Dopamine receptor D4 gene variation predicts preschoolers’ developing theory of mind

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Abstract

Individual differences in preschoolers’ understanding that human action is caused by internal mental states, or representational theory of mind (RTM), are heritable, as are developmental disorders such as autism in which RTM is particularly impaired. We investigated whether polymorphisms of genes affecting dopamine (DA) utilization and metabolism constitute part of the molecular basis of this heritability. Seventy-three 42- to 54-month-olds were given a battery of RTM tasks along with other task batteries that measured executive functioning and representational understanding more generally. Polymorphisms of the dopamine D4 receptor gene (DRD4) were associated with RTM performance such that preschoolers with shorter alleles outperformed those with one or more longer alleles. However, polymorphisms of the catechol-O-methyl transferase gene (COMT) and the dopamine transporter gene (DAT1) genes were not associated with children’s RTM performance. Further tests showed that the association between DRD4 allele length and RTM performance was not attributable to a common association with executive functioning or representational understanding more generally. We conclude that DRD4 receptors, likely via their effects on frontal lobe development and functioning, may represent a neuromaturational constraint governing the stereotypical and universal trajectory of RTM development.

Introduction

Representational theory of mind (RTM) is the understanding that human agentive action is caused by internal mental states (e.g. beliefs, desires, intentions) (Wellman, 1990). Upon seeing agentive action, our first attempts to explain what has happened and predict what will come next are informed by our ability to ascribe to the actor appropriate mental states. RTM reasoning is traditionally assessed through the use of false belief tasks where children are asked to predict or explain what a person will do based upon a belief that no longer matches some true state of affairs (Wellman, Cross & Watson, 2001). The roots of RTM and competence with false belief tasks may emerge during infancy (Onishi & Baillargeon, 2005), though the preschool years are a time of major changes in children’s abilities to explicitly recruit RTM concepts to explain and predict others’ behaviors. Although there are clear environmental effects on the emergence of these explicit RTM skills, they are small and few alter a stereotypical and cross-culturally universal developmental pattern: young 3-year-olds consistently fail false belief tasks while 5-year-olds consistently pass (Liu, Sabbagh, Gehring & Wellman, 2009). Indeed, the most compelling exception to this stereotypical pattern comes from studies of individuals with autism – a neurodevelopmental disorder of genetic etiology (Brieber, Neufang, Bruning, Kamp-Becker, Remschmidt, Herpertz-Dahlmann, Fink & Konrad, 2007; Girgis, Minshew, Melhem, Nutche, Keshavan & Hardan, 2007; Lam, Aman & Arnold, 2006; McAlonan, Suckling, Wong, Cheung, Lienenkaemper, Cheung & Chua, 2008) in which the markers of RTM development are acquired slowly and sometimes out of sequence (Peterson, Wellman & Liu, 2005).

The overall developmental pattern for RTM strongly suggests a critical role for neurogenetic constraints. Initial confirmation of this conjecture has come from Hughes and colleagues (Hughes & Cutting, 1999) who used a monozygotic–dizygotic twin design to show that RTM performance is highly heritable during the preschool years. However, no work to our knowledge has focused on the molecular bases of RTM inheritance. The goal of the present study was to take a first step with this...
question by investigating whether genes responsible for regulating the neurotransmitter dopamine (DA) might constitute part of the molecular basis of RTM development.

We focused on DA-related genes for several reasons. First, recent research has shown that individual differences in DA functioning (as measured by eye blink rates) predict preschoolers’ theory of mind understanding (Lackner, Bowman & Sabbagh, 2010). Second, functional neuroanatomical changes within the dorsal medial prefrontal cortex (dMPFC) have been associated with RTM development in preschoolers (see Liu, Sabbagh et al., 2009; Sabbagh, Bowman, Evraire & Ito, 2009). The dMPFC is a major target of mesocortical DA projections; DA may play a role in fostering cell proliferation in the development of this region (Kalsbeek, Buijs, Hofman, Matthijssen, Pool & Uylings, 1987; Popolo, McCarthy & Bhide, 2004) and in maintaining functionality. Thus, DA might play a role in both developing and maintaining functioning in the prefrontal areas that are important for RTM reasoning in preschoolers.

