mSURJ
McGill Science Undergraduate Research Journal

While the molecular basis receives attention, development of a molecular-based diagnosis is still in a deficit: understanding Attention Deficit Hyperactivity Disorder

Jason Behrmann*

Department of Biochemistry, McGill University, McIntyre Medical Building, 3655 Promenade Sir William Osler, Montréal, Québec, Canada H3G 1Y6.

Abstract

Attention deficit hyperactivity disorder is the most prevalent childhood-onset behavioral disorder, affecting approximately 8% of the population, where a disproportionate amount of males are afflicted. Common symptoms of the disorder include inattentiveness, hyperactivity, and impulsivity. Genomewide linkage analyses have demonstrated that the disorder is likely due to several genetic factors, whereby the dopaminergic, serotonergic, and noradrenergic neurotransmitter systems are highly implicated through various observations. Genetic screens of afflicted individuals have implicated the presence of specific genetic polymorphisms with ADHD, examples being the 10-repeat-40-base-pair allele of the dopamine transporter, DAT-1, and the silent-G861C-substitution allele of the serotonin receptor, 5-HT1B. Evidence is emerging that proteins involved in the release of neurotransmitters from synaptic vesicles, like SNAP-25, may also be involved in the pathology of ADHD. The most common method of treatment is the administration of psychostimulants, like amphetamine derivatives and methylphenidate (Ritalin®), drugs which target the dopaminergic system. New therapies that target other neurotransmitter systems, like the selective noradrenaline transport inhibitor, atomoxetine, are gaining recognition as effective treatments. Common methods to diagnose ADHD reside in psychological assessments. As more insight is gained into the genetic basis for the disorder, it appears likely that a clinical diagnostic test based on genetic screening for these factors, such as specific genetic polymorphisms, could serve as an additional means of diagnosis.

Keywords

ADHD, genomewide linkage scan, genetic loci, genetic polymorphisms, psychostimulants, neurotransmitters.

List of abbreviations

ADHD: attention deficit hyperactivity disorder
DA: dopamine
5-HT: serotonin
MCP: meta-chlorophenylpiperazine
NE: norepinephrine
AMP: dextroamphetamine
MET: methylphenidate (Ritalin®)

Attention deficit hyperactivity disorder (ADHD) is a disorder with a strong genetic component and is observed to have many symptoms that often vary amongst individuals. The few commonalities of the disorder, as stated in the DSM-IV definition (American Psychiatric Association 1994), are that it generally becomes apparent in early to mid-childhood where the symptoms of inattentiveness, hyperactivity, and impulsivity are present. ADHD symptoms have now been sub grouped into three categories: inattentive individuals, those who are hyperactive, and those who possess a combination of both (combined ADHD). More specifically, ADHD individuals are described as constantly fidgeting, impatient, incessantly talkative, interruptive, distractible, engaging in physically dangerous activities, and impatient. Often, a combination of some but not all of these behavioral attributes are observed in ADHD individuals.

Many populations of school-age children from around the world have been assessed for the prevalence of the disorder. The results show that approximately 5-10% of children and adolescents are affected, making this the most common childhood-onset behavioural disorder (Wolraich, Hannah et al. 1996). It has been observed that out of the population diagnosed with ADHD, young males were apparently 4 to 8 times more abundant than young females (Anderson, Williams et al. 1987; Zametkin, Nordahl et al. 1990).

ADHD has been documented since the early nineteen hundreds, when a successful treatment for the disorder was first observed in 1937 by Charles Bradley upon administering amphetamine derivatives to hyperactive children (Bradley 1937). Today, the most commonly employed method of treatment is the administration of psychostimulants (Solanto 1998), such as methylphenidate (Ritalin®), where such treatments have been shown to be effective for the long-term without demonstrating long-term adverse effects (Stevenson and Wolraich 1989; Wilens and Biederman 1992; Gillberg, Melander et al. 1997). Interestingly, since 1990 the use of psychostimulants for the treatment of ADHD has been observed to have more than tripled (Seeman and Madras 1998).

Numerous studies have had this psychological disorder as their focal point but despite modern genetic, psychological, and neurophysiological studies, relatively little is understood concerning the exact molecular mechanisms of ADHD and the specific mechanisms of action of several therapeutic drugs used as treatment (Solanto 1998). The purpose of this review is to provide a general summary of the neuropathology of the disease with focus placed on abnormalities at the biochemical level and review current knowledge on therapeutic drugs for the disorder. The initial section will provide a summary of the genes and gene products associated with ADHD, while the subsequent section will focus on pharmacological

*Corresponding author.. E-mail: jbehrm@po-box.mcgill.ca

treatments for the disorder and what is known about their modes of action. The general conclusion will address areas of interest for future research and critical suggestions for a method to develop a diagnostic test for ADHD that is based on biochemical markers for the disorder.

	Dopamine receptor			Dopamine transporter	Serotonin receptor		Neurotransmitter release (SNARE Proteins)		
Biochemical Factor Implicated in ADHD Pathology	DRD-1	DRD-4	DRD-5	DAT-1	5-HT1	5-HT2	SNAP-25	Syntaxin-8	RIM1
Corresponding Chromosomal Loci	5q35.1	11p15.5	4p16.1 -p15.3	5p15.3	6q13	13q14	20p11.2 -q21	17p12	6q12 -q13
Similar Loci Previously Implicated?	Yes Chr.5	No	No	Yes 5p13	Yes 6q14	No	No	Yes 17p11	Yes 6q14
List of Other Previously Implicated Loci	7p, 9q, 11q25, 15q, 16p13, 20q13								

Table 1. Summary of genetic loci and biochemical factors implicated in ADHD. Listed above are biochemical factors, grouped by their general function, implicated in the pathology of ADHD and their known genetic loci. Gray boxes denote genetic loci previously implicated through genome linkage analysis, some of which roughly superimpose to the genetic loci of the aforementioned biochemical factors. It is noted that the SNARE proteins involved in neurotransmitter release, Syntaxin-8 and RIM1, have not been formally implicated in ADHD. 'Chr.5': chromosome 5.

