantly enhanced positive affect after the rejection game ($t(122) = -5.20$, $p < 0.001$).

In line with previous investigations of rs1800955 (Munafò et al., 2008; Thomson et al., 2013), we compared individuals homozygous for the C allele (C/C) with those carrying at least one T allele (C/T and T/T individuals). Within each Study, data were analyzed using a repeated-measures ANOVA (genotype × film clip, Study 1; genotype × game, Study 2) and Bonferroni post-hoc analyses using IBM SPSS Statistics (v. 26.0.0.0, IBM Corp., Armonk, NY). Significance was set a priori at $p < 0.05$, and mean positive affect scores were graphed as mean ± S.E.M. using GraphPad Prism (v. 9.3.1 (350), GraphPad Software, LLC., La Jolla, CA).

3 | RESULTS

Study 1 involved emotion elicitation through use of validated film clips (Gilman et al., 2015, 2017). Specifically, film clips alternating in negative and positive valences were presented. Mean positive affect after each film clip was quantified (Figure 1), and a repeated measures ANOVA indicated a significant main effect of genotype for the *DRD4* -521C/T SNP ($F(1,118) = 7.20$, $p = 0.008$, partial $\eta^2 = 0.06$). Pairwise comparisons with Bonferroni post-hoc testing revealed that mean positive affect during the baseline "Big Cats" film clip ($p = 0.032$) and the negatively valenced clip from "The Champ" involving social loss and sadness ($p = 0.009$), were higher in individuals homozygous for the C allele as compared to T carriers. A similar nonsignificant trend ($p = 0.054$) for C/C

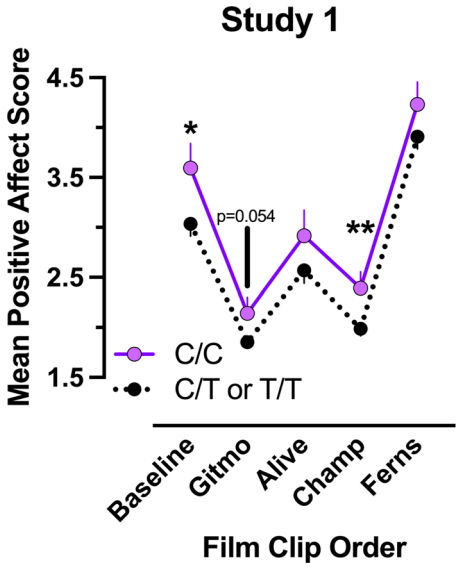

FIGURE 1 Mean self-reported positive affect scores in response to film clips in Study 1. Film clips were shown to participants in the following sequence: (1) "Big Cats" (Baseline); (2) "The Road to Guantanamo (Gitmo); (3) Alive; (4) The Champ (Champ); (5) Between Two Ferns (Ferns). Purple circles (refer to online version for color) with solid lines indicate individuals with C/C genotypes ($N = 28$); black circles with dotted lines indicate individuals with at least one T allele ($N = 92$). Data are graphed as mean ± S.E.M. *$p = 0.032$; **$p = 0.009$ comparing C/C with C/T or T/T for that specific film clip. Scores between genotypes for the Gitmo film clip approached significance ($p = 0.054$, vertical line)

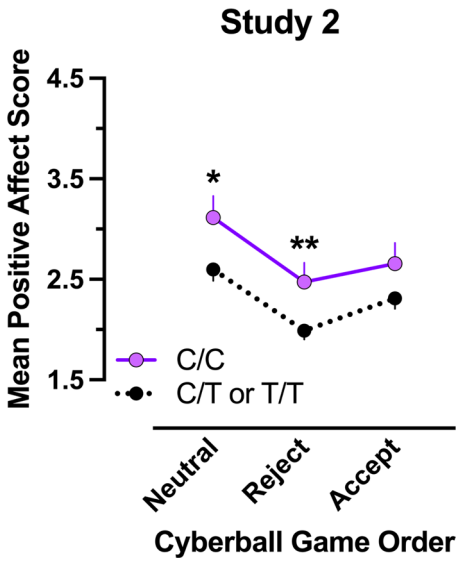

FIGURE 2 Mean self-reported positive affect scores in response to Cyberball games in Study 2. Participants engaged in a Cyberball task that involved three consecutive games: (1) a baseline game (Neutral); (2) a game designed to simulate social rejection (Reject); (3) a game designed to simulate social acceptance (Accept). Purple circles (refer to online version for color) with solid lines indicate individuals with C/C genotypes ($N = 35$); black circles with dotted lines indicate individuals with at least one T allele ($N = 87$). Data are graphed as mean ± S.E.M. *$p = 0.024$; **$p = 0.009$ comparing C/C with C/T or T/T for that specific game

individuals exhibiting more positive affect than C/T and T/T individuals was noted for the other negatively valenced clip, "Road to Guantanamo," that shows treatment of war prisoners. No significant differences between these two genotypes were detected for either of the positively valenced film clips, "Alive" ($p = 0.20$) and "Between Two Ferns" ($p = 0.20$).

