Double Dissociation Between Lab Measures of Inattention and Impulsivity and the Dopamine Transporter Gene (DAT1) and Dopamine D4 Receptor Gene (DRD4)

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Studies examining the biological and neuropsychological processes underlying attention-deficit/hyperactivity disorder (ADHD) suggest that error indices from the A-X Continuous Performance Test (A-X CPT) might represent useful endophenotypes for ADHD. The current study extended such findings by evaluating the utility of these putative endophenotypes in the context of a molecular genetic study. One hundred and forty-eight clinic-referred ADHD probands and 56 siblings were recruited as part of an ongoing study. Between- and within-family tests of association were conducted to test for relations between polymorphisms in two candidate genes, the dopamine transporter gene (DAT1) and the dopamine D4 receptor gene (DRD4), and indices of inattention and impulsivity derived from the A-X CPT. Association analyses of these polymorphisms with the A-X CPT indices suggested a double dissociation such that an index of inattention was associated with DAT1 but not DRD4, and an index of impulsivity was associated with DAT1 and not DRD4. Further analyses suggested that an A-X CPT index of impulsivity partially mediated previously observed associations between hyperactive-impulsive ADHD symptoms and DAT1. Additionally, an A-X CPT index of inattention moderated the relation between inattentive ADHD symptoms and DRD4 such that children with high levels of the endophenotype showed a stronger association between inattentive symptoms and DRD4. The potential utility of endophenotypes derived from the A-X CPT in molecular genetic studies of ADHD is discussed.

Keywords: attention deficit/hyperactivity disorder, endophenotype, Continuous Performance Test, executive functions, dopamine

Attention-deficit/hyperactivity disorder (ADHD) is characterized by inattention, hyperactivity, and impulsivity, and is estimated to occur in approximately 3–7% of children (American Psychiatric Association, 2000). Due to its high prevalence and negative sequelae, including substance abuse and juvenile delinquency (Barkley, Fischer, Edelbrock, & Smallish, 1990), ADHD represents a serious public health concern (Pelham, Foster, & Robb, 2007). Though the specific etiology of ADHD continues to be investigated, quantitative genetic studies of ADHD suggest substantial genetic influences, with heritability estimates ranging from 60–90% (Waldman & Rhee, 2002).

Replicable genetic associations with ADHD have been reported for several candidate genes, including the dopamine transporter gene (DAT1) and the dopamine D4 and D5 receptor genes (DRD4 and DRD5) (Faraone & Khan, 2006; Waldman & Gizer, 2006). Nonetheless, these associations are relatively small in magnitude, with odds ratios of 1.1–1.4 (Faraone et al., 2005; Gizer, Ficks, & Waldman, 2009). In reaction, some researchers have argued that valid intermediate phenotypes (i.e., endophenotypes) may prove useful in identifying susceptibility loci for complex diseases by more directly assessing the biological mechanisms assumed to underlie the disorder, which should be more directly related to the genes that confer risk for the disorder (Almasy & Blangero, 2001; Doyle et al., 2005; Gottesman & Gould, 2003; Leboyer et al., 1998; Waldman, 2005). For example, given that an inhibition deficit has been hypothesized to underlie the hyperactive-impulsive symptoms of ADHD (Barkley, 1997), it could be argued that performance on a measure of response inhibition would show stronger relations with ADHD candidate genes than the ADHD diagnosis itself. Thus, such constructs could strengthen the evidence for association between ADHD and specific genetic loci.

The A-X Continuous Performance Test (A-X CPT) is a neuropsychological measure hypothesized to assess two cognitive domains suggested to be involved in the etiology of ADHD, inattention and impulsivity (Losier, McGrath, & Klein, 1996). The A-X CPT requires subjects to attend to a series of letters presented in
rapid succession and respond whenever they observe the target sequence of an A followed by an X (Rosvold, Mirsky, Sarason, Bransome, & Ismand, 1956). Traditionally, responses to nontarget sequences (i.e., commission errors) are hypothesized to index impulsivity, whereas missed targets (i.e., omission errors) are hypothesized to index inattention (Losier et al., 1996). Alternatively, Halperin et al. (1988) provided empirical evidence suggesting that omission errors as well as commission errors made in response to an X not preceded by an A (an X-only error) and commission errors made on trials following a target trial (very long correct errors) index inattention (Halperin, Wolf, Greenblatt, & Young, 1991). In contrast, they found that commission errors made in response to an A trial (an A-only error) and commission errors made in response to an A followed by a letter other than an X (an A-not-X error) index impulsivity. Halperin et al. (1988) argued that summing these errors into inattention and impulsivity scores might provide more valid measures of these constructs than traditional omission and commission errors.

Multiple lines of evidence suggest that CPT error indices, including the Halperin indices, provide valid assessments of inattention and impulsivity (Glosser & Goodglass, 1990; Losier et al., 1996), and thus, might represent useful endophenotype measures for ADHD (Doyle et al., 2005). Neuroimaging studies suggest that CPT error indices provide dissociable measures of brain function in areas hypothesized to be involved in the etiology of ADHD, namely the anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (DLPFC) (Brooks et al., 2006; Dias et al., 2006; Perlstein, Dixit, Carter, Noll, & Cohen, 2003). These findings are supported by further studies suggesting the ACC, which is involved in aspects of motivated behavior (Bronstein & Cummings, 2001) and error monitoring (Cohen, Botvinick, & Carter, 2000), and the DLPFC, which is involved in response inhibition and working memory (Sawaguchi & Goldman-Rakic, 1991; Stuss et al., 2002), may be related, respectively, to the inattentive and hyperactive/impulsive symptoms observed in children diagnosed with ADHD (Pliszka, McCracken, & Maas, 1996; Rubia et al., 2008). Additionally, we have presented evidence from twin analyses suggesting that omission and commission errors and the Halperin inattentive and impulsive errors represent heritable traits that show evidence of a shared genetic etiology with ADHD (Gizer & Waldman, 2011). Such findings suggest the A-X CPT indices may represent useful endophenotype measures for ADHD.