In addition to the experimental evidence, recent work has shown that DA is important for causal learning, which itself is thought to play an important role in RTM development. For instance, DA activity is elicited when animals encounter situations in which their expectations about an event (such as a reward) do not match with what ultimately occurs (Schultz, 2000). It is generally thought that in these cases, DA activity promotes plasticity necessary for adjusting expectations and coming to increasingly refined understandings of the causal structure of a given event (Schultz, 2007). Prominent theories of theory of mind development (e.g. the ‘theory of theory’ theory) suggest that theory of mind development proceeds via similar mechanisms of refining understandings based upon the relations between predicted and observed outcomes in the domain of human behavior (Gopnik & Wellman, 1994). Thus, DA may play a key role in promoting the development of the causal understandings associated with mental state reasoning.

Several genes are known to affect levels of synaptic DA. However, three in particular have been looked at extensively in the psychological literature: the catechol-O-methyl transferase gene (COMT), the dopamine active transporter gene (DAT1), and the dopamine receptor D4 gene (DRD4). Each of these genes contains functional polymorphisms that have been studied extensively for their role in psychological processes. COMT metabolizes DA as well as norepinephrine. A substitution in codon 158 leads to two alleles, one containing methionine and the other having valine at this position. The 158Val form is a less active enzyme and leads to increased extracellular DA concentrations (Lachman, Papolos, Saito, Yu, Szumlanski & Weinshilboum, 1996). Heterozygotes for this allele demonstrate intermediate inactivation of DA. DAT1 encodes the DA reuptake transporter and has a variable number of tandem repeat (VNTR) polymorphism in the 3’ untranslated region (UTR), with the most common alleles being 9 and 10 repeats. The 9-repeat allele is associated with fewer DAT transporter proteins and therefore greater DA in the synapse and greater DA signaling (Miller & Madras, 2002; Van Ness, Owens & Kilts, 2005). DRD4 encodes the dopamine D4 receptor, and has a polymorphic 48 bp VNTR in exon III that codes for the third intracellular loop in the D4 receptor. Compared to 2- and 4-repeat alleles, the 7-repeat allele is about half as potent in inhibiting cyclic AMP (Asghari, Sanyal, Buchwaldt, Paterson, Jovanovic & Van Tol, 1995) and may result in reduced DRD4 expression, influencing RNA stability or translational efficiency (Schoots & Van Tol, 2003).

On the basis of prior research, we propose that these functional polymorphisms in DRD4, COMT, and DAT1 which promote increased DA might also be associated with stronger RTM performance. We reasoned that this might be particularly true for the DRD4 and COMT genes because they are more highly expressed than DAT1 in prefrontal regions that are important for RTM reasoning (see Matsumoto, Weickert, Akil, Lipska, Hyde, Herman, Kleinman & Weinberger, 2003; Moron, Brockington, Wise, Rocha & Hope, 2002; Primus, Thurkauf, Xu, Yevich, McInerney, Shaw, Tallman & Gallagher, 1997).