Study 2 used a Cyberball task to simulate, following a neutral game round, peer rejection followed by peer acceptance (Gilman et al., 2015). Using this task, genotype for the *DRD4* -521C/T SNP was significant for mean positive affect across the Cyberball task using a repeated measures ANOVA ($F(1,120) = 5.30$, $p = 0.023$, partial $\eta^2 = 0.04$) (Figure 2). Bonferroni post-hoc comparisons indicated that C/C individuals had higher mean positive affect during the baseline neutral game ($p = 0.024$) and the social rejection game ($p = 0.009$) relative to T carriers. No significant difference between these two genotypes was detected during the acceptance game ($p = 0.11$). For Studies 1 and 2, main effects for genotype held even when mean negative affect was included as a covariate.

4 | DISCUSSION

Across two separate samples employing different emotional evocation tasks with social stimuli, C/C individuals consistently exhibited higher mean positive affect after baseline phases and following negatively valenced social manipulations than individuals with one or two T alleles. This work is the first to evaluate associations between the *DRD4* -521C/T SNP and emotional responses to social stimuli. Further, the present findings are consistent with evidence that *DRD4* polymorphisms are reliably associated with behavioral and neurophysiological shifts (Abrahams et al., 2019; Agam et al., 2014; Camara et al., 2010; Gizer et al., 2009; Munafò et al., 2008; Pérez-Rubio et al., 2017; Thomson et al., 2013), unlike other candidate genes that have encountered replication challenges (Border et al., 2019).

How the *DRD4* -521C/T SNP affects DRD4 transcription and/or expression remains uncertain. One study observed in vitro that the T allele reduced transcriptional activity by approximately 40% (Okuyama et al., 2000), but this finding has not been replicated. Nonetheless, functional brain

imaging studies (Agam et al., 2014; Camara et al., 2010) suggest this SNP impacts DRD4 translation or posttranslational modifications that affect receptor function, an outcome that is not mutually exclusive of an absence in mRNA level differences. Our findings here provide support for a functional effect of this polymorphism on DRD4 receptors, and provide the first evidence of an impact of the rs1800955 polymorphism on emotion processing in the presence of positively and negatively valenced social stimuli.

Individuals homozygous for the C allele consistently exhibited higher mean positive affect at baseline and during negative emotion manipulations compared to T carriers. By nature of such genotype comparisons, it is presently not possible to determine if T carriers are generally more negative under such emotionally evocative circumstances, or if C/C individuals might be more positive during ambiguous or negative social experiences. Regardless, the consistent observation of a genotype-specific discrepancy across two different studies, restricted to portions of each task that are neutral or negatively valenced, suggests this is a reproducible effect likely to have real world consequences on emotional responses. Indeed, prior research has demonstrated the explicit role of positive emotion in healthy adaptation to stress (Coifman et al., 2021). Considering the uncertainty and myriad adverse outcomes resulting from the ongoing COVID-19 pandemic, it could be enlightening to evaluate if the *DRD4* -521C/T SNP is associated with distinct perceptions regarding this global social stressor. Our study samples limit the generalizability of these findings, given they consist of only of undergraduate students, and both studies have majority Western Eurasian ancestry (see Supporting Information Discussion), non-Hispanic/Latino, female participants. Thus, larger investigations powered for stratification of different social identities and educational access are warranted to verify our results.

Though candidate gene studies as a group have become limited in the literature in favor of genome wide sequencing studies (Border et al., 2019), select polymorphisms exhibiting replicable effects remain worth investigating. Examinations of genetic interactions with environmental manipulations, such as with the *DRD4* -521C/T SNP and emotionally evocative social stimuli, facilitate identification of key molecular players in core behavioral responses known to drive health and psychological adaptation. Fundamental association studies like those presented here are necessary springboards for subsequent evaluations in preclinical rodent studies, as well as for more in-depth longitudinal assessments into how genetics shape neurophysiological processes and lifelong behavioral responses to changing environmental conditions.

AUTHOR CONTRIBUTIONS

Study design: AMJ, KGC; Data collection: TLG, AMJ, KGC; Data analysis & visualization: TLG, MTF, KGC; Manuscript preparation: TLG; Manuscript revisions: TLG, MTF, AMJ, KGC; Funding acquisition: AMJ, KGC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available privacy and ethical restrictions, as stipulated by the Kent State University Institutional Review Board.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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