To evaluate this possibility, we compared and contrasted the A-X CPT scoring indices against the following set of four criteria proposed by Waldman (2005) for evaluating the utility of putative endophenotypes. First, an endophenotype measure should be associated with some of the genes associated with the disorder of interest. Second, an endophenotype measure should show an incremental association with relevant candidate genes over and above those genes’ association with the disorder of interest. Third, an endophenotype measure should mediate the association between the gene and the disorder, either in part or in full. Evidence of mediation would suggest the endophenotype is related to both the candidate gene and to the disorder and explains, either fully or in part, the association between the gene and disorder. Fourth, an endophenotype measure might also moderate the association between the gene and the disorder. Such evidence of moderation would suggest that the endophenotype acts to reduce heterogeneity in the disorder and its symptoms, and thus, strengthens its association with the candidate gene. In the present study, the utility of the A-X CPT scoring indices were evaluated using these four criteria by examining the extent to which they explained and/or strengthened relations between ADHD and two genes associated with ADHD, DAT1, and DRD4.

DAT1 was first suggested as a candidate gene for ADHD given that stimulant medications, such as methylphenidate, prescribed to treat ADHD symptoms bind to the dopamine transporter and block dopamine reuptake (Ritz, Lamb, Goldberg, & Kuhar, 1987; Volkow et al., 1995). Several studies have found evidence of association between ADHD and the 10-repeat allele of a variable number of tandem repeats (VNTR) sequence in the 3′ untranslated region (UTR) of DAT1 (Barr et al., 2001; Cook et al., 1995; Curran et al., 2001; Gill, Daly, Heron, Hawi, & Fitzgerald, 1997; Waldman et al., 1998), though there also have been failures to replicate (Holmes et al., 2000; Palmer et al., 1999; Todd et al., 2001). Nonetheless, recent meta-analyses suggest a significant association between ADHD and this polymorphism in DAT1 (Faraone et al., 2005; Gizer et al., 2009).

Similar findings have emerged suggesting an association between ADHD and DRD4. Initially, DRD4 was shown to be associated with novelty-seeking (Benjamin et al., 1996; Ebstein, 1996), a personality trait compared to the high levels of impulsivity and excitability often seen in ADHD (Faraone et al., 1999), though more recent research has suggested that novelty-seeking is related to both the hyperactive-impulsive and inattentive ADHD symptom dimensions (Faraone, Knuwar, Adamson, & Biederman, 2009; Lynn et al., 2005). Several studies have reported an association between ADHD and a 48-bp VNTR in exon 3 of DRD4 (Faraone et al., 1999; LaHoste et al., 1996; Rowe et al., 1998), though there also have been several failures to replicate (Castellanos et al., 1998; Hawi et al., 2000; Kotler et al., 2000). Meta-analyses of these and additional studies have found DRD4 to be associated with ADHD (Faraone et al., 1999; Faraone et al., 2005; Gizer et al., 2009).

In addition to these results, there is growing evidence that DAT1 and DRD4 may be associated with specific ADHD symptom domains. For example, studies have suggested that DRD4 is more strongly related to the inattentive than the hyperactive-impulsive symptoms of ADHD (Lasky-Su et al., 2007; McCracken et al., 2000; Rowe et al., 1998) and is related to attention problems in the general population (Laucht, Becker, & Schmidt, 2006; Schmidt, Fox, Perez-Edgar, Hu, & Hamer, 2001). Studies testing for association between DAT1 and ADHD suggest DAT1 is strongly associated with hyperactive-impulsive symptoms (Mill et al., 2005; Waldman et al., 1998). Additionally, DAT1 and DRD4 may influence distinct aspects of executive functioning, with evidence suggesting DAT1 is related to response inhibition (Cornish et al., 2005; Demiralp et al., 2007) and response variability (Bellgrove, Hawi, Kirby, Gill, & Robertson, 2005) whereas DRD4 is related to sustained attention and conflict monitoring (Bellgrove et al., 2005; Demiralp et al., 2007; Kieling, Roman, Doyle, Hutz, & Rohde, 2006; Swanson et al., 2000).

Despite the promise of such studies to further our understanding of the relations between specific genes and ADHD, it is important to briefly review the conflicting results that such studies have yielded. For example, among studies supporting a relation between executive function and sustained attention and DRD4, some studies have suggested reduced performance among participants with
one or more 7-repeat alleles of the exon 3 VNTR (e.g., Herrmann et al., 2007; Kieling et al., 2006; Loo et al., 2008), whereas others have suggested improved performance (e.g., Johnson et al., 2008; Manor et al., 2002; Swanson et al., 2000). A similarly conflicting picture has emerged regarding the relation between the 3’ UTR VNTR of DAT1 and response inhibition (for reviews see Kebir, Tabbane, Sengupta, & Joober, 2009; Rommelse et al., 2008; Turic, Swanson, & Sonuga-Barke, 2010). One potential explanation for the lack of consistent findings is the use of different measures or scoring protocols across studies. For example, Kieling et al. (2006) used omissions from a simplified version of the CPT to assess sustained attention, whereas Johnson and colleagues used variation in response time on the Sustained Attention to Response Task (SART) (Robertson, Manly, Andrade, Baddeley, & Yiend, 1997). As a result, additional studies are needed to clarify the associations between performance on executive function and sustained attention measures and these candidate genes for ADHD.

