DRD4 and striatal modulation of the link between childhood behavioral inhibition and adolescent anxiety

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Behavioral inhibition (BI), a temperament characterized by vigilance to novelty, sensitivity to approach-withdrawal cues and social reticence in childhood, is associated with risk for anxiety in adolescence. Independent studies link reward hyper-responsivity to BI, adolescent anxiety and dopamine gene variants. This exploratory study extends these observations by examining the impact of DRD4 genotype and reward hyper-responsivity on the BI-anxiety link. Adolescents (N = 78) completed a monetary incentive delay task in the fMRI environment. Participants were characterized based on a continuous score of BI and the 7-repeat allele (7R+) of the DRD4 functional polymorphism. Parent-report and self-report measures of anxiety were also collected. Across the entire sample, striatal activation increased systematically with increases in the magnitude of anticipated monetary gains and losses. DRD4 status moderated the relation between BI and activation in the caudate nucleus. Childhood BI was associated with parent report of adolescent anxiety among 7R+ participants with elevated levels of striatal response to incentive cues. DRD4 genotype influenced the relations among neural response to incentives, early childhood BI and anxiety. The findings help refine our understanding of the role reward-related brain systems play in the emergence of anxiety in temperamentally at-risk individuals, building a foundation for future larger scale studies.

Keywords: fMRI; caudate; reward; genetics; temperament

INTRODUCTION

Behavioral inhibition (BI), a temperament identified in early childhood, is marked by an early-emerging and persistent tendency to display fear to novelty (Fox et al., 2005), social reticence (Garcia Coll et al., 1984; Coplan et al., 1994; Fox et al., 2005) and sensitivity to approach–avoidance cues (Fox et al., 2008). Children with stable, high levels of BI exhibit increased risk as adolescents for anxiety disorders (Schwartz et al., 1999; Prior et al., 2000; Kagan et al., 2001; Chronis-Tuscano et al., 2009), relative to non-behaviorally inhibited children. Similarities between BI and anxiety (Pérez-Edgar and Fox, 2005) are also evident at the neural level (Schwartz et al., 2003; Schwartz and Rauch, 2004; McClure et al., 2007; Pérez-Edgar et al., 2007), suggestive of common neural mechanisms underlying the phenotypic link between BI and anxiety.

Individuals with a history of BI also display unique patterns of striatal response to anticipated gains and losses (Guyer et al., 2006; Bar-Haim et al., 2009; Helfinstein et al., 2011, 2012). In a monetary incentive delay (MID) task, adolescents with a history of BI showed striatal hyper-reactivity to incentive cues relative to non-BI peers (Guyer et al., 2006). In a related task, this hyper-reactivity was specifically evident when incentive delivery was contingent on performance (Bar-Haim et al., 2009). In parallel, adolescents with social anxiety disorder (SAD) showed a similar pattern of hyper-reactivity to incentives, relative to adolescents with generalized anxiety disorder (GAD) and healthy adolescents (Guyer et al., 2012), suggesting that comparable alterations in striatal response to incentives may be at play for both BI and SAD. Although BI imparts an elevated risk for SAD (Chronis-Tuscano et al., 2009), most BI children do not manifest this disorder in adolescence (Degnan and Fox, 2007; Fox and Pine, 2012). Instead, there is often a broad pattern of elevated anxiety symptoms or internalizing difficulties across this generally healthy population (Williams et al., 2009). Thus, it is important to delineate factors that modulate the BI–anxiety link in order to eventually identify individuals more vulnerable to developing an anxiety disorder. Differential developmental trajectories are likely shaped by multiple mechanisms operating across a wide spectrum from genes to environment. In order to lay the foundation for future large-scale research and refine the targets of subsequent studies, the current exploratory work examines a genetic marker previously linked to both temperament and reward reactivity that may help better understand the relation between early BI and later anxiety.

Dopamine neurotransmission plays a fundamental role in reward functioning (Luciana et al., 2012) and can be indexed by dopamine-related genes. To date, the exon III polymorphism of the dopamine receptor D4 subtype (DRD4) has received the greatest empirical support, in terms of a specific gene associated with temperament-linked individual differences (Schmidt et al., 2007, 2009). In addition, DRD4 gene variants are directly linked to individual variations in reward sensitivity (Marco-Pallares et al., 2009; Camara et al., 2010). Specifically, the 7-repeat, long-allele variant of DRD4 (7R+) predicts weaker transmission of intracellular dopamine signals (Robbins and Everitt, 1999), and 7R+ carriers have been shown in previous work to display increased reward-related ventral striatum reactivity (Forbes et al., 2009) and risk for substance use in adolescence (Ellis et al., 2011). Tying these areas of research together, we also found that adolescents with a history of BI show higher levels of substance use...
Adolescence is a unique developmental window marked by peak incidence of psychopathology, particularly anxiety and substance use (Steinberg et al., 2006; Paus et al., 2008). Neurodevelopmental models (Ernst and Fudge, 2009; Somerville and Casey, 2010) suggest that this observed developmental pattern may be due to acute limbic and striatal sensitivity to salient stimuli, coupled with relatively immature regulatory mechanisms centered in the prefrontal cortex. This model suggests that the adolescent period may be particularly sensitive to differential gene effects on reward processing. Thus, this study, via its focus on adolescents, is well positioned to examine the contribution of dopamine gene effects and the neural correlates of reward sensitivity to previously observed patterns of socioemotional functioning in individuals with a history of BI.