Isolation of genetic factors for ADHD

Numerous studies have demonstrated that ADHD is often familial, just like many other psychological disorders (Smalley 1997). One notable group of studies demonstrating the strong genetic link for the disorder were concordance studies between twins. Two large studies (Goodman and Stevenson 1989; Sherman, McGue et al. 1997) found that monozygotic twins had a concordance rate for ADHD of 51 and 58 % while dizygotic twins had a concordance rate for ADHD of only 33 and 31%, respectively. These studies reported a heritability estimate of 64 and 79%, respectively. Several adoption studies have also demonstrated that ADHD is determined far more by one's heredity rather than the environment in which children were raised. For example, ADHD children who were adopted and raised in separate homes from their biological siblings had higher rates of hyperactivity similar to their biological siblings, but unlike their adoptive siblings (Safer 1973).

The mode of inheritance of ADHD is complex and non-Mendelian since it appears to be a polygenetic disorder that shows incomplete penetrance **(Table 1)**. For instance, a systematic genomewide linkage scan on affected siblings that was performed by Fisher et al. (2002) implicated several genetic loci. One focus of this study was a meticulous screen of the X chromosomes of affected pairs of brothers. From this scan they concluded that the apparent excess of affected males with the disorder was not due to an X-linked recessive factor. A second genome linkage scan performed by Bakker et al. (2003) on siblings implicated five loci, all on different chromosomes. Each region was assessed by a multipoint maximum likelihood score (MLS) where a 15q locus had the maximum MLS value of 3.54, which was obtained from a sample of sibling pairs that met a standard, broad phenotype definition for ADHD. Loci located on the chromosome regions 7p and 9q also had notable MLS values, where these values were obtained from a sample of sibling pairs that met a narrower definition for the ADHD phenotype. Both Fisher et al. (2002) and Bakker et al. (2003) implicated chromosome 5 for possessing a possible factor for ADHD. A third analysis of affected sibling pairs performed by Smalley et al. (2002) found that a 12 centiMorgan region on chromosome 16p13 was a major locus for ADHD. A fourth linkage analysis of affected sibling pairs was performed by Ogdie et al. (2003) where five new regions, each on a separate chromosome, were found to have significant MLS values. These regions were 20q13, 17p11, 11q25, 6q14, and 5p13. The regions on chromosome 17 had the

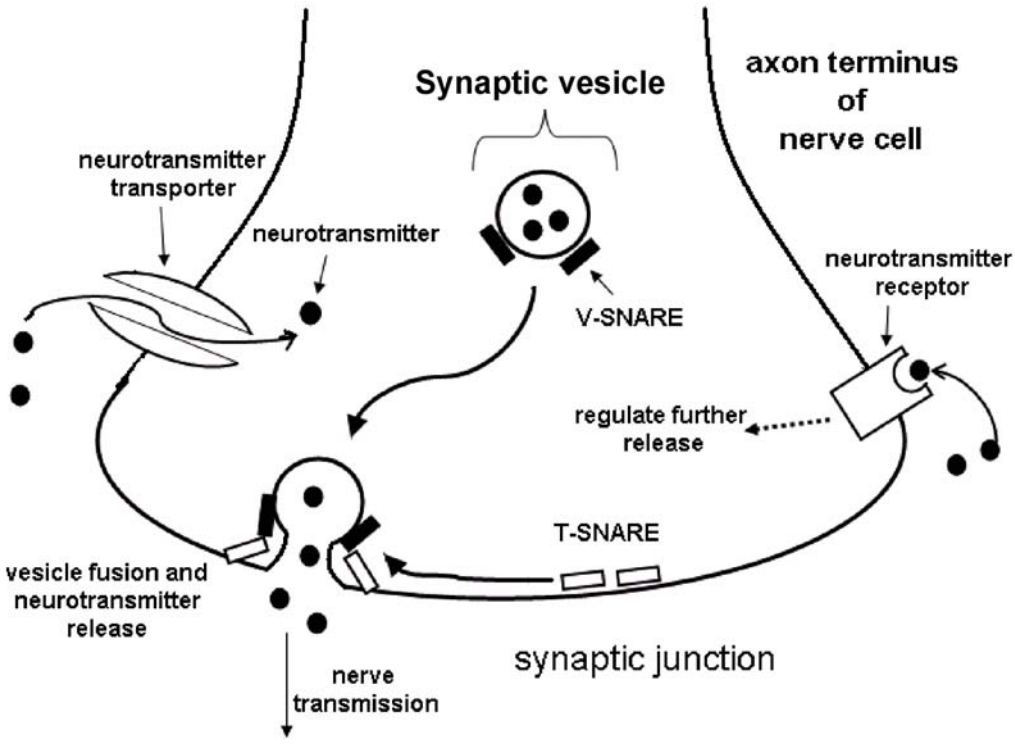

Figure 1. Basic overview of biochemical factors involved in neurotransmitter release that will be pertinent to our discussion of ADHD pathology. Within nerve cells, neurotransmitters that mediate nerve transmission are packaged into vesicle bodies known as synaptic vesicles. Synaptic vesicles are targeted to the presynaptic membrane through SNARE proteins, v-SNARE members being associated with the vesicle and t-SNARE members with the presynaptic membrane. Upon stimulus, membrane fusion occurs and the release of the vesicle cargo into the synaptic space results in nerve transmission. Once nerve transmission is conveyed, neurotransmitter concentration dissipates in the synaptic space by passive diffusion or by being returned into the axon terminus of the presynaptic nerve cell through a specific transporter. Additionally, neurotransmitters in the synaptic space may bind to specific receptors that serve to regulate subsequent neurotransmitter release.

highest MLS score (2.98) of the set. In conclusion, the aforementioned linkage analyses strongly implicate chromosome regions 17p11, 16p13, and 15q in ADHD and these should be the focus of further study. Although regions from chromosome 5 had less significant MLS scores, the fact that this chromosome was identified in three separate analyses is quite noteworthy. Since the disorder is most likely due to a combination of genes, the effect of an individual locus on the appearance of the disorder is likely to be small (Hawi, Dring et al. 2002).

