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Review

Genetic targeting of the amphetamine and methylphenidate-sensitive dopamine transporter: On the path to an animal model of attention-deficit hyperactivity disorder

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ABSTRACT

Alterations in dopamine (DA) signaling underlie the most widely held theories of molecular and circuit level perturbations that lead to risk for attention-deficit hyperactivity disorder (ADHD). The DA transporter (DAT), a presynaptic reuptake protein whose activity provides critical support for DA signaling by limiting DA action at pre- and postsynaptic receptors, has been consistently associated with ADHD through pharmacological, behavioral, brain imaging and genetic studies. Currently, the animal models of ADHD exhibit significant limitations, stemming in large part from their lack of construct validity. To remedy this situation, we have pursued the creation of a mouse model derived from a functional nonsynonymous variant in the DAT gene (*SLC6A3*) of ADHD probands. We trace our path from the identification of these variants to *in vitro* biochemical and physiological studies to the production of the DAT Val559 mouse model. We discuss our initial findings with these animals and their promise in the context of existing rodent models of ADHD.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is the most commonly diagnosed neuropsychiatric disorder of childhood, affecting an estimated 4–12% of school-age children (Biederman and Faraone, 2005; Polanczyk et al., 2007; Willcutt, 2012). Adult ADHD is also fairly common, estimated at 4–5% of adults (Fayyad et al., 2007; de Graaf et al., 2008; Kessler et al., 2006). More recent studies suggest that the rates of adult ADHD may actually be greater than 10% (Cahill et al., 2012; Garnier-Dykstra et al., 2010). Indeed, though ADHD is often considered a disorder of childhood and adolescence, studies suggest that ADHD symptoms persist into adulthood in 60–70% of cases (Biederman et al., 2000; Kessler et al., 2005). Like all neuropsychiatric disorders, ADHD presents with a spectrum of behavioral alterations, with features of motor hyperactivity, impulsivity, and/or inattention providing the diagnostic criteria used for diagnosis (American Psychiatric Association, 1994). There are no biomarkers for ADHD, and therefore diagnoses are based on clinical observation, as well as parent and teacher reports (Visser et al., 2013; Wolraich et al., 2003, 2013). ADHD diagnoses exhibit an ~3:1 male:female bias (Gaub and Carlson, 1997; Getahun et al., 2013). Whether this sex bias arises from cultural or biological factors (or both) that impact ADHD risk is unknown. Interestingly, rates of ADHD diagnosis are consistent among different cultural groups, as studies of populations in Africa (Bakare, 2012), Asia (Chien et al., 2012), and Europe (Bianchini et al., 2013; Ezpeleta et al., 2013) report similar prevalence and sex bias. These findings suggest that although environmental factors may be shared across communities, strong biological risk factors likely drive features and risk of the disorder and ultimately, diagnosis.

1.1. Support for a DA Connection to ADHD

A large body of research demonstrates that the dopamine (DA) system underlies the hallmark symptoms of ADHD. For example, locomotor hyperactivity, a main feature of ADHD as well as ADHD animal models, can be induced by treatment with a DA D1 receptor agonist (Brent, 1991; Dreher and Jackson, 1989; Tirelli and Terry, 1993) or psychostimulants that block DAT and increase synaptic DA concentrations (van Rossum and Hurkmans, 1964; Smith, 1964; Zubrycki et al., 1990). Conversely, depletion of DA with

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reserpine (Johnels, 1982; Sugita et al., 1989) or lesion of DA neurons with 6-hydroxydopamine (Erinoff et al., 1979; Joyce and Koob, 1981) or MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Colotla et al., 1990; Gnanalingham et al., 1995; Sahgal et al., 1984; Tsai et al., 1991) leads to a hypokinetic state. Similar hypokinetic characteristics are observed in Parkinson's disease (Morris et al., 1994; Sian et al., 1999), a brain disorder characterized by death of DA neurons in the substantia nigra.

In addition to locomotor hyperactivity, differences in the DA system have been reported to underlie impulsivity. Work in rodents (Puumala and Sirviö, 1998; Winstanley et al., 2005) and humans (Buckholtz et al., 2010) have demonstrated that differences in DA and DA receptor levels correlate with impulsive traits. Consistent with these observations, animal studies indicate that DA receptor antagonists can reduce impulsivity, whereas AMPH can increase impulsivity (Burton and Fletcher, 2012; Wade et al., 2000). With respect to human studies, striatal DAT (Costa et al., 2013a) and D2-like receptor (Ghahremani et al., 2012; Trifilieff and Martinez, 2014) availability correlate with impulsive traits in healthy human subjects, whereas treatment with DA receptor agonists increases impulsivity (Barake et al., 2013; Ondo and Lai, 2008; Voon et al., 2010). Consistent with these findings, genetic variation in a number of components of DA signaling have been associated with impulsivity (Dalley and Roiser, 2012; Forbes et al., 2009).