Taken together, the literature suggests that the DRD4 gene might modulate previously seen links between BI and striatal hyper-reactivity. Striatal hyper-reactivity to incentives, in turn, has been documented in socially anxious adolescents (Guyer et al., 2012), further implicating this response pattern in the BI-anxiety association. Thus, the current exploratory study examines the inter-relations among striatal reactivity to incentives, BI and DRD4 variants. It also examines the potential impact of striatal response and gene variants on anxiety levels in an at-risk sample.

We predict that (i) BI will be associated with striatal hyper-reactivity to incentive cues; (ii) this effect will be most pronounced in adolescents with the 7R+ allele and (iii) DRD4 status and striatal response to incentives will moderate the BI-anxiety relation.

**METHODS**

**Participants**

We recruited 78 adolescents (mean age = 16.33, s.d. = 2.83) from a larger longitudinal study of BI who had available DRD4 genotype and fMRI data (Tables 1 and 2). Data from subsets of the current participants have been published in previous studies involving the MID task (Guyer et al., 2006; Lahat et al., 2012).

Initially, 433 four-month-old infants were screened for reactivity to novel sensory stimuli; 153 infants were retained based on their scores (Fox et al., 2001). Individual differences in BI were assessed at 14 and 24 months and individual differences in social reticence were observed at 4 and 7 years of age. Laboratory assessments at 14 and 24 months presented children with novel and unfamiliar objects and people (Fox et al., 2001). At 4 and 7 years, children’s reticent behavior with unfamiliar peers was measured using Rubin’s Play Observation Scale (Rubin, 1989). Maternal ratings of their child’s social fear were collected at 14 and 24 months with the Toddler Behavior Assessment Questionnaire (Goldsmith, 1996). Mothers rated their child’s shyness at ages 4 and 7 years with the Colorado Child Temperament Inventory (Rowe and Plomin, 1977).

Individual behavioral and maternal report scores across the four testing waves were taken from the entire cohort (regardless of participation in this study). Past research has shown that high, stable levels of BI are most closely linked to elevated levels of anxiety (Chronis-Tuscano et al., 2009). Thus, we standardized and then averaged the individual Z-scores to create a composite measure of BI (mean = −0.008, s.d. = 0.641, range = −1.33 to 2.82). To capitalize on our large sample and examine the broad spectrum of BI, we used the continuous scores in our analyses below.

The participants included in this study were representative of the larger cohort with respect to mean BI scores overall (−0.04 for participants vs 0.02 for the cohort, P = 0.53).

Participants and their parents provided written informed assent/consent and were compensated for their participation.

**DRD4 genotyping**

Genomic DNA was prepared from saliva samples collected in the laboratory using Oragene®DNA kits (DNA Genotek, Ottawa, Ontario, Canada). DRD4 48-bp VNTR Polymorphism in exon 3 was amplified from 20 ng genomic DNA using the primer sequences: forward 5′- (GACCGGACTAGTTGCTTG)-3′ and reverse 5′- (CTCTTGACGCTGCGCCG)-3′ (Monuteaux et al., 2008).

Due to the high GC content in the VNTR region, amplification was performed using GC-rich PCR system (Roche Applied Science, 68298 Mannheim, Germany). PCR conditions were 98°C (3 min), 40 cycles of 98°C (30 s), 60.5°C (30 s), 68°C (45 s) and a final elongation, 68°C (10 min). The forward primer was labeled with the fluorescent dye 6-FAM, amplicons were visualized with GeneScan-1200 LIZ Size Standard (Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI 3730 capillary sequencer. Allele sizes (allele 2, 404 bp; allele 3, 452 bp; allele 4, 500 bp; allele 5, 548 bp; allele 6, 596 bp; allele 7, 642 bp; allele 8, 692 bp; allele 9, 740 bp; allele 10, 785 bp and allele 11, 836 bp) were determined using GeneMapper v4.0 (Applied Biosystems).

Genotyping accuracy was determined empirically by duplicate genotyping of 25% of the samples selected randomly. The error rate was less than 0.005, and the completion rate was greater than 0.95. Genotype distributions for the full cohort, including adolescents not retained for the final analyses presented, were 2R/7R (N = 8, 6.0%), 3R/7R (N = 1, 0.7%), 4R/7R (N = 42, 31.3%), 7R/7R (N = 7, 4.7%), 7R/8R (N = 1, 0.7%), 2R/2R (N = 1, 0.7%), 2R/4R (N = 15, 11.2%), 3R/4R (N = 7, 5.2%), 4R/4R (N = 52, 38.8%) and 4R/5R (N = 1, 0.7%). The distribution was in line with the Hardy–Weinberg equilibrium (χ² = 1.65, P = 0.20).

For the final sample, participants were classified as 7-repeat carriers (7R+; N = 32; 25 heterozygous and 7 homozygous) or non-carriers.