Biochemical and molecular genetic aspects of ADHD

From a biochemical perspective, the best known causative factors of ADHD mainly involve the dopaminergic, noradrenergic, and the serotonergic neurotransmitter systems (Figure 1). The association of the dopaminergic system with ADHD has been, by far, the most intensively studied (Solanto 1998). The concentration of brain dopamine (DA) shows biphasic action concerning locomotion, where an overabundance of DA release during nerve transmission stimulates abnormal movement (Hornykiewicz 1966), as in patients afflicted with Parkinson's disease, and decreased DA release during nerve transmission reduces movement (Stromberg and Svensson 1975). Certain studies that have monitored DA levels in the brain have concluded that elevated DA release can be correlated with the severity of ADHD symptoms (Castellanos, Elia et al. 1994; Ernst, Zametkin et al. 1997). Two types of dopaminergic system proteins have been associated to ADHD pathology: the DA receptors and DA transporters, where both function as key regulators of DA concentration in the synaptic space (Seeman and Madras 1998). Certain receptors can regulate levels of DA by moderating its release during future nerve impulses while the transporters deplete its concentration through reuptake of DA into the nerve cell.

Five DA receptors, termed DRD-1 to DRD-5, have been identified, and genetic polymorphisms of specific receptors have been implicated in ADHD. A 148-base pair (bp) allele of the DRD-5 receptor containing a dinucleotide repeat was identified as a possible susceptibility locus (Daly, Hawi et al. 1999), and one study found a correlation between the density of DRD-1 receptors and ADHD in primates (Goldman-Rakic 1992). The DRD-4 receptor has been found to be highly polymorphic, where a 48-bp segment in its third exon may be repeated 2 to 11 times. The number of the repeat is known to be highly variable between different ethnicities, where the 7-repeat allele shows a high frequency in American populations, but a low frequency in Asian populations (Chang, Kidd et al. 1996). Numerous studies have implicated the 7-repeat polymorphism in ADHD (Swanson, Flodman et al. 2000), but no information was found on whether Asian populations show a lower incidence of the disorder. Additionally, the location of the DRD-4 gene was found to be at the 11p15.5 locus (Van Tol, Bunzow et al. 1991), which was not implicated in the previously mentioned linkage analyses.

The exact mechanism for the induction of ADHD symptoms by these polymorphisms is unknown, but a possible explanation has been found for DRD-4. DA receptors belong to a group of G-protein coupled receptors that have 7-transmembrane domains. The polymorphic region of DRD-4 corresponds to the third intracellular loop of the protein (Lichter, Barr et al. 1993) that is involved in G-protein coupling. The 7-repeat form of the gene probably has altered coupling capacities that would alter its ability in regulating DA concentrations. This form of the receptor has been found to respond differently to DA antagonists and agonists such that its response has been described as being "blunted" (Asghari, Schoots et al. 1994; Asghari, Sanyal et al. 1995) and has also been suspected of being less sensitive to DA (Seeman and Madras 1998).

The specific DA transporter, DAT-1, also has different polymorphic forms where a 40-bp repeat exists in 3 to 13 copies within the 3′ untranslated region of the gene (Vandenbergh, Persico et al. 1992; Sano, Kondoh et al. 1993). The 10-repeat allelic form has been implicated in ADHD and also corresponds to the most prevalent allelic form of the gene (Swanson, Flodman et al. 2000). It has been proposed that this allele encodes an overactive transporter that would over reuptake DA from the synaptic space (Swanson, Flodman et al. 2000). One particular study (Fuke, Suo et al. 2001) found that the 10-repeat allele significantly increased the expression of the gene in a manner that was not observed with the other allelic forms. This observation indicates that an overabundance of the transporter may be a factor in ADHD, one which would also have the effect of depleting DA in the synaptic space. The genomic locus for the transporter was found to be 5p15.3 (Vandenbergh, Persico et al. 1992), which roughly corresponds to the 5p13 locus implicated in ADHD by Ogdie et al. (2003).

The norepinephrine (NE) system exerts widespread regulatory effects since its terminals are found throughout the brain (Solanto 1998). The NE system is known to be important in attentional processes such as selective attention and vigilance (Aston-Jones, Chiang et al. 1991), where evidence has suggested the possibility that overactivity of the NE system is responsible for ADHD symptoms which may be related to its association with the dopaminergic system (Solanto 1998). Hyperactivity was also found to be induced in animal models through chemically induced legions in the brain which lead to the depletion of cerebral NE (Shaywitz, Cohen et al. 1977). To summarize, it appears that, like DA, NE may display biphasic action where either too little or too much of the neurotransmitter in the synaptic junction may produce symptoms that resemble traits seen with ADHD. The relevance of NE to ADHD will be expanded in a subsequent section that describes the NE transport inhibitor, atomoxetine.

Serotonin (5-hydroxytryptamine; 5-HT) has numerous physiologic functions that range from appetite to sexual behaviour. Upon release into the synaptic junction, 5-HT binds to numerous specific receptors to exert its effects. 5-HT receptors fall into four main groups: 5-HT1, 5-HT2, 5-HT3, and 5-HT4, where each can be subdivided into numerous subgroups. DNA variations in genes of the serotonin system and the abnormal functioning of serotonergic system proteins in relation to their influence on the doaminergic system have been implicated with the pathophysiology of ADHD (Hawi, Dring et al. 2002), where it has been found that both neurotransmitter systems exert regulatory control over one another (Kelland and Chiodo 1996). Much of this evidence has been acquired from studies involving animal models. One such model was of mutant mice that possessed a deletion in the 5-HT1B receptor. They were noted to be far more impulsive and displayed more aggressive behaviour than wild-type controls (Saudou, Amara et al. 1994). This receptor also showed associations with hyperactivity, where controlled stimulation of the

receptor by a specific agonist induced hyperactive tendencies in wild-type mice (Hawi, Dring et al. 2002). This same agonist had no effect on mice possessing a knock out mutation for the receptor. Additional observations specifically pointed to the 5-HT1B receptor as the causative agent for induced hyperactivity (Heisler and Tecott 2000). Hyperactivity was induced in mice containing a knock-out mutation in the 5-HT2C gene upon administration of the non-specific serotonin receptor agonist, meta-chlorophenylpiperazine (MCP). When the same mice were pre-treated with a specific antagonist for the 5-HT1B receptor, no hyperactivity was observed following administration of MCP. Studies on mice involving the 5-HT2A receptor showed similar results where induced hyperactivity was found to be attenuated by 5-HT2A antagonists (O'Neill, Heron-Maxwell et al. 1999).