Finally, we designed our study to address the possible specificity of the relations between these DA-related genes and RTM development. Prior research has shown that RTM development is associated with two more general cognitive skills: response-conflict executive functioning (EF) and an ability to reason about non-mental representations (NMR) (Carlson & Moses, 2001; Sabbagh, Moses & Shiverick, 2006). Response-conflict executive functioning performance is measured in tasks that require the participant to make a novel response while simultaneously suppressing a prepotent response (i.e. Stroop-like tasks). NMR performance is measured in tasks that are structured like false belief tasks, but require children to reason about non-mental rather than mental representations (i.e. signs instead of beliefs). A critical question is whether the selected DA-related genes are related to RTM directly, or whether the relationship is mediated by factors common to these more general cognitive skills. The importance of investigating EF in this regard is underscored by a growing literature showing that EF performance is associated with variation in the DA-related genes we chose to include in our current investigation (Diamond, Briad, Fossella & Gehlbach, 2004; Egan, Goldberg, Kolachana, Callicott, Mazzanti, Straub, Goldman & Weinberger, 2001; Karama, Grizenko, Sonuga-Barke, Doyle, Biederman, Mbekou, Polotsjaia, Ter-Stepanian, De Guzman, Bellingham, Sengupta & Jouber, 2008; Lipsky, Sparling, Ryan, Xu, Salazar, Goldman & Warden, 2005; Rybakowski, Borkowska, Czerski, Dmitrzak-Weglacz, Skibinska, Kapelski & Hauser, 2006). Evidence that DA is associated with RTM independent of these common associations will support the contention that some aspects of
DA functioning are especially important for the development of RTM reasoning.

**Method**

**Participants**

Participants were 73 healthy, monolingual, preschool-aged children ($M_{age} = 47.25$ months, range = 42–54 months, 40 female) with no family history of autism that were recruited from a database of interested families in a primarily Caucasian, middle-class community in South-eastern Ontario. Because some prior research suggests that allelic distributions of dopamine differ by ethnicity (Lichter, Barr, Kennedy, Van Tol, Kidd & Livak, 1993), it was important that we examine this as a potential influence on our results. Ninety-seven percent of our sample ($n = 71$) were Caucasian. The two non-Caucasian children in the sample were not excluded as their behavioral performance and genetic variation was not atypical for our sample. Seventeen additional children were recruited but excluded from the final sample because they failed to complete the task batteries ($n = 16$) or because of equipment malfunction ($n = 1$). Participants received their choice of a gift certificate or a T-shirt and a small toy at the end of the testing session.

**Measures and materials**

The behavioral measures consisted of RTM, NMR, and EF tasks. Additionally, saliva samples were collected and DNA extracted for genotyping of polymorphisms of COMT, DAT1, and DRD4.

**RTM battery**

*False Belief Contents* (Gopnik & Astington, 1988). Children were shown a familiar sweets box (i.e. Smarties) with unfamiliar contents (i.e. pencil crayons). Children were asked both what they believed was inside the box before its true contents were revealed and what a character who had never seen inside the box would believe that it contains (Score: 0–2).

*False Belief Location* (Wimmer & Perner, 1983). Children were introduced to two characters, James and Sarah, who play together with a toy. Sarah leaves the room and places the toy in one location. James removes the toy from this location and places it somewhere else. The child is asked where Sarah will look for the toy when she returns (Score: 0–1).

*Deceptive Pointing* (Carlson, Moses & Hix, 1998). Children watched as the experimenter placed a ball inside one of two boxes. The child was introduced to a puppet that did not see where the toy was placed and were asked to play a trick on the puppet by making her look in the wrong box. In order to succeed at this task, children must point to the empty box (Score: 0–1).

**Non-Mental Representation (NMR) battery**

All tasks were presented in a storybook format with one scene and event per page.

*Conventional False Sign Location* (Sabbagh et al., 2006). Children were introduced to a character, Chester, who plays inside one of two houses and indicates to a friend which house he is in through the use of a sign. Chester then switches houses but fails to switch the sign. Two control questions were included, as well as the test question, ‘Where does the sign say Chester is?’ (Score: 0–1).

*Conventional False Sign Contents* (Sabbagh et al., 2006). Children were introduced to Betty, who is shown putting her cat in a box prior to leaving. She indicates that the cat is in the box by putting out a sign. In her absence, the cat jumps out of the box and a dog jumps in instead. The child is asked what the sign says is inside the box versus what is actually inside the box (Score: 0–1).

*Natural False Sign Location*. Children were introduced to a character that likes to play in two different locations and indicates his location through the use of a gate. The character switches locations but fails to switch the direction in which the gate is pointing. The experimenter asks the child to indicate the character’s true location (Score: 0–1).

