**Supplementary Material for**:

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

**Running title**: *DRD4* genotype and sustained positive affect in presence of negative social cues

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**Supplementary Introduction**

While much *DRD4* polymorphism research has centered around impulsivity, addiction, and other externalized behaviors (for example, (Balestri et al., 2014)), 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). Such studies evaluating the DRD4 exon III VNTR predominantly focused on negative – but not positive – emotion manipulations (Gehricke et al., 2015; Roussos et al., 2009; Wells et al., 2013), or evaluated self-reported emotion regulation (Su et al., 2018) or positivity bias (Tompson et al., 2018) in the absence of an experimental manipulation. Collectively, these studies reported that the long (7 repeat) DRD4 exon III VNTR was associated with increased brain activity but decreased behavioral responsivity to negatively valenced stimuli unless previously primed by negative cues (Gehricke et al., 2015; Roussos et al., 2009; Wells et al., 2013). Further, such protections interact both with sociocultural and adverse life experiences (Su et al., 2018; Tompson et al., 2018).

**Supplementary Materials and Methods**

The present results are secondary data analyses of data collected between 2012-2015 for a parent project (Gilman et al., 2015; Latsko et al., 2016; Nylocks et al., 2018), 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. Genotypes for Study 1 (c2=8.65.5, p=0.013) and Study 2 (c2=7.64, p=0.022) were not within Hardy-Weinberg equilibrium as evaluated with a goodness of fit chi-square test, as has been observed for this polymorphism previously (Abrahams et al., 2019; Bhowmik et al., 2017). As others have indicated (Pérez-Rubio et al., 2017), disequilibrium of *DRD4* genotypes does not necessarily invalidate findings, particularly when observations are consistent across multiple samples as is the case here. Concern regarding the absence of Hardy-Weinberg equilibrium predominates when genes are associated with disease or risk (Schaid & Jacobsen, 1999; Trikalinos et al., 2006; Wittke-Thompson et al., 2005), unlike the present study in which the polymorphism is being associated with neither a disease nor a risk. Moreover, there is considerable variation in the reported allele frequency and dominance for rs1800955, not only across populations varying in nationality and/or social identities of race/ethnicity (Bookman et al., 2002; Huppertz et al., 2014; Mitaki et al., 2013; Okuyama et al., 1999, 2000), but also within research reports sampling from the same population (Thomson et al., 2013). Nonetheless, this disequilibrium should be kept in mind when assessing the present findings.

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). Where appropriate, Greenhouse-Geisser corrections were used for within-subjects analyses. 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).

**Supplementary Results**

Supplementary Table S1. Positive Emotions

|  |  |  |  |
| --- | --- | --- | --- |
| **Study 1 Film Clip** | **C/C** | **C/T or T/T** |  |
| *Baseline* | 3.60 ± 1.29 | 3.03 ± 1.17 |  |
| *The Road to Guantanamo* | 2.14 ± 0.85 | 1.85 ± 0.64 |  |
| *Alive* | 2.92 ± 1.36 | 2.57 ± 1.23 |  |
| *The Champ* | 2.39 ± 0.86 | 1.99 ± 0.66 |  |
| *Between Two Ferns* | 4.23 ± 1.19 | 3.91 ± 1.15 |  |
|  |  |  |  |
| Film Clip × Genotype | (F(3.31,391)=0.31, p=0.87, partial η2=0.003) | | |
| Film Clip | (F(3.31,391)=79.2, **p<0.001,** partial η2=0.40) | | |
| Genotype | (F(1,118)=7.20, **p=0.008,** partial η2=0.06) | | |
|  |  |  |  |
| **Study 2 Game** | **C/C** | **C/T or T/T** |  |
| *Neutral* | 3.11 ± 1.26 | 2.60 ± 1.07 |  |
| *Rejection* | 2.47 ± 1.14 | 1.99 ± 0.81 |  |
| *Acceptance* | 2.66 ± 1.22 | 2.31 ± 0.99 |  |
|  |  |  |  |
| Game × Genotype | (F(1.97,236)=1.03, p=0.36, partial η2=0.009) | | |
| Game | (F(1.97,236)=49.6, **p<0.001,** partial η2=0.29) | | |
| Genotype | (F(1,120)=5.30, **p=0.023,** partial η2=0.04) | | |
|  |  |  |  |

Means ± standard deviations (SDs) for positive emotions self-reported by participants after each film clip (Study 1) or Cyberball game (Study 2). Outcomes of repeated measures ANOVAs are reported for each Study, including Greenhouse-Geisser corrections for within-subjects analyses.