These observations were extrapolated to humans whereby the orthologous 5-HT1B gene was also shown to be linked with ADHD. The function of this autoreceptor is to adjust the release of 5-HT from the presynaptic serotonergic neurons and has been implicated in the control of movement such that it is predominantly expressed in regions of the brain involved in motor control (Demchyshyn, Sunahara et al. 1992; Quist and Kennedy 2001). This receptor, through the action of 5-HT, has also demonstrated an inhibitory (Sarhan, Cloez-Tayarani et al. 1999) as well as stimulatory (Ng, Lee et al. 1999) role in the release of DA in the brain. Over four polymorphisms of the receptor have been isolated and studies have implicated specific alleles with ADHD. The allele containing the silent G861C substitution was observed to be preferentially transmitted in a sample of ADHD individuals (Quist, Barr et al. 2000; Hawi, Dring et al. 2002). Why a silent mutation would be linked to the disorder is unknown but it has been suggested that a disease-causing-genetic variation may be found close to the location of this silent mutation and would thus also show preferential transmission with the pathologic genetic variation (Hawi, Dring et al. 2002). Additional evidence linking the 5-HT1B gene with the disorder originates from its chromosomal location, 6q13 (Jin, Oksenberg et al. 1992; Lappalainen, Dean et al. 1995). This region roughly coincides with 6q14, a previously implicated locus found through linkage analysis (Ogdie, Macphie et al. 2003).

The human orthologue of 5-HT2A has also been linked to ADHD. Hawi et al. (2002) stated that serotonergic agonists inhibit neuronal firing, possibly due to a decrease in synaptic DA resulting from lack of synthesis or release of the neurotransmitter. They further stated that this effect may be mediated by the 5-HT2A receptor. Several polymorphisms have also been identified with this serotonergic receptor. One allele that encodes an amino acid substitution of histidine for tyrosine shows preferential transmission in ADHD individuals (Quist, Barr et al. 2000; Hawi, Dring et al. 2002). This allelic variation appears to produce a desensitized receptor that may alter the balance of serotonergic transmission (Ozaki, Manji et al. 1997). The 5-HT2 receptor has been mapped to chromosome 13 (Hsieh, Bowcock et al. 1990), a locus not previously implicated with the disorder.

To conclude, the controlled release of DA through the action of 5-HT on its associated receptors is probably the relevant cause of dopamine-mediated symptoms that are seen in ADHD. Interestingly, genes encoding serotonergic receptors may be the reason why there is a disproportion of males with the disorder. There is evidence of genomic imprinting of the 5-HT2 gene where the gene was found to be expressed only from the maternal allele (Kato, Shimizu et al. 1996). Hawi et al. (2002) also implicated genomic imprinting from noting preferential transmission of the 5-HT1B G861C allele from fathers to their offspring. Evidence of genomic imprinting has been found for the 6q27 locus (Xu, Goodyer et al. 1993), one that is proximal to the 6q13 locus of the receptor.

Aside from the observations that neurotransmitter receptors and transporters are factors in ADHD, a different type of protein, SNAP-25, has been proposed as a candidate. Recent evidence has found that SNAP-25 plays a crucial role in the release of classical neurotransmitters (Raber, Mehta et al. 1997). More specifically, SNAP-25, along with the protein Syntaxin, are two major t-SNARE proteins located at the plasma membrane of the axon terminus. The complex of these proteins serves as a docking complex for synaptic vesicles through interactions with the vesicle membrane protein VAMP (a v-SNARE protein). Upon localization of the vesicle at the presynaptic membrane, the two membranes fuse, leading to the release of the vesicle's cargo into the synaptic cleft.

SNAP-25 became implicated in ADHD as a result of the development of the Coloboma mutant (Cm) mouse containing a deletion on chromosome 2. This region is known to contain several genes, including that encoding SNAP-25 (Hess, Collins et al. 1994; Hess, Collins et al. 1996). The heterozygous mouse (containing one Cm mutated chromosome 2 and a wild-type chromosome 2) displays a semidominant effect on SNAP-25 with a 50% reduction of the protein throughout the brain and no change in the expression pattern of the remaining gene (Hess, Jinnah et al. 1992). This mutant mouse displayed spontaneous hyperactivity and now serves as an animal model for ADHD, since its hyperactivity could be successfully treated with amphetamines, and genetic complementation of the deficiency with a SNAP-25 transgene produced wild-type behaviour (Hess, Collins et al. 1996). These observations suggest that the behavioral problems are due to a reduction of functional SNAP-25, which causes a reduced release of neurotransmitters at presynaptic terminals (Raber, Mehta et al. 1997). Studies by Raber et al. (1997) have confirmed that this mutant mouse does show abnormalities in the release of neurotransmitters, where, for example, induced depolarization of the synaptic region was found to no longer release DA and the release of 5-HT was significantly lower.

These observations, although preliminary and based on a mouse model, raise the question of whether biochemical pathways involved in the general release of neurotransmitters are key factors in the etiology of ADHD. An interesting link between human SNAP-25 and ADHD can be made from its chromosomal location, 20p11.2 (Maglott, Feldblyum et al. 1996). This locus roughly coincides with the previously stated 20q13 locus found by linkage analysis (Ogdie, Macphie et al. 2003). Furthermore, I note that the human genes encoding syntaxin-8 and RIM1, SNARE proteins involved in the docking and fusion process of synaptic vesicles, have been mapped to 17p12 (Thoreau, Berges et al. 1999) and chromosome 6 (Nagase, Ishikawa et al. 1997), respectively. Both regions roughly coincide with previously implicated loci found through linkage analysis where the 17p11 locus had the highest MLS value (Ogdie, Macphie et al. 2003). In conclusion, it appears that further evidence asserting the associations of

human SNARE proteins in relation to ADHD may uncover new causative agents and drug targets for the disorder. An interesting avenue for investigation would be to confirm if syntaxin-8 and RIM1 are factors in this disorder, as suggested through previous chromosomal linkage analyses **(Table 1)**s.