*Natural False Sign Contents*. Children were introduced to two animals, a black cat and a skunk. The cat is shown to rub its back against a wall of wet paint and consequently get a white stripe down its back. The child is asked what animal the cat now looks like and what animal the cat really is (Score: 0–1).

**Executive functioning battery**

*Grass/Snow Stroop Task* (Carlson & Moses, 2001). Children were asked to point to a green square affixed to a black card whenever the experimenter said ‘snow’, and to a white square affixed to this same card when the experimenter said ‘grass’. They were given three learning trials on each color (reverse scored: 1–6 to index the number of trials required to learn the game) and then 16 test trials in a randomized order (Score: 0–16).

*Dimensional Change Card Sort Task* (Frye, Zelazo & Palfai, 1995; Zelazo, Muller, Frye & Marcovitch, 2003). Children were presented with cards depicting two different shapes (boats and rabbits) in two different colors (red or blue). In a series of five pre-switch trials
the child was asked to sort the cards into two boxes according to shape only. In five post-switch trials children were asked to sort the same cards according to color, ignoring shape. Of most interest were the three post-switch trials in which the shapes and colors were incompatible with one another (i.e. sorting according to color leads to putting the card in one box, but sorting according to shape leads to putting the same card in the other box). Correct responses on these trials required inhibiting the tendency to sort according to the pre-switch rule (Score: 0–3).

Less is More Task (Carlson, Davis & Leach, 2005). Children were introduced to a game where they could win candies as a reward. The candies were presented in small trays of three and five treats. They were introduced to a character, Naughty Monkey, who likes to keep all of the treats for himself. The child was instructed that when they pointed to a tray Naughty Monkey would get the treats in that tray and they would get the treats in the other tray. In order to obtain the maximal amount of treats for themselves, the child must always point to the smaller amount of treats, thereby inhibiting their impulse to point to the larger amount. A maximum of three learning trials were given to ensure that the child understood how the game was played (reverse scored: 1–3 to index the number of trials required to learn the game). This was followed by 12 test trials with the side of presentation of large and small amounts of treats randomized (Score: 0–12).

Hand Game (Hughes, 1998). In five pre-switch trials the child was asked to imitate the experimenter when she made one of two shapes with her hand (i.e. pointed a finger or made a fist). Each trial began with the experimenter made a fist, the child was asked to point a finger. The child was instructed that when they pointed to a tray Naughty Monkey would get the treats in that tray and they would get the treats in the other tray. In order to obtain the maximal amount of treats for themselves, the child must always point to the smaller amount of treats, thereby inhibiting their impulse to point to the larger amount. A maximum of three learning trials were given to ensure that the child understood how the game was played (reverse scored: 1–3 to index the number of trials required to learn the game). This was followed by 12 test trials with the side of presentation of large and small amounts of treats randomized (Score: 0–12).

Genetic testing

After the behavioral data were collected, the child was asked to provide a saliva sample by spitting into a medical grade sterile tube (Oragene, DNA Genotek). The majority of the children (94%) were unable to spit into the tube and so sterile sponges were used to absorb saliva from the cheek pouches.

Genomic DNA was extracted from the saliva samples following the manufacturer’s instructions (Oragene, DNA Genotek). Genotyping of the COMT Val/Met (rs4680) SNP was carried out using validated custom TaqMan SNP Genotyping Assays (http://www.applied-biosystems.com) on an ABI Prism 7900HT, using 384-well plates as previously described (Liu, Novosedlik, Wang, Hudson, Cohen, Chudley, Forster-Gibson, Lewis & Holden, 2009). Duplicate samples were included in each plate to check the accuracy of genotyping. Genotypes were automatically scored with the SDS 2.2.2 software.