**Table 1** Means (s.d.) of demographic and temperament measures for study participants

<table>
<thead>
<tr>
<th>Study Measures</th>
<th>All subjects</th>
<th>7R+</th>
<th>7R-</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>16.33 (2.84)</td>
<td>15.75+ (2.69)</td>
<td>16.84+ (2.80)</td>
<td>0.173</td>
</tr>
<tr>
<td>Gender</td>
<td>3/40</td>
<td>16/16</td>
<td>21/25</td>
<td>0.169</td>
</tr>
<tr>
<td>IQ</td>
<td>114.20 (10.68)</td>
<td>116.30 (9.95)</td>
<td>112.73 (11.14)</td>
<td>0.072</td>
</tr>
<tr>
<td>SCARED parent</td>
<td>9.38 (9.91)</td>
<td>9.72 (9.84)</td>
<td>9.25 (9.62)</td>
<td>0.243</td>
</tr>
<tr>
<td>SCARED child</td>
<td>14.55 (10.99)</td>
<td>14.48 (11.39)</td>
<td>14.69 (10.84)</td>
<td>0.086</td>
</tr>
<tr>
<td>BI composite</td>
<td>−0.04 (0.70)</td>
<td>−0.02 (0.66)</td>
<td>−0.04 (0.73)</td>
<td>—</td>
</tr>
<tr>
<td>N</td>
<td>78</td>
<td>32</td>
<td>46</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are presented for the full sample in the first column, followed separately for DRD4 genotype (7R+, 7R-) in the next two columns. The final column presents the correlation between BI score and the row variable. Gender = male/female. *P < 0.10, **P < 0.05.
Anxiety

BI is associated with increased risk for anxiety disorders (Chronis-Tuscano et al., 2009) and our previous work has linked SAD with an enhanced caudate and putamen response to incentives during the MID task (Guyer et al., 2012). Thus, we examined participants’ anxiety based on parent-report and self-report of anxiety on the Screen for Child Anxiety Related Emotional Disorders-Revised (SCARED-R) (Muris et al., 1999) concurrent with their neuroimaging scan. This study focused on a broad-band anxiety scale because of the low incidence of identified diagnoses, including social anxiety, in the current sample (see below). In addition, we wished to avoid the increased risk of generating Type I errors by running multiple analyses on the five instrument-specific subscales.

Analyses were run separately for the parent and child report scores. Although correlated, $r = 0.59$, $P < 0.001$, prior work has noted discrepancies between informants’ reports (Edelbrock et al., 1986; Salbach-Andrae et al., 2009), particularly in adolescence. Anxiety scores were not associated with age, IQ or sex ($Ps > 0.11$).

Diagnostic status. Experienced clinicians assessed participants using the Schedule for Affective Disorders and Schizophrenia for School Aged Children—Present and Lifetime Version (Kaufman et al., 1997) or the Structured Clinical Interview for DSM Disorders (Spitzer et al., 1992), depending on participant age. Twenty-one participants met criteria for one or more current or past Axis I diagnoses (five SAD, three GAD, seven attention deficit hyperactivity disorder, two tic disorder, two adjustment disorder, one post-traumatic stress disorder, two specific phobia, one major mood disorder and two enuresis). Of these, eight were diagnosed with an anxiety disorder (SAD, GAD or both).

Participants with an anxiety diagnosis ($N = 8$), participants with another diagnosis ($N = 13$) and healthy participants ($N = 57$), were equivalent on all measures ($Ps > 0.09$), except for BI and parent-report SCARED scores. Individuals with current/past anxiety diagnoses had higher BI ($0.21 \pm 0.47$ vs $−0.12$), $F(2,73) = 3.52$, $P = 0.03$, $f = 0.31$, and higher parent-report SCARED ($18.88 \pm 6.29$ vs $6.53$), $F(2,73) = 7.36$, $P = 0.002$, $f = 0.45$, scores vs participants with a non-anxiety diagnosis or no diagnosis, respectively. A similar, albeit non-significant, pattern emerged for self-report SCARED scores ($21.25 \pm 11.93$ vs $11.57$), $F(2,73) = 2.75$, $P = 0.07$, $f = 0.27$. Excluding individuals with current/past diagnoses did not change the pattern of findings presented below; they were therefore retained in the final analyses.

MID task

The task consisted of two runs of 72 contiguous 6 s trials involving presentation of a cue (250 ms), followed by a variable delay (2000–2500 ms) displaying a crosshair point, then a response target (160–260 ms), and finally outcome information (1650 ms) (Figure 1). Three cue types established trial condition: circle cues ($n = 64$) indicated potential monetary gain; square cues ($n = 64$) indicated potential loss and triangle cues ($n = 16$) indicated no incentive (neutral). Magnitude of the incentive value was conveyed via lines within the circle or rectangle: single line for small incentive ($$0.20$; $n = 32$), two lines for medium incentive ($$1; $n = 32$) or three lines for high incentive ($$$5; $n = 32$). Correct responses made during target presentation yielded the anticipated gain or avoidance of potential loss. Participants were notified of their current and cumulative dollar amount. Trial type order was randomized. Participants were told that they would receive a percentage of the dollar amount won.