The primary aim of the current study was to compare and contrast the utility of the traditional A-X CPT scoring indices of omission and commission errors with the Halperin inattentive and impulsive error indices in strengthening and explaining associations between inattentive and hyperactive-impulsive ADHD symptoms and DRD4 and DAT1. It was predicted that omission errors and/or the Halperin inattentive errors would strengthen previously observed relations between DRD4 and inattentive ADHD symptoms (Lasky-Su et al., 2007; McCracken et al., 2000; Rowe et al., 1998), whereas commission errors and/or the Halperin impulsive errors would strengthen previously observed relations between DAT1 and hyperactive-impulsive ADHD symptoms (Mill et al., 2005; Waldman et al., 1998).

**Method**

All assessment procedures were approved by the Emory University Institutional Review Board. Parents read and signed an informed consent form prior to study participation, and verbal assent was obtained from the children.

**Participants**

Participants represent an expanded sample of an ongoing study that has been included in previous publications (Rowe et al., 1998; Waldman et al., 1998), and includes 204 children from 148 families recruited through the Center for Learning and Attention Deficit Disorders (CLADD) at the Emory University School of Medicine and the Emory University Psychological Center in Atlanta, Georgia (see Table 1 for sample characteristics). Both clinics specialize in the assessment and treatment of childhood learning

### Table 1
**Description of Study Sample**

<table>
<thead>
<tr>
<th>Sample characteristics</th>
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<tbody>
<tr>
<td><strong>Sample Size</strong></td>
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<tr>
<td># of families</td>
<td>148</td>
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<tr>
<td>Total children</td>
<td>204</td>
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<tr>
<td># of probands</td>
<td>148</td>
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<tr>
<td># of siblings</td>
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<tr>
<td><strong>Diagnostic Information</strong></td>
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<tr>
<td>Proband</td>
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<tr>
<td>+ ADHD</td>
<td>119 children (I-47 (39%), H-8 (7%), C-64 (54%))</td>
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<tr>
<td>+ ODD</td>
<td>78 (65%)</td>
</tr>
<tr>
<td>+ CD</td>
<td>11 (9%)</td>
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<tr>
<td>Siblings</td>
<td></td>
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<tr>
<td>+ ADHD</td>
<td>19 children (I-9 (47%), H-3 (16%), C-7 (37%))</td>
</tr>
<tr>
<td>+ ODD</td>
<td>17 (89%)</td>
</tr>
<tr>
<td>+ CD</td>
<td>4 (21%)</td>
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<td><strong>Demographic Characteristics</strong></td>
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<tr>
<td>Age</td>
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<tr>
<td>Gender</td>
<td>139 M (68%), 65 F (32%)</td>
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<td>Asian</td>
<td>2 (1%)</td>
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<td><strong>Genotype Frequencies</strong></td>
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<td># of high risk alleles</td>
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<td>0</td>
<td>DAT1: 10-repeat alleles 6 (5%) DRD4: 7-repeat alleles 103 (71%)</td>
</tr>
<tr>
<td>1</td>
<td>DAT1: 43 (35%) DRD4: 37 (26%)</td>
</tr>
<tr>
<td>2</td>
<td>DAT1: 74 (60%) DRD4: 5 (3%)</td>
</tr>
</tbody>
</table>

*Note.* + indicates number of children diagnosed with that disorder; I = Inattentive ADHD subtype; H = Hyperactive-Impulsive ADHD subtype; C = Combined subtype; M = Males; F = Females.
disabilities and externalizing disorders. Probands and their siblings between ages 6 and 18 identified through these clinics were recruited to participate. Siblings were included irrespective of ADHD diagnoses or symptom levels. Probands or siblings diagnosed with autism, traumatic brain injury, or neurological conditions (e.g., epilepsy) were excluded, as were children with IQs <75. Other clinic diagnoses remained confidential and did not influence inclusion in the study. In total, 185 and 210 children were successfully genotyped for the DAT1 3' UTR and DRD4 exon 3 VNTRs, respectively.

Assessment Procedures

Emory Diagnostic Rating Scale (EDRS). Symptom ratings were obtained from mothers and fathers (when the mother was not available) using the EDRS (Waldman et al., 1998). Because participants were not selected with respect to medication status, informants rated their child’s behavior as though the child was not currently taking medication. The EDRS was developed to assess symptoms of the major DSM-IV childhood psychiatric disorders. Parents rated ADHD symptoms on a 0–4 scale, and inattentive and hyperactive-impulsive scores were created by averaging the scores for the 9 items that comprise each symptom dimension. The symptom scales demonstrated acceptable internal consistencies in the current sample and a previously collected control sample (α = .85–.96), and the EDRS has been shown to yield ADHD diagnostic rates similar to the population prevalence (Waldman et al., 1998).

A-X CPT. As described above, previous neuroimaging studies suggest that CPT error indices provide dissociable measures of brain function associated with inattention and impulsivity (e.g., Dias et al., 2006), and twin analyses suggest that error indices from the A-X CPT represent heritable traits that show evidence of a shared genetic etiology with ADHD (Gizer & Waldman, 2011). Thus, the error indices from this task (i.e., omission, commission, inattentive, and impulsive errors) were evaluated as putative endophenotypes for ADHD. All testing was conducted in the subjects’ homes in a quiet room free of distraction using a laptop computer. Parents were instructed to withhold their child’s medication for the day of testing and compliance was confirmed verbally prior to testing. Time of day of testing varied and was not controlled for.

The task was programmed according to parameters outlined by Halperin et al. (1988). Stimuli consisted of 11 letters presented for 200 ms each with an interstimulus interval of 1500 ms. The target stimulus was the letter sequence A-X, and subjects pressed the space bar whenever the sequence appeared. There were 40 target trials distributed across 400 trials during the 12-minute test. Subjects completed a brief practice session prior to the test. Omission and commission and “inattentive” and “impulsive” errors were calculated according to procedures described by Halperin et al. (1988). Because extreme values on the A-X CPT scoring indices may indicate noncompliance (Koelega, 1995), data in the top 5% of the distribution for each scoring index were trimmed from analyses conducted for that index. Significant age and sex effects were observed for each of the A-X CPT indices, and thus, relevant age and sex terms (i.e., age, sex, age², sex × age) served as covariates.