Genotyping of the DAT1 VNTR polymorphism in the 3’ UTR and the DRD4 VNTR in exon III was performed using polymerase chain reaction (PCR) in a volume of 5 μL on a MJ Dyad thermal cycler (MJ Research, Watertown, MA). PCR reactions for DAT1 VNTR contained 10 ng genomic DNA, 1.5 mM MgCl2, 0.2 mM of each dNTP (deoxyribonucleotides), 0.5 μM of each primer (Forward: 5’-TGTGGTGTTAGGGAAACG GCCTGAG; Reverse: 5’-CTTCCTGGAGTGTCACGGC TCAAGG), 10% DMSO (dimethyl sulfoxide) and 0.5 unit of Taq DNA polymerase. The cycling conditions were: initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 65°C for 1 minute and extension at 72°C for 30 seconds, with a final extension step at 72°C for 6 minutes. The PCR reaction mix for the DRD4 48 bp VNTR contained 1 X Q-Solution (Qiagen), 1 X Buffer (Qiagen), 0.5 μM of each primer (Forward: 5’- GCGACTACGTGGTCTACTCG; Reverse: 5’-AGGACCCTATGGCCCTTG), 0.2 mM of each dNTP, 0.2 units HotStar Taq polymerase (Qiagen) and 10 ng of DNA template. The Cycling conditions were: 15 min at 95°C to activate the enzyme and denature the DNA, 38 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 55°C, 1.5 minute extension at 72°C, followed by 10 minutes of extra extension at 72°C. The PCR products were separated on 2% agarose gels with ethidium bromide staining and visualized under ultraviolet illumination. The genotypes were scored from these gels.

Following other research, we compared children with two short DRD4 alleles (both alleles ≤ 4 repeats) to those with at least one long allele (≥ 6 repeats). While some research has singled out the 7-repeat allele for investigation, we focused on the overall DRD4 VNTR genotypes. These participants were excluded from analyses including that particular gene but in order to maximize statistical power were included in all other analyses.

Procedure

All children were tested in the Early Experience Lab at Queen’s University during one 75 minute session. Participants were asked to work with the lab and informed consent was obtained from parents and assent obtained from the
children. Testing proceeded in the following fixed order: (1) False Sign Location, (2) False Belief Contents, (3) Natural False Sign Location, (4) False Belief Location, (5) Natural False Sign Contents, (6) False Sign Contents, (7) Deceptive Pointing, (8) Deceptive Pointing, (9) Dimensional Change Card Sort, (10) Less is More, and (11) Hand Game. After behavioral task administration, we collected the saliva sample. The use of such a fixed task order helps ensure that all participants’ experiences are as similar as possible to one another, therefore strengthening the possibility that differences found are due to true individual differences rather than to variance in task administration.

Twenty percent of the video tapes for RTM and RC-EF tasks were re-coded by a second observer. Inter-rater agreement was greater than 95% in all cases, Kap- pa = .969, p < .001. Where there was disagreement, the raters discussed until a mutual decision could be made.

Results

The descriptive statistics for each of the individual RTM, EF, and NMR tasks and the task battery composites are summarized in Table 1. We initially planned on using the False Belief Location task, the False Belief Contents task and the Deceptive Pointing task in our RTM composite. However, the internal consistency of this battery was quite poor (α = .095). Removing the False Belief Contents task from the battery resulted in a substantial increase in alpha (α = .43; Φ = .277, p = .022), therefore we elected to remove the False Belief Contents task from the battery. Task battery composites were created by summing z-scores on each of the individual tasks. Using such an approach rather than simply summing the individual scores allows for each individual’s score to be weighted relative to the performance of the rest of the sample. Table 2 shows the intercorrelations among each of the task composites, and their individual associations with age. In line with previous research (e.g. Carlson & Moses, 2001), the task batteries showed strong intercorrelations and all were positively correlated with age. Independent samples t-tests revealed no significant sex differences on any of the behavioral task batteries, all ts < .79, all ps > .44. Table 3 contains information on the sample distribution of allelic variants.

Our focal interest was whether allelic variations in DA-related genes would predict RTM performance and, if so, whether possible associations exist independent of common associations with either EF or NMR performance. To answer the first question, we conducted a set of ANCOVA analyses testing for effects of allele types for each of the three genes on each of the three behavioral tasks, including age as a covariate. The means for these analyses are presented in Figure 1.