Participants completed a practice MID task, providing an estimate of their reaction time (RT). Task difficulty was standardized to an $\sim 66\%$ success rate by adjusting target duration (five difficulty levels) to minimize potential confounds from large performance differences across participants. After the task, participants rated each of the cues (large, medium and small potential gains and equivalent potential losses) on a scale from $−5$ (dislike very much) to $+5$ (like very much).

Behavioral data analysis

Dependent variables included mean difficulty level, accuracy, RT and post-scan ratings. Repeated-measures analyses of covariance (ANCOVAs) employed DRD4 group and continuous BI score, with valence and incentive magnitude as within-subjects factors. To parallel the imaging analyses, which used $0$ trials as a baseline, RTs to $0$ cues were also included in the models as a covariate.

fMRI acquisition

Scanning occurred in a Signa 3 tesla scanner (General Electric, Waukesha, WI, USA) for the first 64 participants. For the final 14 participants, scanning occurred in a GE 3 tesla Excite HDX scanner. Both groups used the same GE head coil. The two scanning groups did not differ on major demographic or research factors ($Ps > 0.11$), other than age ($P < 0.01$). To assess for any potential impacts of scanner, we first carried out our analyses (below) with scanner type as a between-subjects factor and again as a covariate. We found no significant effect of scanner on blood oxygen level-dependent (BOLD) signal ($P > 0.12$). We then replicated the reported analyses with only the first 64 participants. One critical finding for this study was a three-way BI-by-DRD4-by-incentive interaction (below), which remained significant ($P = 0.03$) in the participants studied on the initial scanner. The equivalent analyses in the remaining 14 participants could not be completed due to sample size.

A Cedrus Lumina response box (Psychological Software Tools, San Pedro, CA, USA) recorded behavioral data. Task stimuli were projected onto a screen at the foot of the scanner bed and viewed with mirrors mounted on the head coil. Foam padding constrained head movement.

Each brain volume consisted of 30 interleaved slices, 4-mm thick, acquired in the sagittal plane using a T2*-weighted echo-planar sequence with a repetition time (TR) of 2500 ms, echo time (TE) of $23$ ms and flip angle of $90^\circ$. Voxel dimension was $3.75 \times 3.75 \times 4.0$ mm. Matrix size was $64 \times 64$, and field of view...
(FOV) was 24 cm. To allow for signal stabilization, four acquisitions were obtained before task onset. A high-resolution structural image was also acquired for each subject using a T1-weighted standardized magnetization prepared spoiled gradient recalled echo sequence: 124 1-mm slices, 8100 ms TR, 32 ms TE, 15° flip angle, 256 x 256 matrix and 24 cm FOV.

**fMRI data analysis**

Analysis of functional and neural images (AFNI) software was used for the analyses (Cox, 1996). As with the behavioral data, error trials were removed from processing. Standard preprocessing of echo-planar data included slice time correction, motion correction and spatial smoothing with a 6-mm full-width half-maximum smoothing kernel. Signal deviations >2.5 s.d. from the mean were removed using an AFNI despiking algorithm applied on a voxel-wise basis and a band-pass filtering algorithm to remove cyclical fluctuations in signal (either >0.01 or <0.15 Hz) not temporally indicative of a hemodynamic response. We then normalized BOLD signal intensity to percentage signal change using each subject’s voxel-wise time series mean as a baseline.

Preprocessed time series data for each individual were analyzed by multiple regression (Neter et al., 1996). In line with previous work with the task (Guyer et al., 2006, 2012), the regression model included both cues and feedback, although only the cue period was analyzed for this study. Regressors modeled effects attributable to residual motion (using the motion correction factors in the x, y and z planes and in the yaw, pitch and roll dimensions), as well as baseline and linear trends for each of the two runs. Regressors of interest included cues signaling trial type (e.g., large, medium and small potential gains and equivalent potential losses) and were convolved with a gamma variate function that modeled a prototypical hemodynamic response (Cohen, 1997). Idealized signal time courses were estimated based on onset time of different event types during the task.

The first analysis examined the effects of BI and DRD4 on BOLD signal during the MID task. The second set of analyses treated anxiety levels of BOLD activity in this region (peak t = -4.18).

**BI and DRD4 modulation of striatal activity**

Contrasts of BOLD activation were created for each subject for cues signaling magnitude of (i) large, (ii) medium and (iii) small vs no incentive. Our main analysis predicting levels of BOLD activity targeted the interaction of BI score with DRD4 group on striatal activation in response to incentive anticipation. Based on our a priori hypothesis focused on striatal activation, and our previous findings with this task (Guyer et al., 2006, 2012), we used a region of interest (ROI) approach to assess bilateral caudate (body and head), putamen and nucleus accumbens activation. Previous BI and anxiety studies (Guyer et al., 2006, 2012; Bar-Haim et al., 2009; Helfenstein et al., 2011) have found varying patterns of results across ROIs. Therefore, we examined each ROI separately.