Pharmacological treatments for ADHD and their modes of action

Intensive research on the pathology of ADHD has led to the development of numerous pharmacological treatments, of which most exert their activities on specific neurotransmitter systems. Since the dopaminergic system has been the most intensely studied in relation to ADHD, the mechanisms of drugs affecting this system are most understood. The most widely used drugs for the treatment of ADHD are the psychostimulants dextroamphetamine (AMP), methylphenidate (Ritalin®, MET), and pemoline. These drugs both promote the release of DA and block its reuptake into the neuron via the DA transporter, where MET plays a greater role in blocking the transporter (Volkow, Ding et al. 1995) and AMP plays a greater role in enhancing the release of DA (Seeman and Madras 1998). In brief, both drugs increase the DA concentration in synaptic junction. These drugs show molecular homology with DA, which allows them to enter the transporter much like DA but are molecularly structured so that they block, rather than pass through it, as would DA **(Figure 2**). Like DA and suggested for NE, MET and AMP also display a biphasic action where low doses of the drugs promote a calming effect and elevated doses produce stimulation (Seeman and Madras 1998).

Ironically, increased amounts of DA release during nerve transmission are known to increase impulsive movements (as in Parkinson's disease), thus the effects of psychostimulants on increasing DA concentrations in synaptic junctions must have a more complicated mechanism in order to produce a calming effect. A potential mechanism will now be presented.

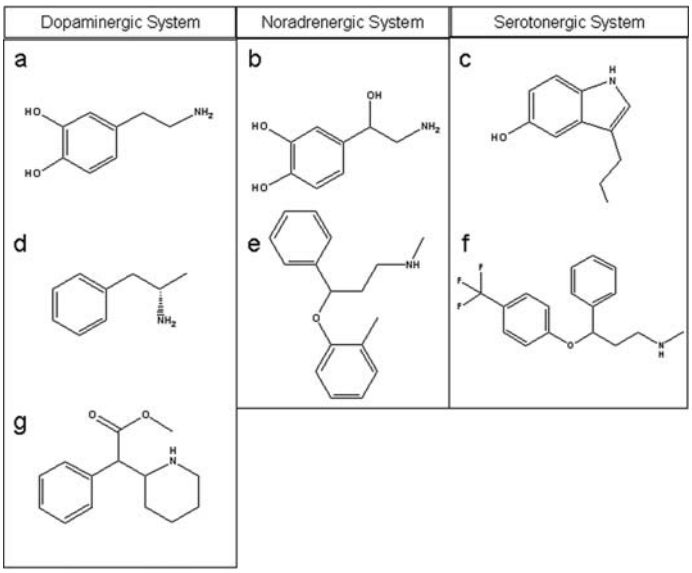

Figure 2. Molecular structures of neurotransmitters and pharmacological therapeutics for ADHD. The therapeutics display structural similarities to the neurotransmitter of the neurotransmitter system they act upon. a) dopamine and the psychostimulants d) dextroamphetamine and g) methylphenidate (Ritalin®). b) norepinephrine and NE transport inhibitor e) atomoxetine. c) serotonin and the 5-HT transporter inhibitor f) fluoxetine (Prozac®).

During a normal nerve impulse, the extracellular level of DA in the synaptic junction may increase to over 60 times the basal pre-stimulatory level (Kawagoe, Garris et al. 1992). Return to the basal level occurs by diffusion and reuptake of DA by DA transporters **(Figure 1)** (Seeman and Madras 1998). Low doses of psychostimulants have been found to increase both basal levels of DA within the synaptic junction and the amount of DA released upon nerve transmission. More specifically, the increase in the basal level of DA in the synaptic junction is several times that of the increase in the amount of DA released upon nerve transmission(Seeman and Madras 1998). Thus, the calming effect of low doses of psychostimulants may reside in the fact that the relative increase of DA in the synaptic junction before and after nerve transmission is reduced compared to the amount obtained in the absence of psychostimulants. Simply put, this appears to mimic a situation where less DA is released during nerve transmission. Additionally, the elevated basal amount of DA in the synaptic junction can mediate subsequent DA release by saturating DA receptors that regulate the release of this neurotransmitter, thus resulting in a decrease of DA release during future nerve impulses. This lowering of pulsatile DA concentrations will result in less activation of DA receptors that mediate the initiation of psychomotor activity (Silvia, King et al. 1994; Seeman and Madras 1998). Furthermore, it has been documented that DA receptors that mediate the initiation of psychomotor activity can lower their affinity for DA and become desensitized through elevated associations with DA (Seeman, Watanabe et al. 1985). It has been suggested that the elevated basal level of DA in the synaptic junction may also cause such DA receptors compensate by becoming desensitized (Seeman and Madras 1998), which would also decrease their ability to initiate psychomotor activity. On the contrary, sudden administration of elevated concentrations of psychostimulants (as is seen with recreational drug use) do not produce the same effect since the basal level of DA within the synaptic junction under this situation becomes extreme and thus produces general stimulation (Seeman and Madras 1998).

Another therapeutic drug that has been found to attenuate ADHD symptoms is fluoxetine (Prozac®), which is a selective inhibitor of 5-HT reuptake. The drug blocks 5-HT transporters, causing an increase in extracellular 5-HT (Gainetdinov, Wetsel et al. 1999). Fluoxetine was noted to diminish ADHD symptoms in DAT-1 gene knockout mice, whereas the drug MET showed no effect (Hawi, Dring et al. 2002).