Data Analysis

General Procedures

Prior to conducting the association analyses, parental genotypes were evaluated for departures from Hardy-Weinberg equilibrium (HWE) to evaluate genotyping quality. No departures were detected (DAT1 – p = .88; DRD4 – p = .25). Relations between A-X CPT indices and DAT1 and DRD4 were then examined using between- and within-family tests of association to capitalize on the strengths of each design. Between-family designs, which are based on the classic case versus control design, are often more statistically powerful than within-family designs. Nonetheless, a major limitation of this design is the potential for a false positive result due to population stratification, which occurs when diverse groups of individuals who vary both in symptom level and allele frequency are included in the study sample. Within-family designs, while they may be slightly less statistically powerful, can control for spurious findings due to population stratification as well as any additional variables that have not been controlled for (Pericak-Vance, 1998). Briefly, within-family designs test the frequency with which parents heterozygous at a given locus transmit allele a versus allele b to an affected offspring. Under the null hypothesis of no genetic association or linkage, we would expect the frequency of transmission for each allele to be 50% according to Mendelian genetics. Thus, a transmission rate >50% for either allele would lead us to reject the null hypothesis and conclude that genetic association and linkage were present. When interpreting results from both analytic approaches, statistical tests with p values < .05 were described as significant, tests with p values < .10 were described as trends, and tests with p values > .10 that followed study predictions were described as yielding “limited” evidence for association.

Between-family tests of association were conducted using ordinal logistic regression, which makes no assumptions regarding the distribution of the explanatory variables. The number of DAT1 or DRD4 “high-risk” alleles (i.e., 0, 1, or 2) served as the criterion variable, and ADHD symptom scores, the A-X CPT indices, and interactions between these variables served as predictors. Recent studies indicate that self-reported ethnicity is highly correlated with ancestry inferred from genotype data (Tang et al., 2005), thus subjects’ parent-reported ethnic background was included as a covariate in the between-family analyses to control for potential population stratification. Sex and the linear and curvilinear effects of age and the interactions between sex and the age terms were also included as covariates when significant. Ordinal logistic regression yields a Likelihood Ratio $\chi^2$ statistic that indexes the overall fit of the regression model as well as goodness of fit indices referred to as “pseudo-$R^2$” statistics. The Nagelkerke $R^2$ (Nagelkerke, 1991) is one such statistic that provides a specific advantage over other pseudo-$R^2$ statistics in terms of interpretation because it is scaled to range from 0 (indicating poor fit) to a maximum of 1 (indicating perfect fit). Thus, it is on the same scale as the OLS $R^2$ statistic, whereas other pseudo-$R^2$ statistics are not (Cohen, Cohen, West, & Aiken, 2003). Ordinal logistic regression also yields a Wald’s chi-square that tests the unique contribution of each of the individual explanatory variables in the model. If single predictors are entered into a regression model hierarchically, the Wald’s chi-square for each added predictor represents a specific
test of the incremental contribution of the added predictor (Cohen, Cohen, West, & Aiken, 2003) and the accompanying change in pseudo-$R^2$ represents the effect size attributed to the added predictor. This permits tests of association of the candidate genes with ADHD symptom scores or A-X CPT indices uniquely and in combination, thus facilitating analyses of mediation and moderation as described in detail below.

Within-family tests of association were conducted using the extension of the transmission disequilibrium test (TDT) ( Spielman, McGinnis, & Ewens, 1993) implemented in the Tools for Family-Based Association Tests (PBAT and FBAT) software programs (Laird, Horvath, & Xu, 2000; Lange, DeMeo, Silverman, Weiss, & Laird, 2003; Lange & Laird, 2002). PBAT also permits tests of interactions between phenotypic variables, thus allowing for tests of moderation.

**Evaluation of the Utility Criteria**

First, between- and within-family tests of association were conducted between DAT1 and DRD4 and the ADHD symptom dimensions and A-X CPT indices. Previous studies suggested the 10-repeat allele of the DAT1 VNTR and the 7-repeat allele of the DRD4 VNTR are associated with increased risk for ADHD (Faraone et al., 2005; Gizer et al., 2009). Thus, it was predicted that these would represent the “high-risk” alleles, and one-tailed $p$ values were reported. Second, tests of the incremental associations of DAT1 and DRD4 with the A-X CPT indices over and above the ADHD symptom dimensions were conducted using between-family analyses. These tests were conducted by comparing the results of two multiple regression analyses, the first in which genotype is regressed on ADHD symptom level and the second in which genotype is regressed on both ADHD symptom level and the endophenotype (i.e., the A-X CPT index). The increase in effect size (e.g., $\Delta R^2_{inc}$) and Wald’s chi-square and $p$ value corresponding to the endophenotype were used to test for incremental associations. Parallel within-family analyses could not be conducted given that FBAT and PBAT do not provide tests of the incremental contributions of hierarchically entered phenotypes.