ROIs were defined by anatomical boundaries provided by AFNI after spatial normalization (Talairach and Tournoux, 1988), again in line with previous work with this task (Guyer et al., 2006, 2012). Specifically, we extracted values from the ROIs to reflect the main effects of task for our contrasts of interest (large, medium and small vs no incentive). Thus, three contrast values were generated per subject for each ROI, thereby minimizing Type I errors associated with multi-voxel testing.

Individual contrast values were extracted from the ROIs and entered into group-level repeated-measures ANCOVA performed in SPSS (SPSS 19.0, Chicago, IL, USA). The initial model included DRD4 group (7R+, 7R−) and BI score as the main predictors. Incentive magnitude (small, medium and large), valence (gain and loss) and laterality (right and left) served as within-subjects factors. To minimize Type I errors, the Greenhouse-Geisser (G–G) procedure was applied when appropriate. The degrees of freedom indicated in the text are those before the G–G correction. However, epsilon (ε) is noted when less than 1.0.

The initial analyses revealed only the expected linear main effect of incentive level in the nucleus accumbens, F(1,73) = 7.38, P = 0.008, d = 0.64, ε = 0.84. Similarly, for the putamen, there were linear main effects of incentive level, F(1,73) = 22.84, P < 0.001, d = 1.12, ε = 0.93, and valence, F(1,73) = 5.54, P = 0.02, d = 0.55, with greater activation for gain cues than loss cues. As neither was associated with either BI or DRD4 genotype, they were not included in the full analyses.

The central findings for the caudate are presented below. Our focus on the caudate was also confirmed by a whole-brain analysis (threshold of P = 0.001 with a 10-voxel-size minimum) which found significant activation in this region (peak t = -4.18).

**Predicting anxiety levels with BI, DRD4 and caudate activation**

To test hypothesized DRD4 genotype and caudate activation modulation of the BI–anxiety link, we regressed SCARED scores in adolescence on early-childhood BI score (continuous), DRD4 (7R+, 7R−) and adolescent BOLD activation at each incentive level (high, medium and low) in the caudate. Continuous predictive measures were mean centered before use in the regressions. The regressions were run separately for the parent-report and self-report SCARED scores.

Predictors were initially entered into a hierarchical linear regression in six sequential blocks: (i) scanner, age and task difficulty level; (ii) BI, DRD4, high, medium and low; (iii) BI-by-DRD4; (iv) BI-by-high, BI-by-medium and BI-by-low; (v) DRD4-by-high, DRD4-by-medium and DRD4-by-low and (vi) BI-by-DRD4-by-high, BI-by-DRD4-by-medium and BI-by-DRD4-by-low. None of the predictors in Step 1 contributed significantly to the model, as such they were removed from the subsequent analysis presented here. Given the large number of predictors, we then completed a backward elimination procedure, removing predictors failing to reach probability cut-offs, in order to create a more parsimonious model for comparison. As a final confirmatory analysis, we repeated the model using 5000 bootstrapping samples.

**RESULTS**

**Behavioral data**

**Post-scan ratings of likeability**

Behavioral data acquired after scanning demonstrated the expected patterns of performance, confirming that adolescents understood cue manipulations. Both incentive values and valence influenced the post-scan ratings, F(2,146) = 6.94, P < 0.001, f = 0.94, which increased linearly from a large potential loss to a large potential win.

There was a significant BI by valence interaction, F(1,73) = 5.76, P = 0.02, f = 0.28, such that BI was positively associated with ratings of loss cues (r = 0.32, P = 0.007) and negatively associated with ratings of gain cues (r = -0.30, P = 0.012).

**Task performance scores**

BI was positively associated with difficulty level, F(1,70) = 5.58, P = 0.02, f = 0.28. As such, we initially included difficulty level as a nuisance covariate for all analyses. There were no significant effects involving this covariate for DRD4, P = 0.24.

Performance did not differ across BI scores or DRD4 groups on RTs (Ps > 0.10) or accuracy (Ps > 0.09). Thus, with the individualized task
parameters, the groups attained comparable levels of success for both the gain and loss trials.

**fMRI data**

**BI and DRD4 modulation of caudate activity**

The initial hypothesis was tested in a multi-factorial omnibus ANCOVA. Laterality and valence showed no significant effects. In addition, age, sex and difficulty level had no significant impact when included as covariates. We removed non-significant factors and the presented analysis is restricted to the three main factors of interest: incentive, BI and DRD4.

As expected, caudate activity varied by incentive value, indicating a linear increase in caudate activation with increased monetary value (0.050 vs 0.099 vs 0.126), \( F(1,73) = 27.60, P < 0.001, d = 1.23, \varepsilon = 0.85. \) We also found a three-way incentive-by-BI-by-DRD4 interaction, \( F(1,73) = 7.26, P = 0.009, d = 0.63. \) The finding is illustrated in Figure 2 using the GroupAna procedure within AFNI (Cox, 1996).