A recent study has shown that a selective NE transport inhibitor, atomoxetine, was able to alleviate ADHD symptoms (Bymaster, Katner et al. 2002). This study investigated the mechanism of action of the drug on DA, 5-HT, and NE transporters and its effect on the extracellular concentrations of these neurotransmitters. Atomoxetine displayed specificity for NE transporters, causing increased extracellular levels of NE in the brain but having no effect on 5-HT concentrations. Interestingly, extracellular levels of DA were found to have also increased in certain regions of the brain. The extent of this increase was found to be comparable to that produced with the administration of MET. Thus, the therapeutic effects of atomoxetine were concluded to be due to the induced increase in DA and NE. The action of blocking NE transporters by atomoxetine is mimicked by certain tricyclic anti-depressants like desipramine (Delgado, Miller et al. 1993), which have also

been proposed as treatments for ADHD (Spencer, Biederman et al. 1996) but are considered to be less effective than psychostimulants (Pliszka 1987). To conclude, evidence is emerging that atomoxetine may be a favourable alternative to psychostimulant treatments in certain conditions and has the added benefit of not increasing extracellular levels of DA in regions of the brain that are associated with addiction, which is the case with other psychostimulants (Kuczenski and Segal 1997; Bymaster, Katner et al. 2002).

Concluding statements

ADHD is a complex disorder that is linked to multiple neurotransmitter systems. Associations of the disorder with the dopaminergic, noradrenergic, and serotonergic systems are relatively well known. Conversely, associations between the disorder and SNARE proteins, notably SNAP-25, and neurotransmitter release are in their infancy and probably hold much research potential, especially since many SNARE genes appear to have been previously implicated with the disorder through linkage analyses. For example, it is noted in this review that the SNARE proteins RIM1 and syntaxin-8 are of interest for the aforementioned reason. How exactly SNARE proteins may be linked to the disorder remains nebulous and should be the focus of investigation. It is interesting to note that hyperactivity in the Coloboma mouse was due to the 50% reduction in SNAP-25 protein expression. This suggests that depletion or loss-of-function of certain SNARE proteins could be a general trait of ADHD whereby knowledge into the specifics as to how this might occur, such as through genetic polymorphisms as seen with DAT-1, would be invaluable to our understanding of this condition. The genetics of ADHD also show several ambiguities and require focussed investigation; especially why the disorder appears to be predominantly observed in males and whether genomic imprinting is indeed a factor in this discrepancy. Further understanding as to why ADHD shows prominence in males may divulge very specific factors that cause the disorder and their mechanisms in pathology. For example, if genomic imprinting results in the silencing of certain genomic loci, depletion of certain genetic products may induce the disorder whereby therapies to correct this condition may favour alternatives to pharmaceuticals. Increasing knowledge into the specific molecular mechanisms of the disorder is broadening options for successful treatments and is exposing new potential drug targets. It appears that after using psychostimulants for decades, new therapies are providing other options to these compounds as seen with atomoxetine. Research concerning specific factors of ADHD that are not directly related with the dopaminergic system and DA, namely serotonergic receptors and possible factors within the noradrenergic system, appear to be ones that will provide the revolutionary discoveries into this disorder in the years to come. Since several drugs have been observed to be effective (psychostimulants, atomoxetine, fluoxetine), or somewhat effective (tricyclic anti-depressants) in the treatment of ADHD, future investigations into the effectiveness of administering combinations of these drugs might prove valuable. Identification of effective combinations of drug therapies may result in lowering the necessary doses of any one particular therapy, thus reducing the possibility of adverse drug responses, especially since these treatments are often administered well into adulthood with ADHD sufferers.

It has been noted that many studies have focused on the themes of drug development and the identification of causative biological factors associated with ADHD, yet have not focused on the development of a diagnostic test for the disorder that is based on such biological factors. Most ADHD individuals are diagnosed through psychological assessments, while it appears that few are diagnosed through methods concerning genetic or biochemical factors, such as genetic screening. The lack of such tests is probably due to the complexity of the disorder where a successful clinical diagnosis would probably involve numerous biochemical and/or physiological variables, many of which have yet to be confirmed as agents in ADHD pathology. The analysis of specific genomic loci appears to be the most promising approach for the development of a genetics-based clinical test. Several genes implicated in ADHD, their polymorphisms, and their genomic locations are known. Therefore genetic screening of specific genomic locations could be used to identify such polymorphisms or allelic variations, such as through Southern blotting or DNA microarray analysis. Such an approach for diagnosis of the disorder has its flaws, since some polymorphisms are known to be more predominant in certain populations and absent in others. Since ADHD is a polygenetic disorder, several loci would have to be analyzed in order to produce a diagnosis with an appreciable level of certainty. While it is not to be construed that diagnosing ADHD via psychological tests is inefficient or inferior, additional means to diagnose the disorder have their merit. For example, if ambiguities are obtained from a psychological assessment, a subsequent analysis through genetic testing may help to confirm the accurate diagnosis of an individual. Plus, since ADHD is the most prevalent childhood-onset behavioral disorder, developing tests that employ routine procedures requiring a relatively short time per analysis (i.e.: Southern blot analysis to identify specific genetic polymorphisms) may increase the efficiency with which ADHD is diagnosed in large patient populations.

Acknowledgements

The author would like to thank McGill University for funding support throughout his Undergraduate studies from a generous J. W. McConnel Entrance Scholarship and would like to thank Dr. Imed Gallouzi of McGill University for initial comments on this work.

References

1. *Diagnostic and statistical manual of mental disorders, 4th edition*. (1994). Washington D.C., American Psychiatric Association.
2. Abel, L. and B. Muller-Myhsok (1998). *"Robustness and power of the maximum-likelihood-binomial and maximum-likelihood-score methods, in multipoint linkage analysis of affected-sibship data."* Am J Hum Genet 63(2): 638-47.
3. Anderson, J. C., S. Williams, et al. (1987). *"DSM-III disorders in preadolescent children. Prevalence in a large sample from the general population."* Arch Gen Psychiatry 44(1): 69-76.
4. Asghari, V., S. Sanyal, et al. (1995). *"Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants."* J Neurochem 65(3): 1157-65.
5. Asghari, V., O. Schoots, et al. (1994). *"Dopamine D4 recep-*