Third, analyses of mediation were conducted using a two-step process. In the first step, two multiple regression analyses were conducted to provide an initial index of mediation according to the steps described by Baron and Kenny (1986). The first regression analysis estimated the direct relation between genotype and symptom level, whereas the second regression analysis reestimated the direct relation between genotype and symptom level after controlling for the endophenotype (i.e., a particular A-X CPT index). A decline in the magnitude of $\beta$ from the first to the second regression ($\Delta \beta_{med}$) as well as a decline in the $R^2$ contributed uniquely by ADHD symptom levels between the two regression models ($\Delta R^2_{med}$) provided an initial index of mediation. Specifically, if the $R^2$ and $\beta$ attributed uniquely to ADHD symptom level in the second regression model decrease relative to the $R^2$ and $\beta$ attributed to ADHD symptom level in the first model, this suggests that the association of the candidate gene with the disorder is partially or fully mediated by the endophenotype (Baron & Kenny, 1986). If any such declines were observed, the second step in evaluating the presence of mediation was performed by conducting a formal statistical test of mediation using structural equation modeling (SEM) and the Sobel test (1982) as implemented in Mplus Version 6 (Muthén & Muthén, 1998–2010). In this procedure, the total relation of the explanatory variable (i.e., hyperactive-impulsive or inattentive symptom levels) with the criterion variable (i.e., the number of high-risk alleles for DAT1 or DRD4) is partitioned into the direct relation versus the indirect relation via the particular A-X CPT index. Standardized regression coefficients ($\hat{\beta}$), $t$ tests, and their $p$ values are presented separately for the total, direct, and indirect relations. To test for the presence of mediation, we used $t$ tests of the $\hat{\beta}$s and their associated $p$ values as well as a $\chi^2$ difference test that contrasted the $\chi^2$ value for a model in which the indirect relation was freely estimated with the $\chi^2$ value for a nested model in which this indirect relation was omitted by constraining the regression of the genotype on the endophenotype to equal 0. Parallel within-family analyses could not be conducted as FBAT and PBAT have yet to incorporate tests of mediation.

Fourth, tests of moderation were conducted. Waldman (2005) argued that an endophenotype might also act to moderate associations between the disorder and genotype, such that the effects of the candidate gene on the disorder are strongest in those individuals who display both high symptom levels and impairment on the endophenotype. Thus, evidence of moderation was further explored to evaluate whether the observed interaction was consistent with or counter to this hypothesis. For between-family analyses, the evaluation of an interaction term representing the product of the mean-centered putative endophenotype and symptom level variables in a regression model containing the main effects of each variable provided a test of moderation. To limit the number of statistical tests conducted, only significant between-family tests of moderation were followed-up in the within-family analyses using PBAT.

**Results**

**Tests of Association Between ADHD Symptoms and A-X CPT Indices and DAT1 and DRD4**

Tests of association were conducted between DAT1 and DRD4 polymorphisms and the ADHD symptom dimensions (see Table 2). For the DAT1 VNTR, there was a trend toward an association between the 10-repeat allele and hyperactive-impulsive symptoms in the between-family analyses ($\text{Wald } \chi^2(n = 123) = 1.88, p = .085, \text{Nagelkerke } R^2 = .03$) that was significant in the within-family analyses ($Z = 1.92, p = .027, R^2 = .05$). In contrast, there was little evidence to suggest an association between DAT1 and inattentive symptoms in either the between- or within-family analyses. For the DRD4 VNTR, there was also a significant association between the 7-repeat allele and hyperactive-impulsive symptoms in the between-family analyses ($\text{Wald } \chi^2(n = 145) = 3.00, p = .042, \text{Nagelkerke } R^2 = .03$) and a trend toward an association in the within-family analyses ($Z = 1.31, p = .095, R^2 = .04$). There was also a significant association between the 7-repeat allele and inattentive symptoms in the between- ($\text{Wald } \chi^2(n = 145) = 3.78, p = .026, \text{Nagelkerke } R^2 = .04$) and within-family analyses ($Z = 2.15, p = .016, R^2 = .10$).

Tests of association were then conducted between the A-X CPT indices and DAT1 and DRD4. For the DAT1 VNTR, between-family analyses only showed a significant association between the 10-repeat allele and Halperin impulsive errors ($\text{Wald } \chi^2(n = 101) = 2.92, p = .044, \text{Nagelkerke } R^2 = .04$). Within-family
analyses showed a significant association between the 10-repeat allele and the Halperin impulsive errors \( (Z = 1.76, p = .039, R^2 = .04) \) as well as commission errors \( (Z = 1.82, p = .034, R^2 = .05) \). For the DRD4 VNTR, between-family analyses showed a trend toward an association between the 7-repeat allele and the Halperin inattentive errors \( [Wald \chi^2(n = 118) = 2.55, p = .052, Nagelkerke R^2 = .02] \), whereas within-family analyses of the Halperin inattentive errors showed a significant association \( (Z = 1.99, p = .023, R^2 = .05) \) as well as trends for an association of the 7-repeat allele with omission \( (Z = 1.44, p = .075, R^2 = .03) \) and commission errors \( (Z = 1.47, p = .071, R^2 = .03) \). Differences in results across analytic methods for both polymorphisms were likely influenced by limitations in sample size, and would be expected to converge as sample sizes increased (Evangelou, Trikalinos, Salanti, & Ioannidis, 2006).

To evaluate the robustness of these results, the analyses were repeated under three conditions. First, Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD) symptom counts were included as covariates to evaluate whether the findings were independent of these frequently co-occurring symptoms. This yielded only minor changes with the most notable being a strengthening of the association between the DRD4 7-repeat allele and the A-X CPT inattentive errors [Wald \( \chi^2(n = 117) = 3.21, p = .036 \), Nagelkerke \( R^2 = .03 \)]. Second, the analyses were rerun as logistic regressions (DAT1: individuals with 0 or 1 10-repeat alleles vs. individuals with 2 copies; DRD4: individuals with 0 7-repeat alleles vs. individuals with 1 or 2 copies) to ensure that the results were not driven by the low frequency genotypes. Again, only minor changes were observed with the most notable being a slight weakening of the association between the DRD4 7-repeat allele and A-X CPT inattentive errors, though the magnitude of the effect remained unchanged [Wald \( \chi^2(n = 118) = 1.54, p = .107 \), Nagelkerke \( R^2 = .02 \)]. Third, analyses were conducted using only Caucasian participants to ensure the results were not due to population stratification. Excluding this 25% of the sample (see Table 1) produced a significant decline in statistical power and caused a uniform increase in \( p \) values, but no declines in effect sizes were observed suggesting the results were robust to the exclusion of ethnic minority participants. Notably, the follow-up analyses conducted for the within-family analyses were not conducted for the family based analyses given that the latter are robust to stratification effects and do not rely on low frequency genotype groups to test an additive model.