Supplementary Table S2. Negative Emotions

|  |  |  |  |
| --- | --- | --- | --- |
| **Study 1 Film Clip** | **C/C** | **C/T or T/T** |  |
| *Baseline* | 1.12 ± 0.25 | 1.05 ± 0.13 |  |
| *The Road to Guantanamo* | 3.01 ± 1.15 | 2.87 ± 1.25 |  |
| *Alive* | 1.28 ± 0.41 | 1.15 ± 0.28 |  |
| *The Champ* | 2.50 ± 0.80 | 2.18 ± 0.88 |  |
| *Between Two Ferns* | 1.17 ± 0.30 | 1.11 ± 0.33 |  |
|  |  |  |  |
| Film Clip × Genotype | (F(1.93,228)=0.62, p=0.54, partial η2=0.005) | | |
| Film Clip | (F(1.93,228)=155, **p<0.001,** partial η2=0.57) | | |
| Genotype | (F(1,118)=2.37, p=0.13, partial η2=0.02) | | |
|  |  |  |  |
| **Study 2 Game** | **C/C** | **C/T or T/T** |  |
| *Neutral* | 1.17 ± 0.56 | 1.14 ± 0.30 |  |
| *Rejection* | 1.30 ± 0.76 | 1.25 ± 0.51 |  |
| *Acceptance* | 1.12 ± 0.51 | 1.07 ± 0.17 |  |
|  |  |  |  |
| Game × Genotype | (F(1.35,162)=0.032, p=0.92, partial η2<0.001) | | |
| Game | (F(1.35,162)=10.4, **p<0.001,** partial η2=0.08) | | |
| Genotype | (F(1,120)=0.31, p=0.58, partial η2=0.003) | | |
|  |  |  |  |

Means ± SDs for negative emotions self-reported by participants after each film clip (Study 1) or Cyberball game (Study 2). Outcomes of repeated measures ANOVAs are reported for each Study, including Greenhouse-Geisser corrections for within-subjects analyses.

Supplementary Table S3. Comparison of positive emotion analyses between inclusive samples and samples restricted to those participants self-reporting as “white”.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study 1 Film Clip** | **All Participants** | **Mean Estimated Difference**  **(C/C – T carrier)** | **White Participants Only** | **Mean Estimated Difference**  **(C/C – T carrier)** |
| Film Clip × Genotype | (F(3.31,391)=0.31  p=0.87  partial η2=0.003) | 0.386  95% CI: 0.101−0.670 | (F(3.31,308)=0.24  p=0.89  partial η2=0.003) | 0.258  95% CI:  -0.049−0.565 |
| Film Clip | (F(3.31,391)=79.2  **p<0.001**  partial η2=0.40) | (F(3.31,308)=67.1  **p<0.001**  partial η2=0.42) |
| Genotype | (F(1,118)=7.20  **p=0.008**  partial η2=0.06) | (F(1,93)=2.78  p=0.099  partial η2=0.03) |
|  |  |  |  |  |
| **Study 2 Game** |  |  |  |  |
| Film Clip × Genotype | (F(1.97,236)=1.03  p=0.36  partial η2=0.009) | 0.448  95% CI: 0.063−0.834 | (F(1.95,189)=0.89  p=0.41  partial η2=0.009) | 0.288  95% CI:  -0.142−0.718 |
| Film Clip | (F(1.97,236)=49.6  **p<0.001**  partial η2=0.29) | (F(1.95,189)=43.6  **p<0.001**  partial η2=0.31) |
| Genotype | (F(1,120)=5.30  **p=0.023**  partial η2=0.04) | (F(1,97)=1.77  p=0.19  partial η2=0.018) |
|  |  |  |  |  |

Study 1: 25 participants removed (resulting N=95). Study 2: 16 participants removed (resulting N=99). These analyses restricted to participants that self-reported as “white” were performed at the request of the journal. Per journal instructions, participants that self-reported as “white” were re-labeled according to journal instructions as having “Western Eurasian” biogeographic ancestry.