1.2. Support for a DAT connection to ADHD: pharmacological considerations

A major connection between DAT and ADHD is the utility of DAT-targeted agents for treatment of the disorder (Bitter et al., 2012; Vaughan and Kratochvil, 2012). Thus, both methylphenidate (MPH) (e.g. Ritalin®) and amphetamine (AMPH) formulations (e.g. Adderall®) are effective in the treatment of subjects with ADHD (Faraone et al., 2002; Janols et al., 2009; Minzenberg, 2012; Treuer et al., 2013). Psychostimulant treatment in ADHD is often labeled as "paradoxical", since, as indicated by their categorical definition, ADHD medications, particularly D-amphetamine, can produce motor activation (Glick and Milloy, 1973; Ralph et al., 2001) and disruption of cognitive performance (Ornstein et al., 2000; Sanday et al., 2013; Stefani and Moghaddam, 2002), even psychosis (Bramness et al., 2012; Grant et al., 2012; Segal and Kuczenski, 1997; Wallis et al., 1949). Some researchers discount differences in response between ADHD and normal adolescents to psychostimulants, reporting that pre-pubertal adolescents respond to psychostimulants oppositely to that seen in adults (Rapoport et al., 1978; Zahn et al., 1980), and thus the "paradoxical" effect of psychostimulants in ADHD may be more a bias based on expectations from adult actions of the drugs. However, this position seems to be at odds with continued medication benefits seen by adults with the disorder. With respect to animal models, psychostimulant drugs increase locomotor activity in both adult and adolescent rodents (Cirulli and Laviola, 2000; Gainetdinov et al., 1999; Good and Radcliffe, 2011; Kameda et al., 2011). Whether a "normal animal" is an appropriate model for the actions of these drugs in ADHD is certainly debatable. As we discuss later in this report, psychostimulants decrease locomotor hyperactivity in a number of current animal models of ADHD (Gainetdinov, 2010; Russell, 2011). But such observations may be misleading if the underlying causes of ADHD are not mirrored in the model, leading to convergent behavioral characteristics that appear related to the human disorder but that may diverge when it with respect to utility in understanding ADHD mechanisms.

Support for a role of altered DA signaling also comes from the actions of ADHD medications. MPH is a competitive DAT antagonist like cocaine, though more slowly acting, whereas AMPH perturbs DA signaling through multiple mechanisms (Sulzer et al., 2005). First, AMPH is a competitive substrate for DAT, precluding normal DA clearance. Second, the drug acts to trigger depletion of vesicular DA stores by a weak-base action on the intravesicular pH gradient that is required to concentrate DA. Recently, this action has been reported to differentially impact readily releasable versus reserve pool vesicles (Covey et al., 2013), where depletion was observed to occur with a loss of DA from reserve pool vesicles and an enhancement of vesicular release involving the readily releasable vesicle pool. The latter observation may involve the ability of AMPH to elevate intracellular Ca^{2+}, known also to support the phosphorylation of DAT on the transporter's N-terminus (Fog et al., 2006; Gnegy et al., 2004; Khoshbouei et al., 2004; Wei et al., 2007). Phosphorylation (evidence implicates CaMKII, and possibly PKCβ) then leads to an increased probability for DAT-dependent efflux of cytoplasmic DA. Fourth, AMPH is an MAO antagonist, and through a lack of DA catabolism leads to further elevation of DA cytoplasmic levels.

1.3. Support for a DAT connection to ADHD: brain imaging studies

Positron emission tomography (PET) methods have afforded a direct inspection of DAT levels in the brain of human ADHD subjects (Varrone and Halldin, 2010; Zimmer, 2009). However, the findings with this approach have been mixed, possibly due to prior drug exposure in some studies (Fusar-Poli et al., 2012). Thus, whereas DAT binding in the basal ganglia of both children (Cheon et al., 2005) and adults (Dougherty et al., 1999; Dresel et al., 2000; Krause et al., 2000) has been reported to be increased (Spencer et al., 2007) in ADHD, others have seen no change (van Dyck et al., 2002) or decreased DAT density in ADHD (Volkow et al., 2007).

Subsequent studies have focused on brain imaging abnormalities within specific domains of ADHD. Volkow and colleagues correlate reduced DAT and D2-like receptor availability in ADHD subjects with motivational deficits stemming from dysfunction in dopamine reward pathways (Volkow et al., 2011). However, other groups report that increased DAT availability contributes to impulsivity (Costa et al., 2013a; Forbes et al., 2009). Studies examining DAT variability (namely the DAT 3′ VNTR, discussed in Section 2.4) have similarly disparate findings, with a study suggesting an association between the DAT VNTR 10-repeat allele and frontal, medial, and parietal activation during a response inhibition task (Braet et al., 2011), and meta-analysis of SPECT studies reporting no effect of the DAT VNTR on striatal DAT availability (Costa et al., 2011).

Some of the conflicting reports of DAT availability in ADHD may reflect a differential role for DAT in specific ADHD traits. However, methodological limitations, including inadequate sensitivity and imaging ligands affected by endogenous DA (Weyandt et al., 2013), may restrict our ability to interpret imaging studies. Further research is required to improve DAT imaging approaches and clarify our understanding of DAT in ADHD.

1.4. Support for a DAT connection to ADHD: genetic studies

Many lines of evidence implicate DAT and DA receptors in ADHD. For example, genetic studies have repeatedly demonstrated an association between ADHD and D1 (Bobb et al., 2005; Ribases et al., 2012), D2 (Nyman et al., 2007), D4 (Roman et al., 2001; Bidwell et al., 2011) and D5 (Manor et al., 2004) receptors, though how the genetic variants perturb receptor function in ADHD is unclear. Several studies have also observed association between core components of ADHD and variation in the DAT gene, including spatial working memory function (Brehmer et al., 2009; Li et al., 2012; Shang and Gau, 2013), attentional asymmetry (Bellgrove et al., 2005; Newman et al., 2012), impulsivity (Costa et al., 2013a; Forbes et al., 2009; Paloyelis et al., 2010), response inhibition (Cornish

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