Tests of Incremental Associations

For the DAT1 VNTR, between-family analyses suggested that the original association with the Halperin impulsive errors [Wald \( \chi^2(n = 101) = 2.92, p = .044, Nagelkerke R^2 = .04 \)] was only slightly weakened and remained significant after controlling for inattentive ADHD symptoms [Wald \( \chi^2(n = 101) = 2.74, p = .049, Nagelkerke \Delta R^2_{inc} = .03 \)] and remained a trend after controlling for hyperactive-impulsive ADHD symptoms [Wald \( \chi^2(n = 101) = 2.47, p = .058, Nagelkerke \Delta R^2_{inc} = .03 \)] (see Table 3). For the DRD4 VNTR, between-family analyses suggested that the original trend for an association with the Halperin inattentive errors [Wald \( \chi^2(n = 118) = 2.55, p = .052, Nagelkerke R^2 = .02 \)] was weakened after controlling for inattentive [Wald \( \chi^2(n = 118) = 1.53, p = .108, Nagelkerke \Delta R^2_{inc} = .02 \)] or hyperactive-impulsive symptoms [Wald \( \chi^2(n = 118) = 1.70, p = .096, Nagelkerke \Delta R^2_{inc} = .02 \)] (see Table 4).

Tests of Mediation

For the DAT1 VNTR, there was evidence suggesting associations with hyperactive-impulsive ADHD symptoms and Halperin impulsive errors, allowing for the possibility of mediation. When restricting the analysis to those children who completed the CPT, between-family analyses suggested that DAT1 was nonsignificant and showed only limited evidence of an association with
Tests of Mediation and Moderation by A-X CPT Indices of the Association Between the ADHD Symptom Dimensions and DAT1

| Table 3 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Omissions (n = 111) | Commissions (n = 110) | Inattentive errors (n = 98) | Impulsive errors (n = 101) |
| Inattentive Symptoms | Incremental contribution? | No | No | No | Yes |
|                    | $\Delta R^2_{inc}$ | .00 | .01 | .01 | .03 |
| Mediates association? | No | No | No | Yes |
|                    | $\Delta \beta_{med}$ | .01 | .02 | .01 | -.01 |
|                    | $\Delta R^2_{med}$ | .00 | .00 | .00 | .00 |
| Moderates Association?* | No | No | No | Yes* |
|                    | $p$ | .909 | .676 | .364 | .626 |
| Hyperactive-Impulsive Symptoms | Incremental contribution? | No | No | No | Yes |
|                    | $\Delta R^2_{inc}$ | .00 | .00 | .00 | .04 |
| Mediates association? | No | No | No | Partial |
|                    | $\Delta \beta_{med}$ | .01 | .00 | .01 | -.04 |
|                    | $\Delta R^2_{med}$ | .00 | .00 | .00 | .00 |
| Moderates Association?* | No | No | No* | No |
|                    | $p$ | .606 | .834 | .727 | .819 |

Note. $\Delta R^2_{inc}$—indicates the increase in $R^2$ attributable to the endophenotype over and above the ADHD symptom dimension; $\Delta \beta_{med}$, $\Delta R^2_{med}$, and $\Delta R^2_{inc}$—indicates the decline in $p$ value and $R^2$, respectively, for the two regression analyses used to test for mediation, for incremental contribution and moderation analyses, bold text indicates significant result at $p < .05$; italicized text indicates a trend towards significance at $p < .10$; *—indicates that test was two-tailed, remaining tests were one-tailed, †—test of parallel lines was violated for these analyses, thus the displayed results are from binary logistic regressions that were conducted in parallel with the ordinal regressions.

hyperactive-impulsive symptoms [Nagelkerke $R^2 = .02$, $\beta = .20$, Wald $\chi^2(n = 101) = 1.188$, $p = .368$]. This relation was diminished after controlling for the effects of the Halperin impulsive errors [Nagelkerke $R^2 = .01$, $\beta = .16$, Wald $\chi^2(n = 101) = 0.689$, $p = .204$] yielding a $\Delta R^2_{med} = .01$ (see Table 3). The results of the SEM analyses also supported the presence of mediation. First, the total relation between the hyperactive-impulsive symptoms and DAT1 genotype was estimated, and similar to the results described above, yielded limited evidence of an association between the DAT1 genotype and inattentive ADHD symptoms, as well as the Halperin inattentive errors, allowing for the possibility of mediation. For children who completed the CPT, between-family analyses suggested that DRD4 showed a trend toward an association with the hyperactive-impulsive symptoms [Nagelkerke $R^2 = .02$, $\beta = .26$, Wald $\chi^2(n = 118) = 2.167$, $p = .070$]. Similar to the results above, this relation was weakened after controlling for the effects of the Halperin inattentive errors [Nagelkerke $R^2 = .01$, $\beta = .22$, Wald $\chi^2(n = 118) = 1.431$, $p = .116$] yielding a $\Delta R^2_{med} = .01$ (see Table 4). The results of the SEM analyses partially supported the presence of mediation. First, the total relation between the hyperactive-impulsive symptoms and DRD4 genotype was estimated, and similar to the results described above, yielded a trend toward an association ($\beta = .16, t = 1.42, p = .077$). When this relation was partitioned into the direct and indirect relations, the direct relation was weakened ($\beta = .13, t = 1.11, p = .322$), and the Sobel test of the indirect relation via the Halperin inattentive errors yielded a trend toward significance ($\beta = .03, t = 1.53, p = .064$). Further, the $\chi^2$ difference test contrasting a model estimating the indirect relation against a nested model in which this indirect relation was constrained to 0 also yielded a trend toward significance [$\chi^2(1) = 3.58, p = .058$], suggesting that the indirect relation could not be dropped from the model.