One-way ANCOVAs provided no evidence that the genotypes at DAT1 or COMT were associated with RTM performance controlling for children’s age. However, there was an association between the 48 bp VNTR in DRD4 and RTM performance such that children with two short DRD4 alleles (≤ 4 repeats) had stronger RTM performance than children with at least one long DRD4 allele (≥ 6 repeats), F(1, 68) = 8.19, p = .006, η² = .107. These results remained significant even when two non-Caucasian children were removed from the sample.

As expected based upon prior research, a one-way ANCOVA showed that DRD4 genotype was also associated with EF performance, F(1, 68) = 5.09, p = .027, η² = .070. DRD4 genotype was not associated with NMR performance, F(1, 68) = 0.129, p = .721, η² = .002. These findings raise the possibility that EF may be mediating the association between RTM and DRD4 genotype. To test this possibility, we conducted a regression mediation analysis. This analysis showed that after controlling for age, although the initial magnitude of the relation between DRD4 genotype and RTM (b = −.293, t = 2.86, p = .006) was significantly reduced by including EF in the model (Sobel’s test, z = 1.97, p = .048), mediation was only partial as the relation between DRD4 genotype and RTM remained significant (b = − .202, t = 2.053, p = .044). Thus, DRD4 genotype makes a unique independent contribution to RTM rea-

Discussion

Our study is the first to explore the molecular bases of individual differences in RTM performance. We found evidence that the variable 48 bp VNTR in exon III of the DRD4 gene predicted RTM performance such that individuals with two shorter alleles (4 or fewer repeats)

Table 1 Descriptive statistics for behavioral tasks

<table>
<thead>
<tr>
<th>Measure</th>
<th>Possible range</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>False Belief Location</td>
<td>0–1</td>
<td>.50</td>
<td>.50</td>
</tr>
<tr>
<td>False Belief Contents</td>
<td>0–2</td>
<td>1.10</td>
<td>.73</td>
</tr>
<tr>
<td>Deceptive Pointing</td>
<td>0–1</td>
<td>.75</td>
<td>.44</td>
</tr>
<tr>
<td>Grass/Snow Stroop task learning</td>
<td>1–6</td>
<td>3.87</td>
<td>1.44</td>
</tr>
<tr>
<td>trials reverse scored</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test trials on Grass/Snow Stroop task</td>
<td>0–100</td>
<td>66.72</td>
<td>34.16</td>
</tr>
<tr>
<td>Less is More task learning trials reverse scored</td>
<td>1–3</td>
<td>2.14</td>
<td>.78</td>
</tr>
<tr>
<td>Test trials on Less is More task</td>
<td>0–100</td>
<td>71.5</td>
<td>31.34</td>
</tr>
<tr>
<td>Hand Game</td>
<td>0–10</td>
<td>6.58</td>
<td>2.20</td>
</tr>
<tr>
<td>Dimensional Change Card Sort</td>
<td>0–3</td>
<td>1.52</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Table 2 Intercorrelations among task batteries and age

<table>
<thead>
<tr>
<th>Age</th>
<th>EF</th>
<th>RTM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>.326**</td>
<td>.521***</td>
</tr>
<tr>
<td>EF</td>
<td>.449***</td>
<td>.438***</td>
</tr>
<tr>
<td>RTM</td>
<td>.514***</td>
<td></td>
</tr>
<tr>
<td>NMR</td>
<td>.415***</td>
<td></td>
</tr>
</tbody>
</table>

*** p < .001; ** p < .01.
outperformed those with one or two longer alleles (6 or more repeats). This relationship was independent of common associations with skills previously linked to DA functioning (i.e. EF tasks) and similarly structured control tasks (i.e. NMR tasks). These findings raise the possibility that the dopamine D4 receptor plays an especially important role in social reasoning.