tor repeat: analysis of different native and mutant forms of the human and rat genes." Mol Pharmacol 46(2): 364-73.
6. Aston-Jones, G., C. Chiang, et al. (1991). *"Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance."* Prog Brain Res 88: 501-20.
7. Bakker, S. C., E. M. van der Meulen, et al. (2003). *"A whole-genome scan in 164 Dutch sib pairs with attention-deficit/hyperactivity disorder: suggestive evidence for linkage on chromosomes 7p and 15q."* Am J Hum Genet 72(5): 1251-60.
8. Bradley, C. (1937). *"The behavior of children receiving Benzedrine."* Am. J. Psychiatry 94: 577-585.
9. Bymaster, F. P., J. S. Katner, et al. (2002). *"Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder."* Neuropsychopharmacology 27(5): 699-711.
10. Castellanos, F. X., J. Elia, et al. (1994). *"Cerebrospinal fluid monoamine metabolites in boys with attention-deficit hyperactivity disorder."* Psychiatry Res 52(3): 305-16.
11. Chang, F. M., J. R. Kidd, et al. (1996). *"The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus."* Hum Genet 98(1): 91-101.
12. Daly, G., Z. Hawi, et al. (1999). *"Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children."* Mol Psychiatry 4(2): 192-6.
13. Delgado, P. L., H. L. Miller, et al. (1993). *"Monoamines and the mechanism of antidepressant action: effects of catecholamine depletion on mood of patients treated with antidepressants."* Psychopharmacol Bull 29(3): 389-96.
14. Demchyshyn, L., R. K. Sunahara, et al. (1992). *"A human serotonin 1D receptor variant (5HT1D beta) encoded by an intronless gene on chromosome 6."* Proc Natl Acad Sci U S A 89(12): 5522-6.
15. Ernst, M., A. J. Zametkin, et al. (1997). *Presynaptic dopaminergic activity in ADHD adults and children.* A F18 fluorodopa emission tomographic study International Society for Research in Children and Adolescent Psychopathology, Paris, France.
16. Fisher, S. E., C. Francks, et al. (2002). *"A genomewide scan for loci involved in attention-deficit/hyperactivity disorder."* Am J Hum Genet 70(5): 1183-96.
17. Fuke, S., S. Suo, et al. (2001). *"The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression."* Pharmacogenomics J 1(2): 152-6.
18. Gainetdinov, R. R., W. C. Wetsel, et al. (1999). *"Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity."* Science 283(5400): 397-401.
19. Gillberg, C., H. Melander, et al. (1997). *"Long-term stimulant treatment of children with attention-deficit hyperactivity disorder symptoms. A randomized, double-blind, placebo-controlled trial."* Arch Gen Psychiatry 54(9): 857-64.
20. Goldman-Rakic, P. S. (1992). *"Topography of cognition: parallel distribution networks in primate association cortex."* Ann Rev Neurosci 11: 137-156.
21. Goodman, R. and J. Stevenson (1989). *"A twin study of hyperactivity--II. The aetiological role of genes, family relationships and perinatal adversity."* J Child Psychol Psychiatry 30(5): 691-709.
22. Hawi, Z., M. Dring, et al. (2002). *"Serotonergic system and attention deficit hyperactivity disorder (ADHD): a potential susceptibility locus at the 5-HT(1B) receptor gene in 273 nuclear families from a multi-centre sample."* Mol Psychiatry 7(7): 718-25.
23. Heisler, L. K. and L. H. Tecott (2000). *"A paradoxical locomotor response in serotonin 5-HT(2C) receptor mutant mice."* J Neurosci 20(8): RC71.
24. Hess, E. J., K. A. Collins, et al. (1994). *"Deletion map of the coloboma (Cm) locus on mouse chromosome 2."* Genomics 21(1): 257-61.
25. Hess, E. J., K. A. Collins, et al. (1996). *"Mouse model of hyperkinesis implicates SNAP-25 in behavioral regulation."* J Neurosci 16(9): 3104-11.
26. Hess, E. J., H. A. Jinnah, et al. (1992). *"Spontaneous locomotor hyperactivity in a mouse mutant with a deletion including the Snap gene on chromosome 2."* J Neurosci 12(7): 2865-74.
27. Hornykiewicz, O. (1966). *"Dopamine (3-hydroxytyramine) and brain function."* Pharmacol Rev 18(2): 925-64.
28. Hsieh, C. L., A. M. Bowcock, et al. (1990). *"The serotonin receptor subtype 2 locus HTR2 is on human chromosome 13 near genes for esterase D and retinoblastoma-1 and on mouse chromosome 14."* Somat Cell Mol Genet 16(6): 567-74.
29. Jin, H., D. Oksenberg, et al. (1992). *"Characterization of the human 5-hydroxytryptamine1B receptor."* J Biol Chem 267(9): 5735-8.
30. Kato, M. V., T. Shimizu, et al. (1996). *"Genomic imprinting of the human serotonin-receptor (HTR2) gene involved in development of retinoblastoma."* Am J Hum Genet 59(5): 1084-90.
31. Kawagoe, K. T., P. A. Garris, et al. (1992). *"Regulation of transient dopamine concentration gradients in the microenvironment surrounding nerve terminals in the rat striatum."* Neuroscience 51(1): 55-64.
32. Kelland, M. D. and L. A. Chiodo (1996). *Serotonergic modulation of midbrain dopamine systems*. In: Charles R., Ashby J. The Modulation of Dopaminergic Neurotransmission by other Neurotransmitters Boca Raton, Florida, CRC Press Inc. p.87-122.
33. Kuczenski, R. and D. S. Segal (1997). *"Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine."* J Neurochem 68(5): 2032-7.
34. Lappalainen, J., M. Dean, et al. (1995). *"Mapping of the serotonin 5-HT1D beta autoreceptor gene on chromosome 6 and direct analysis for sequence variants."* Am J Med Genet 60(2): 157-61.
35. Lichter, J. B., C. L. Barr, et al. (1993). *"A hypervariable segment in the human dopamine receptor D4 (DRD4) gene."* Hum Mol Genet 2(6): 767-73.
36. Maglott, D. R., T. V. Feldblyum, et al. (1996). *"Radiation hybrid mapping of SNAP, PCSK2, and THBD (human chromosome 20p)."* Mamm Genome 7(5): 400-1.
37. Nagase, T., K. Ishikawa, et al. (1997). *"Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro."* DNA Res 4(2): 141-50.