Tests of Moderation

For the DAT1 VNTR, only a single instance of moderation was observed (see Table 3). Between-family analyses suggested a trend for the Halperin impulsive errors to moderate the association with the inattentive symptoms such that the association between DAT1 and inattentive ADHD symptoms was strengthened among subjects who committed high numbers of impulsive errors. The ordinal regression analysis testing this interaction could not be interpreted due to a violation of the test of parallel lines, $\chi^2(9, n = 101) = 17.329$, $p = .044$, but a logistic regression contrasting children with 0 or 1 copies of the 10-repeat allele against children with 2 copies was marginally significant [Wald $\chi^2(n = 101) = 3.49, p = .062$, Nagelkerke $R^2 = .04$] supporting the presence of moderation. Nonetheless, the within-family test of moderation was nonsignificant ($p = .807$), suggesting that this result may not be
robust or that there were too few informative parent-offspring transmissions to yield a test with sufficient statistical power.

For the DRD4 VNTR, two examples of moderation were observed (see Table 4). First, the Halperin inattentive errors significantly moderated the relation with the inattentive symptoms [Wald $\chi^2(n = 118) = 3.91, p = .048$, Nagelkerke $R^2 = .04$], such that the association between DRD4 and inattentive ADHD symptoms was strengthened among children who committed high numbers of inattentive errors (see Figure 1). This result remained significant when using logistic regression contrasting children with 0 copies of the 7-repeat allele against children with 1 or 2 copies [Wald $\chi^2(n = 118) = 3.18, p = .036$, Nagelkerke $R^2 = .04$]. This moderator effect showed some evidence of robustness across analytic methods, as the within-family analysis showed evidence for an association that was in the same direction of that reported for the between-family analyses, though it failed to reach significance ($p = .107$).

Second, omission errors showed a trend toward moderating the association between the hyperactive-impulsive symptoms and DRD4. The ordinal regression analysis testing this interaction violated the test of parallel lines, $\chi^2(9, N = 131) = 84.82, p < .001$, but a logistic regression analysis contrasting children with 0 copies of the 7-repeat allele against children with 1 or 2 copies yielded a statistical trend [Wald $\chi^2(n = 131) = 2.84, p = .092$, Nagelkerke $R^2 = .02$]. Notably, the pattern of results suggested the association between DRD4 and hyperactive-impulsive symptoms was strengthened for children who committed low rather than high numbers of omission errors, which was contrary to the expected moderation effect. Further complicating the interpretation of this result, a test of this interaction using a within-family design was nonsignificant ($p = .344$), suggesting that this finding may not be robust or that there were too few informative parent-offspring transmissions to yield a test with sufficient statistical power.

Discussion

In the present study, we compared and contrasted the utility of traditional CPT omission and commission errors with alternative CPT indices of inattention and impulsivity described by Halperin et al. (1988) as endophenotype measures for ADHD. The Halperin indices yielded evidence for association with DAT1 and DRD4 using between- and within-family methods that was suggestive of a double dissociation. Specifically, the Halperin inattentive errors showed a significant association with the DRD4 exon 3 VNTR 7-repeat allele, but not with the DAT1 3’ UTR VNTR 10-repeat allele. In contrast, the Halperin impulsive errors showed a significant association with the DAT1 10-repeat allele, but not with the DRD4 7-repeat allele. Further, the Halperin impulsive errors continued to show a trend toward an association with DAT1 after controlling for the presence of inattentive or hyperactive-impulsive ADHD symptoms, and the Halperin inattentive errors continued to show a trend toward an association with DRD4 after controlling for the presence of hyperactive-impulsive ADHD symptoms. The traditional CPT omission and commission errors showed associations only in the within-family analyses and did so in a nonspecific manner, as omissions showed a trend toward an association with DRD4, while commissions yielded a significant association with DAT1 and a trend toward an association with DRD4.

This contrast in findings between the traditional omission and commission errors of the A-X CPT and the Halperin inattentive
and impulsive errors clearly demonstrates the need to carefully construct and evaluate phenotypes from neuropsychological lab measures if they are to be incorporated into molecular genetic studies. Given the small effect sizes that have been observed between specific genetic polymorphisms and psychological disorders (Visscher, Goddard, Derks, & Wray, 2011), careful measurement of the constructs under study are crucial. The original studies conducted by Halperin and colleagues (Halperin et al., 1991; Halperin et al., 1988) provided initial evidence of the increased validity of the inattentive and impulsive errors relative to omission and commission errors, and the findings of the present study demonstrate their utility in molecular genetic studies of ADHD. Nonetheless, additional measures of attention and impulsivity derived from attention tasks such as the A-X CPT, including variability in reaction time (RT), have been investigated as endophenotypes for ADHD (e.g., Bellgrove, Hawi, Kirly et al., 2005; Kuntsi et al., 2010), demonstrating the complexity in conducting such research. Notably, this type of measurement issue is not specific to the A-X CPT as a recent study used electrophysiological data to demonstrate that indices of impulsivity and sustained attention derived from another commonly used neuropsychological measure, the Go/No-Go task, can be refined in a similar manner (O’Connell et al., 2009).

These findings may also explain, in part, the conflicting findings that have been previously reported regarding genetic associations between measures of impulsivity and sustained attention and DAT1 and DRD4 polymorphisms. For example, the association between increased inattentive CPT errors and the 7-repeat allele of the DRD4 VNTR described in the present study is supported by previous studies (Kieling et al., 2006; Loo et al., 2008), though others have found that the 7-repeat allele is associated with decreased sustained attention (Bellgrove, Hawi, Lowe, et al., 2005; Manor et al., 2002; Swanson et al., 2000). Similarly conflicting findings have been reported for the described association between increased impulsive CPT errors and the 10-repeat allele of the DAT1 VNTR (see Rommelse et al., 2008 for a review).