To interpret the three-way interaction, we decomposed the initial ANCOVA and repeated the analysis separately for the DRD4 groups. For the 7R+ group, we found a significant BI-by-incentive interaction, \( F(1,29) = 4.10, P = 0.05, d = 0.75. \) There was little relation between BI and caudate activity for the high (\( r = 0.01, P = 0.95 \)) and low (\( r = 0.03, P = 0.86 \)) incentive cues. The significant interaction was likely driven by the BI-activation relation for medium incentives, although the individual correlation was non-significant, \( r = 0.28, P = 0.13. \) In contrast, the 7R− group showed no significant interaction, \( F(1,44) = 2.41, P = 0.13, d = 0.47. \)

Unlike our initial BI study (Guyer et al., 2006), the main effect of BI on striatal activation was not significant in the initial omnibus ANCOVA, \( P = 0.80 \) (see also Table 2). This may be due to the fact that the original study employed an extreme-groups approach, while here we relied on a larger, broader sample of the BI distribution. The inclusion of participants with a wide range of scores on BI may have diluted direct temperament-related differences, thus suggesting the need to closely examine potential moderators.

**Predicting anxiety levels with BI, DRD4 and caudate activation**

We first regressed parent report SCARED scores on childhood BI scores, DRD4 genotype group and adolescent BOLD activity at each incentive level (Table 3). The full model accounted for 35.5% of the total variance, \( F(15,74) = 2.16, P = 0.018. \) Although there were multiple significant contributors, the highest order predictors were the BI-by-DRD4-by-low interaction, the BI-by-DRD4-by-medium interaction and the BI-by-high interaction. Given the large number of predictors relative to sample size in the initial regression, we then repeated the analysis using a backward removal process. The three higher order predictors were retained in the final model. Finally, we completed a confirmatory analysis with 5000 bootstrapping samples, again confirming three core predictors, as each was found to be significant and the 95% confidence intervals did not include zero. We then examined the individual contributions of these three predictors to the model.

The BI-by-DRD4-by-low interaction accounted for 8.3% of the variance. The zero-order correlations between BI and SCARED scores were then calculated separately by DRD4 group and activation level (median split creating strong-BOLD and weak-BOLD groups). For adolescents with 7R+ and strong-BOLD activation, BI predicted anxiety, \( r = 0.59, P = 0.04 \) (Figure 3). In contrast, the relation was non-significant for adolescents with 7R+ and weak-BOLD activation, \( r = 0.46, P = 0.08. \) For 7R−, neither BOLD group showed a significant relation, \( r < 0.07, P > 0.75. \)

The BI-by-DRD4-by-medium interaction accounted for 4.9% of the variance. Again, the zero-order correlation between BI and SCARED scores was significant, \( r = 0.60, P = 0.02, \) for adolescents with 7R+ and strong-BOLD activation. The relation was non-significant for the 7R−/ 0.85. We also found a three-way incentive-by-BI-by-DRD4 interaction, \( F(1,73) = 7.26, P = 0.009, d = 0.63. \) The finding is illustrated in Figure 2 using the GroupAna procedure within AFNI (Cox, 1996).

The second regression used self-report SCARED scores as the outcome measure. In the end, the model did not reach significance, \( F(15,74) = 1.01, P = 0.46. \) To compare with the parent report regression, we examined the three interactions that emerged in that analysis. Together, they accounted for only 7.4% of the total variance, \( F(3,74) = 1.81, P = 0.15. \)

In summary, this analysis revealed that BOLD response to high levels of reward modulated the relation between early BI and anxiety in adolescence, independently of DRD4 allelic status. However, for low and medium magnitude incentives, the relation between BI and anxiety emerged only for individuals with both a large striatal response and the 7R+ DRD4 allele.

**DISCUSSION**

The current exploratory study examined the relations between early childhood BI and neural sensitivity to anticipated reward in adolescence. Based on the central role of dopamine in reward processing (Luciana et al., 2012) and its previous association with temperament-linked behaviors (Schmidt et al., 2007, 2009; Ellis et al., 2011), we examined whether the BI-reward processing association was modulated by variations in DRD4 allelic status. Finally, given the phenotypic link between BI and anxiety (Pérez-Edgar and Fox, 2005; Fox and Pine, 2012) and recent neuroimaging work showing parallel patterns of neural functioning during the MID task for behaviorally inhibited and anxious adolescents (Guyer et al., 2006, 2012), we examined whether the BI-anxiety link was modulated by variations in DRD4 and the neural response to reward.

This study examines the moderating effects of allelic status on neural activation associated with both temperament and reward processing. It also examines the relation between neural activation and anxiety symptomatology within a sample selected for early temperamental variations. Thus, these data examine the functional significance of the reward-related variations seen across previous studies of temperament (Guyer et al., 2006; Bar-Haim et al., 2009; Helfinstein et al., 2011). These preliminary results will allow future larger scale studies to more effectively target individuals most vulnerable to anxiety and suggest mechanisms that can modulate the long-term consequences of early temperament.

No relations were observed between BI and striatal activation when relying on continuous scores in our full sample. The participants in our study were characterized based on BI and social reticence scores derived from multiple laboratory and questionnaire-based observations over a 7-year period. This continuous score and our large sample reflected a broad spectrum of BI, suggesting that our previous finding directly linking BI to heightened striatal activation is not distributed linearly across the population. Debate within the literature has centered on whether temperament is best characterized as gradual variation across a broad spectrum or discrete shifts in functioning across qualitatively distinct subgroups (Rothbart, 2004; Kagan et al., 2007). The current data suggest that while extreme groups that reflect the prototypic markers of a trait may show significant differences in an
A significant interaction pattern was evident for the medium cues (peak differences were found at Talairach coordinates $-5, -16, 0, P < 0.001$).