The findings that common polymorphisms in \textit{DRD4}, but not \textit{COMT} or \textit{DAT1}, were associated with RTM performance provides some insight into the nature of the relation between DA and RTM. Prior work had established that general levels of DA are associated with RTM reasoning in preschoolers (Lackner \textit{et al.}, 2010), but left questions about the precise neurobiological mechanisms for that association. Though all three gene products can affect levels of synaptic DA, the different DA genes we tested played different roles within the DA system (\textit{DRD4}: receptor binding; \textit{COMT}: metabolism; \textit{DAT1}: dopamine reuptake). Thus, the current pattern of findings suggests that DA binding may be particularly important for RTM development. Given prior research showing that \textit{DRD4} is preferentially expressed in frontal lobes, we might speculate that frontal DA binding makes an especially important contribution to RTM development. This speculation dovetails nicely with recent findings showing that particular aspects of medial frontal lobe development make unique contributions to theory of mind development (Liu, Sabbagh \textit{et al.}, 2009; Sabbagh \textit{et al.}, 2009).

An important question concerns how, specifically, RTM development might be affected by dopamine D4 receptors and frontal DA binding. As noted above, we focused our initial investigation on DA genes because of the role that they play in both the development and function of the regions of medial prefrontal cortex that are important for RTM reasoning. Thus, it is possible that genes affecting DA levels provide one source of neurodevelopmental constraint on the development of RTM skills in preschool children. Understanding the sources of neurodevelopmental constraint on RTM development may provide important insight into reasons why there is an essentially universal broad developmental timetable and trajectory for this foundational social cognitive skill. That is, DA and genes that affect its utilization and metabolism may play a critical role in pacing the maturation of the cortical regions that are ultimately important for RTM development.

A second possibility is that DA may also help children learn from RTM-relevant experiences. As noted from the

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Gene & Polymorphism & Total N & Males & Females & Mean Age (SD) \\
\hline
\hline
\textit{COMT} & Val/Val & 13 & 9 & 4 & 47.08(3.30) \\
& Val/Met & 43 & 20 & 23 & 46.95(3.53) \\
& Met/Met & 16 & 8 & 8 & 48.19(3.35) \\
\hline
\textit{DAT1} & 10/10 & 42 & 18 & 24 & 47.59(3.58) \\
& 10/9 & 21 & 13 & 8 & 46.86(3.23) \\
& 9/9 & 7 & 5 & 2 & 46.71(3.45) \\
\hline
\textit{DRD4} & All \leq 4 repeat & 43 & 23 & 20 & 47.38(3.32) \\
& \geq 6 repeats & 28 & 14 & 14 & 47.11(3.65) \\
\hline
\end{tabular}
\caption{Distribution of allele variations and relevant demographic information}
\end{table}

\begin{figure}
\centering
\includegraphics{Figure1}
\caption{Mean standardized task battery performance controlling for age (± 1 SE) by genotypes at (a) \textit{COMT}, (b) \textit{DAT1} and (c) \textit{DRD4}.}
\end{figure}

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outset, in addition to parent–child conversation, there are a number of experiential factors that affect the trajectory of RTM development. It is generally presumed that these factors have their effects by providing children with the experiences that allow them to notice when their nascent conceptualizations of the mind make incorrect predictions about human behavior and to revise those conceptualizations in line with available evidence. There is burgeoning evidence that this process of revising internal models based on feedback following erroneous predictions is associated with DA functioning (Schultz, 2007). Thus, DA may help children to capitalize on experiences that promote RTM development. In addition, DRD4 may exert its effects on RTM most strongly through a direct interaction effect with the environment. Some recent research has suggested that DRD4 interacts with environmental variables such as maternal sensitivity to predict behaviors related to EF (Bakermans-Kranenburg & van Ijzendoorn, 2006), and therefore the possibility exists that similar gene × environment interactions will be uncovered for RTM development.