Despite these conflicting results, the genetic double dissociation described in the present study has been previously suggested in the literature. Studies have shown that DAT1 is highly expressed in the striatum, caudate, and putamen (Ciliax et al., 1999), whereas DRD4 is predominantly expressed in frontal lobe regions, such as the orbitofrontal cortex and anterior cingulate (Noaín et al., 2006). Neurons in the caudate and striatum that rely on DAT1 to metabolize dopamine project to neurons in regions of the prefrontal cortex including the dorsolateral prefrontal cortex (DLPFC), which is rich in D1 receptors (Hurd, Suzuki, & Sedvall, 2001). In addition to connections from the caudate and striatum, prefrontal regions also receive connections from the ventral tegmental area (VTA) and amygdala. The primary regions receiving connections from the VTA are the anterior cingulate cortex (ACC) and orbitofrontal cortex, which are rich in D2 and D4 receptors (Floresco & Tse, 2007). This suggests that DAT1 and DRD4 are differentially expressed in these overlapping yet distinct cortical-subcortical circuits. Such a conclusion is supported by recent molecular genetic studies suggesting that DAT1 and DRD4 may differentially influence brain regions involved in these circuits, with DAT1 influencing caudate volume and DRD4 influencing prefrontal volume (Durston et al., 2005; Shaw et al., 2007).

In addition to an anatomical dissociation, the prefrontal regions associated with these two circuits may reflect unique aspects of executive functioning, suggesting a possible functional dissociation as well. For example, behavioral studies suggest the DLPFC is associated with working memory and inhibitory control in both animals and humans (Sawaguchi & Goldman-Rakic, 1991; Stuss et al., 2002), whereas the ACC is associated with motivated behavior (Bronstein & Cummings, 2001) and error monitoring (JD Cohen et
al., 2000). Neuroimaging studies of executive functioning provide additional support for such a dissociation (Dias et al., 2006; MacDonald, Cohen, Stenger, & Carter, 2000). Of direct relevance to the current study, a recent molecular genetic study presented imaging data collected during a target detection task and suggested that DAT1 and DRD4 may differentially influence working memory/inhibitory control and conflict monitoring, respectively (Demiralp et al., 2007). The double dissociation observed in the current study thus adds to this literature and may serve to further explain the differential relations between DAT1 and DRD4 and the ADHD symptom dimensions.

The observed double dissociation strongly supported the utility of the Halperin CPT indices as endophenotype measures for ADHD, and subsequent tests of mediation and moderation reaffirmed this conclusion. For example, only the Halperin indices appeared to mediate associations between ADHD symptoms and either candidate gene. The Halperin impulsive errors showed significant evidence of partially mediating the association between the hyperactive-impulsive symptoms and DAT1, thus explaining some, though not all, of the relation between hyperactive-impulsive ADHD symptoms and DAT1. Similarly, the Halperin inattentive errors showed a trend toward partially mediating the association between the hyperactive-impulsive symptoms and DRD4.

The tests of moderation provided further evidence supporting the utility of the Halperin scoring indices as endophenotype measures for ADHD. Across analytic methods, the Halperin inattentive errors moderated the relation between DRD4 and the inattentive ADHD symptom dimension, suggesting a stronger association between DRD4 and inattentive symptoms among children exhibiting high rather than low levels of inattentive errors. These results demonstrate the utility of the Halperin scoring indices as endophenotype measures and their potential to further our understanding of the relations between susceptibility genes and ADHD.

Although the presented findings have important implications for molecular genetic studies of ADHD, there are limitations that should be noted. First, although 204 children participated in the present study, only a subset of 165 children completed the A-X CPT and were successfully genotyped for one of the two polymorphisms. Though this represents a large sample relative to many published candidate gene studies of endophenotype variables, larger samples are typically desired for providing sufficient statistical power (Lohmueller, Pearce, Pike, Lander, & Hirschhorn, 2003). Thus, it is possible that meaningful associations were not detected due to insufficient power. Nonetheless, an examination of the effect sizes for the tests of main effects presented in Table 2 shows that many of the $R^2$ values for tests that failed to reach significance were <.02, suggesting that even with an increase in sample size these tests would remain nonsignificant. This was particularly true of the analyses examining the association between DAT1 and the inattentive errors and DRD4 and the impulsive errors. Second, $p$ values were not adjusted for multiple testing due to concerns regarding statistical power as stated above. This raises the possibility that spurious associations may have been interpreted as substantive. As a result, we attempted to protect against potential false positive results in three ways. Specific a priori predictions regarding the nature and direction of the associations between the A-X CPT indices and DAT1 and DRD4 were made based on prior research. Additionally, multiple methods were used to test for association (i.e., between- and within-family analyses) with the assumption that findings supported by both analytic methods would be more robust and have a greater likelihood of successful replication. Lastly, for the between-family analyses, follow-up analyses were conducted to ensure the results were not due to population stratification, the presence of co-occurring symptoms of ODD and CD, or driven by a small number of individuals with low frequency genotypes. Third, the analyses were conducted using a clinic-referred sample of children ascertained for ADHD and their siblings, and thus, need to be replicated in other clinically referred and nonreferred samples before our findings can be extended to the general population or other clinical populations. Due to these limitations, the need for replication in large independent samples clearly remains.

In conclusion, the current study suggests that the Halperin A-X CPT scoring indices represent useful endophenotype measures for molecular genetic studies of ADHD. Further, the observed double dissociation in the pattern of genetic associations between the Halperin impulsive and inattentive errors and DAT1 and DRD4 represents a novel contribution to the literature. As the neural substrates underlying performance on the A-X CPT are better understood, these findings have the potential to explain the mechanisms by which these candidate genes confer risk for ADHD.

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