**Fig. 2** Changes in BOLD signal for the BI by $DRD4$ (7R+; 7R–) interaction using the GroupAna procedure in AFNI. Activation patterns are presented separately for the small, medium and large incentive cues.

**Table 2** Correlations between the central measures and outcomes incorporated in this study

<table>
<thead>
<tr>
<th>Predictor</th>
<th>BI</th>
<th>DRD4</th>
<th>SCARED parent</th>
<th>SCARED child</th>
<th>High incentive</th>
<th>Medium incentive</th>
<th>Low incentive</th>
</tr>
</thead>
<tbody>
<tr>
<td>BI</td>
<td>0.02</td>
<td></td>
<td>0.11</td>
<td>0.14</td>
<td>0.58**</td>
<td>0.72**</td>
<td>0.31</td>
</tr>
<tr>
<td>DRD4</td>
<td></td>
<td>0.02</td>
<td>0.11</td>
<td>0.14</td>
<td>0.58**</td>
<td>0.72**</td>
<td>0.31</td>
</tr>
<tr>
<td>SCARED parent</td>
<td>0.24*</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED child</td>
<td>0.09</td>
<td>-0.01</td>
<td>0.59**</td>
<td>0.07</td>
<td>0.77**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High incentive</td>
<td>-0.01</td>
<td>-0.02</td>
<td>-0.00</td>
<td>-0.03</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium incentive</td>
<td>0.03</td>
<td>-0.02</td>
<td>0.11</td>
<td>0.07</td>
<td>0.77**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low incentive</td>
<td>0.02</td>
<td>-0.17</td>
<td>0.05</td>
<td>0.14</td>
<td>0.58**</td>
<td>0.72**</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*P < 0.05. **P < 0.01.

**Table 3** Predicting SCARED scores using childhood BI, $DRD4$ group and concurrent BOLD activation levels

<table>
<thead>
<tr>
<th>Predictor</th>
<th>$\beta$</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BI</td>
<td>0.151</td>
<td>0.980</td>
<td>0.331</td>
</tr>
<tr>
<td>DRD4</td>
<td>-0.228</td>
<td>-1.728</td>
<td>0.089</td>
</tr>
<tr>
<td>BI-by-DRD4</td>
<td>-0.327</td>
<td>-1.532</td>
<td>0.131</td>
</tr>
<tr>
<td>High incentive</td>
<td>-0.250</td>
<td>-0.992</td>
<td>0.325</td>
</tr>
<tr>
<td>Medium incentive</td>
<td>0.833</td>
<td>2.591</td>
<td>0.012</td>
</tr>
<tr>
<td>Low incentive</td>
<td>0.622</td>
<td>2.326</td>
<td>0.023</td>
</tr>
<tr>
<td>BI-by-high incentive</td>
<td>0.319</td>
<td>1.971</td>
<td>0.050</td>
</tr>
<tr>
<td>BI-by-medium incentive</td>
<td>0.173</td>
<td>0.699</td>
<td>0.487</td>
</tr>
<tr>
<td>BI-by-low incentive</td>
<td>0.048</td>
<td>0.241</td>
<td>0.810</td>
</tr>
<tr>
<td>DRD4-by-high incentive</td>
<td>-0.227</td>
<td>-0.936</td>
<td>0.353</td>
</tr>
<tr>
<td>DRD4-by-medium incentive</td>
<td>0.030</td>
<td>0.104</td>
<td>0.917</td>
</tr>
<tr>
<td>DRD4-by-low incentive</td>
<td>0.026</td>
<td>0.109</td>
<td>0.914</td>
</tr>
<tr>
<td>BI-by-DRD4-by-high incentive</td>
<td>0.133</td>
<td>0.790</td>
<td>0.432</td>
</tr>
<tr>
<td>BI-by-DRD4-by-medium incentive</td>
<td>0.528</td>
<td>1.976</td>
<td>0.050</td>
</tr>
<tr>
<td>BI-by-DRD4-by-low incentive</td>
<td>0.642</td>
<td>2.693</td>
<td>0.009</td>
</tr>
<tr>
<td>$F(15,74) = 2.17,$ P = 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table presents the predictors from the initial hierarchical regression model. The equivalent model for self-report SCARED scores was not significant. Standardized $\beta$ are presented. Significant predictors are in bold ($p < 0.05$).

outcome of interest (Guyer et al., 2006), additional moderators may need to be incorporated in order for similar patterns to emerge across a wider, more heterogeneous, spectrum.

In this study, $DRD4$ modulated the relation between BI and caudate activation. The current data support this previous work (Zink et al., 2004) suggesting that striatal activity tracks stimulus salience rather than the absolute value of a cue. Adolescents with a history of BI were previously found to exhibit elevated striatal response to reward only when the outcome was contingent on performance (Bar-Haim et al., 2009) and thus more salient, in line with data noting increased levels of performance monitoring in BI (McDermott et al., 2009; Henderson, 2010). This hypothesis could be tested in future work with larger samples, investigating the unique conditions during which individuals with a history of BI invest stimuli with salience, and whether salience is indeed the main parameter responsible for distinctive striatal responses in BI.