Supplementary Table S4. Comparison of negative emotion analyses between inclusive samples and samples restricted to those participants self-reporting as “white”.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study 1 Film Clip** | **All Participants** | **Mean Estimated Difference**  **(C/C – T carrier)** | **White Participants Only** | **Mean Estimated Difference**  **(C/C – T carrier)** |
| Film Clip × Genotype | (F(1.93,228)=0.62  p=0.54  partial η2=0.005) | 0.142  95% CI:  -0.041−0.324 | (F(1.88,174)=0.64  p=0.52  partial η2=0.007) | 0.155  95% CI:  -0.046−0.355 |
| Film Clip | (F(1.93,228)=155  **p<0.001**  partial η2=0.57) | (F(1.88,174)=127  **p<0.001**  partial η2=0.58) |
| Genotype | (F(1,118)=2.37  p=0.13  partial η2=0.02) | (F(1,93)=2.34  p=0.13  partial η2=0.03) |
|  |  |  |  |  |
| **Study 2 Game** |  |  |  |  |
| Film Clip × Genotype | (F(1.35,162)=0.032  p=0.92  partial η2<0.001) | 0.043  95% CI:  -0.108−0.193 | (F(1.60,155)=2.40  p=0.11  partial η2=0.024) | -0.033  95% CI:  -0.197−0.131 |
| Film Clip | (F(1.35,162)=10.4  **p<0.001**  partial η2=0.08) | (F(1.60,155)=8.63  **p=0.001**  partial η2=0.082) |
| Genotype | (F(1,120)=0.31  p=0.58  partial η2=0.003) | (F(1,97)=0.16  p=0.69  partial η2=0.002) |
|  |  |  |  |  |

Study 1: 25 participants removed (resulting N=95). Study 2: 16 participants removed (resulting N=99). These analyses restricted to participants that self-reported as “white” were performed at the request of the journal. Per journal instructions, participants that self-reported as “white” were re-labeled according to journal instructions as having “Western Eurasian” biogeographic ancestry.

**Supplementary Discussion**

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), whereas another *in vitro* study reported no effects of the SNP on mRNA levels (Kereszturi et al., 2006). An investigation of human post-mortem brain *DRD4* mRNA levels indicated no influence of the SNP (Simpson et al., 2010). Despite inconsistent evidence regarding transcriptional effects of this promoter polymorphism, functional brain imaging studies (Agam et al., 2014; Camara et al., 2010) suggest this SNP impacts DRD4 translation or post-translational modifications that affect receptor function, an outcome that is not mutually exclusive of an absence in mRNA level differences.

Our Study samples limit the generalizability of the present results, given they consist of only of undergraduate students, and both Studies have majority Western Eurasian ancestry, non-Hispanic/Latino, female participants. However, the allele frequencies in our Study 1 (C=0.41, T=0.59) and Study 2 (C=0.46, T=0.54) samples are identical to those reported in participants from Japan (Okuyama et al., 1999) and Canada (Thomson et al., 2013), respectively. As mentioned in our Methods section, reported allele frequency and dominance for rs1800955 varies considerably (C:T of 0.41:0.59 (Okuyama et al., 1999), 0.55:0.45 (Bookman et al., 2002), 0.43:0.57 (Huppertz et al., 2014), and 0.54:0.46-0.46:0.54 (Thomson et al., 2013) in Japanese, Sub-Saharan, Netherlands Twin Register, and Western Eurasian Canadian participants, respectively), so further investigations of populations consisting of different social identities and educational access are certainly warranted.

Because our study samples included participants from multiple biogeographic ancestral origins, and given the possibility that there may be biogeographic ancestry differences in rs1800955 allele frequencies, it is possible that our findings are vulnerable to Type I error. To assess this possibility, on the advice of the journal’s editors, we reanalyzed our data after restricting our sample to only those individuals who self-identified as “white” (Study 1: 25 participants excluded, N=95 remaining; Study 2: 16 participants excluded, N=99 remaining). The outcomes of these analyses are presented in Supplementary Tables S3 and S4. Estimated mean differences for the groups became smaller but remained intact in terms of their direction for positive emotion. If one compares this to the consistent null effects and differences in means for negative emotion, it suggests the effects for positive emotion are stable, even with a markedly smaller sample. These outcomes suggest that biogeographic ancestral differences are not contributing to a false positive interpretation, but of course, additional studies with the power to directly investigate this possibility are needed to determine if our findings are replicable.

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