In addition to helping to better understand the mechanisms by which RTM reasoning emerges in typically developing preschoolers, there are some intriguing clinical implications of our findings. Most directly, one possibility is that identifying genetic factors associated with typical RTM development might point the way to understanding some aspects of the etiology of autism – a heritable neurodevelopmental disorder in which RTM is particularly impaired. To date, no study has found a link between DRD4 variants and autism (Yirmiya, Pilowsky, Nemanov, Arbelle, Feinsilver, Fried & Ebstein, 2001), although one study implicates the DRD1 gene in the etiology of autism spectrum disorders (Hettinger, Liu, Schwartz, Michaelis & Holden, 2008). Also, some general support for the hypothesis that DA is involved in autism comes from research showing that treatments aimed at improving DA functioning have had positive effects in ameliorating symptoms of autism (Lam et al., 2006). Yet, perhaps more promising for understanding molecular genetic risk factors for autism is our more specific speculation that frontal DA binding is important for RTM development. Many genetic factors can affect frontal DA binding directly or indirectly. For instance, genetic variation in the monoamine oxidase A (MAOA) gene can affect the extent to which MAOA can deaminate monoamines such as DA, rendering them functionless. Increased levels of MAOA lead to decreased levels of DA, and thus a reduction in frontal DA binding. Also, the serotonin system inhibits the firing of the dopaminergic system at the midbrain (Jacobs & Azmitia, 1992) therefore decreasing the likelihood of activating the frontal DA system. Our findings lead us to speculate that aspects of the suite of genes that affect frontal DA binding may account for part of the heritability of autism, even if DRD4 is not part of that complex.

These findings also raise questions about whether there might be intrinsic connections between RTM and other aspects of behavior that have been connected with DRD4, such as the personality trait of novelty seeking (Ebstein, Novick, Uriansky, Priet, Osher, Blaine, Bennett, Nemanov, Katz & Belmaker, 1996). Strong associations are typically found in adults between DRD4 and novelty seeking, a personality trait correlated with executive functioning performance (Cassimjee & Murphy, 2010). Specifically, individuals who have longer DRD4 alleles and poorer EF skills tend to be high in novelty seeking. Given this pattern of associations, we might expect that children who score lower on measures of novelty seeking (and thus, have a tendency towards stronger EF performance) might have better RTM skills. We know of no studies that have investigated this question systematically in typically developing individuals. However, one study showed that autistic individuals, who typically have poor RTM skills, tend to score low on standard measures of novelty seeking (Anckarsater, Stahlberg, Larson, Hakansson, Jutblad, Niklasson, Nydén, Wentz, Westergren, Cloninger, Gillberg & Rastam, 2006), thereby suggesting that the link between RTM and novelty seeking may be complicated. Future more direct investigation of the link between novelty seeking and RTM that is hinted at by their common association with DRD4 is necessary to draw firmer conclusions.

It is important to note that our sample was largely Caucasian which may weaken the generalizability of our findings. Allelic variations in the dopamine D4 receptor gene have been shown to vary as a function of ethnogenetic background (Lichter et al., 1993). Although we have no reason to believe that these genes function any differently in differing ethnic populations (i.e. the short alleles of DRD4 should always result in greater binding), future research is warranted investigating the presently reported effects in other ethnically divergent genetic samples. Moreover, we acknowledge that, as in much research in the field of cognitive development, there may have been a potential selection effect in participant recruitment. Families of low socioeconomic status (SES) are less likely to participate in university-centered research, which potentially limits the generalizability of the findings. A much broader investigation that endeavors to include a more diverse sample will help in determining the extent to which the present findings generalize to the wider population.

In conclusion, our goal was to investigate the possibility that the heritable basis of theory of mind development might be in part attributable to genes that affect DA functioning. Our findings provided support for this hypothesis by showing that variations of the dopamine D4 receptor gene (DRD4) are associated with children’s developing performance on theory of mind tasks. These findings specifically suggest that constraints on the processes that affect frontal DA binding may be particularly important for the development of theory of mind, and perhaps social cognition broadly. More generally, these findings point in an exciting new direction for
understanding the nature and source of neurodevelopmental constraints on theory of mind development, and provide a basis for further research in the area.

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