Our focus was on the caudate nucleus, as no significant findings emerged for the nucleus accumbens or putamen. Recent work suggests striatal sub-region dissociations in response to different types of reward, particularly in adolescence (Ernst and Fudge, 2009). The caudate nucleus plays a central role in instrumental responding (Yin and Knowlton, 2006). Children with, or at risk for, anxiety may be particularly sensitive to reward- or loss-related environmental cues and feedback (Fox and Helfinstein, In press). The caudate, as well as the broader dopaminergic system, is integral to learning the reward or punishment values of an environmental cue. This general sensitivity to salient incentive cues may contribute to the sensitivity behaviorally inhibited children show to social feedback. That is, for the temperamentally vulnerable child, this sensitivity may mean that only a relatively few negative social encounters may be needed before strong avoidance patterns become entrenched and lead to the manifestation of anxiety (Helfinstein et al., 2012). Indeed, recent work found an atypical caudate response to social feedback in adolescents with a history of BI (Guyer et al., in press).

In line with the larger literature, early childhood BI was associated with increased levels of parent-reported anxiety. At high levels of incentive, there was a direct effect of striatal activation on anxiety for adolescents with a history of BI, relative to their non-inhibited peers. In contrast, for medium and low incentives, the pattern was only evident when early temperamental inhibition was coupled with the 7R+ variant of $DRD4$. This suggests that these moderate markers of gain and loss may be more open to subjective assessments of salience, as opposed to the clearly significant high-incentive...
To the best of our knowledge, this is the first study to demonstrate the role of caudate functioning, manifest during adolescence, in the emergence of anxiety within a prospectively followed, non-clinical sample selected for a temperamental trait in infancy and early childhood.

We found no relations between early BI and adolescent anxiety for participants lacking the genetic or neural marker of reward sensitivity. Typically, early risk can be mitigated by protective factors that are either environmental (e.g. sensitive parenting) or biological (e.g. more efficient self-regulation mechanisms), such that most behaviorally inhibited children do not go on to show extremely high levels of social anxiety in adolescence. However, individuals who also harbor additional mechanisms of risk for anxiety, such as genetic risk factors, may be ‘resistant’, to these protective mechanisms. A similar argument has been made within the attention literature, suggesting that early BI is associated with later social withdrawal only if also coupled with attention biases to threat (Pérez-Edgar et al., 2010a, 2011). Our current data suggest that sensitivity to incentive cues, manifest in the caudate response and coupled with markers of genetic risk, may also act to link early risk to the later emergence of maladaptation, as in attention (Pérez-Edgar et al., 2010b).

The current findings should be viewed in light of study limitations. The use of an adolescent sample allowed us to focus on a developmental window marked by the peak incidence of both reward sensitivity and psychopathology. However, while the sample size is fairly large for a neuroimaging study, the sub-groups were not large enough to allow for further exploration of additional moderators, such as sex, age or pubertal status. Although sex- and age-related differences in dopamine transmission may influence striatal function (Andersen et al., 1997), the direction of these differences is not well understood (Andersen et al., 2002). In addition, since reward processes were only assessed in adolescence, we cannot comment on any changes in the relation between the core measures over time. A longitudinal study incorporating multiple measures of each predictor over time would be able to address this issue.

DRD4 only indirectly reflects levels of dopaminergic activity. Our use of a healthy adolescent sample precluded more invasive measures or manipulations of dopamine levels. However, there is recent evidence...
evidence that supports our presumptive link between DRD4 allelic status and dopamine variation. Past work has found modulations in striatal activation to reward with dopaminergic medication (Riba et al., 2008) and BOLD signal changes in the striatum have been correlated with dopamine release (Knutson and Gibbs, 2007). We were also unable to address variations across multiple candidate genes that may impact both temperament and reward processing (Smoller et al., 2001).

Finally, the relatively small sample did not allow us to explicitly incorporate psychiatric status into the analyses. Post-hoc analyses found that removal of affected adolescents did not alter the reported findings. A direct comparison of BI and clinically anxious adolescents would allow researchers to assess the extent to which at-risk and affected adolescents parallel each other in striatum- (Guyer et al., 2006, 2012) and amygdala-linked (McClure et al., 2007; Pérez-Edgar et al., 2007) processing. A larger sample would have also allowed us to further explore the differing pattern of results for the parent report and self-report measures of anxiety. Recent work has attempted to determine whether informant discrepancies are simply measurement error or meaningful indicators of differences in intra-personal vs inter-personal assessments (De Los Reyes, 2011).

In summary, this study expands our understanding of the scope of neural functions associated with BI (Fox et al., 2008) and their relations, in turn, to psychological outcomes. In doing so, the data document a common gene variant interacting with temperament measured in the first years of life to modulate neural functioning across childhood into adolescence. In addition, we examine the functional impact of these relations on the emergence of anxiety in an at-risk sample.

Conflict of Interest
None declared.

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