38. Ng, N. K., H. S. Lee, et al. (1999). *"Regulation of striatal dopamine release through 5-HT1 and 5-HT2 receptors."* J Neurosci Res 55(5): 600-7.
39. O'Neill, M. F., C. L. Heron-Maxwell, et al. (1999). *"5-HT2 receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D1 agonist C-APB."* Pharmacol Biochem Behav 63(2): 237-43.
40. Ogdie, M. N., Macphie, I. L., et al. (2003). *"A genomewide scan for attention-deficit/hyperactivity disorder in an extended sample: suggestive linkage on 17p11."* Am J Hum Genet 72(5): 1268-79.
41. Ozaki, N., H. Manji, et al. (1997). *"A naturally occurring amino acid substitution of the human serotonin 5-HT2A receptor influences amplitude and timing of intracellular calcium mobilization."* J Neurochem 68(5): 2186-93.
42. Pliszka, S. R. (1987). *"Tricyclic antidepressants in the treatment of children with attention deficit disorder."* J Am Acad Child Adolesc Psychiatry 26(2): 127-32.
43. Quist, J. F., C. L. Barr, et al. (2000). *"Evidence for the serotonin HTR2A receptor gene as a susceptibility factor in attention deficit hyperactivity disorder (ADHD)."* Mol Psychiatry 5(5): 537-41.
44. Quist, J. F. and J. L. Kennedy (2001). *"Genetics of childhood disorders: XXIII. ADHD, Part 7: The serotonin system."* J Am Acad Child Adolesc Psychiatry 40(2): 253-6.
45. Raber, J., P. P. Mehta, et al. (1997). *"Coloboma hyperactive mutant mice exhibit regional and transmitter-specific deficits in neurotransmission."* J Neurochem 68(1): 176-86.
46. Safer, D. J. (1973). *"A familial factor in minimal brain dysfunction."* Behav Genet 3(2): 175-86.
47. Sano, A., K. Kondoh, et al. (1993). *"A 40-nucleotide repeat polymorphism in the human dopamine transporter gene."* Hum Genet 91(4): 405-6.
48. Sarhan, H., I. Cloez-Tayarani, et al. (1999). *"5-HT1B receptors modulate release of [3H]dopamine from rat striatal synaptosomes."* Naunyn Schmiedebergs Arch Pharmacol 359(1): 40-7.
49. Saudou, F., D. A. Amara, et al. (1994). *"Enhanced aggressive behavior in mice lacking 5-HT1B receptor."* Science 265(5180): 1875-8.
50. Seeman, P. and B. K. Madras (1998). *"Anti-hyperactivity medication: methylphenidate and amphetamine."* Mol Psychiatry 3(5): 386-96.
51. Seeman, P., M. Watanabe, et al. (1985). *"Dopamine D2 receptor binding sites for agonists. A tetrahedral model."* Mol Pharmacol 28(5): 391-9.
52. Shaywitz, B. A., D. J. Cohen, et al. (1977). *"CSF monoamine metabolites in children with minimal brain dysfunction: evidence for alteration of brain dopamine. A preliminary report."* J Pediatr 90(1): 67-71.
53. Sherman, D. K., M. K. McGue, et al. (1997). *"Twin concordance for attention deficit hyperactivity disorder: a comparison of teachers' and mothers' reports."* Am J Psychiatry 154(4): 532-5.
54. Silvia, C. P., G. R. King, et al. (1994). *"Intranigral administration of D2 dopamine receptor antisense oligodeoxynucleotides establishes a role for nigrostriatal D2 autoreceptors in the motor actions of cocaine."* Mol Pharmacol 46(1): 51-7.
55. Smalley, S. L. (1997). *"Genetic influences in childhood-onset psychiatric disorders: autism and attention-deficit/hyperactivity disorder."* Am J Hum Genet 60(6): 1276-82.
56. Smalley, S. L., V. Kustanovich, et al. (2002). *"Genetic linkage of attention-deficit/hyperactivity disorder on chromosome 16p13, in a region implicated in autism."* Am J Hum Genet 71(4): 959-63.
57. Solanto, M. V. (1998). *"Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration."* Behav Brain Res 94(1): 127-52.
58. Spencer, T., J. Biederman, et al. (1996). *"Pharmacotherapy of attention-deficit hyperactivity disorder across the life cycle."* J Am Acad Child Adolesc Psychiatry 35(4): 409-32.
59. Stevenson, R. D. and M. L. Wolraich (1989). *"Stimulant medication therapy in the treatment of children with attention deficit hyperactivity disorder."* Pediatr Clin North Am 36(5): 1183-97.
60. Stromberg, U. and T. H. Svensson (1975). *"Differences between (+)- and (-)-amphetamine in effects on locomotor activity and L-dopa potentiating action in mice."* Naunyn Schmiedebergs Arch Pharmacol 287(2): 171-9.
61. Swanson, J. M., P. Flodman, et al. (2000). *"Dopamine genes and ADHD."* Neurosci Biobehav Rev 24(1): 21-5.
62. Thoreau, V., T. Berges, et al. (1999). *"Molecular cloning, expression analysis, and chromosomal localization of human syntaxin 8 (STX8)."* Biochem Biophys Res Commun 257(2): 577-83.
63. Van Tol, H. H., J. R. Bunzow, et al. (1991). *"Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine."* Nature 350(6319): 610-4.
64. Vandenbergh, D. J., A. M. Persico, et al. (1992). *"Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR."* Genomics 14(4): 1104-6.
65. Volkow, N. D., Y. S. Ding, et al. (1995). *"Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain."* Arch Gen Psychiatry 52(6): 456-63.
66. Wilens, T. E. and J. Biederman (1992). *"The stimulants."* Psychiatr Clin North Am 15(1): 191-222.
67. Wolraich, M. L., J. N. Hannah, et al. (1996). *"Comparison of diagnostic criteria for attention-deficit hyperactivity disorder in a county-wide sample."* J Am Acad Child Adolesc Psychiatry 35(3): 319-24.
68. Xu, Y., C. G. Goodyer, et al. (1993). *"Functional polymorphism in the parental imprinting of the human IGF2R gene."* Biochem Biophys Res Commun 197: 747-754.
69. Zametkin, A. J., T. E. Nordahl, et al. (1990). *"Cerebral glucose metabolism in adults with hyperactivity of childhood onset."* N Engl J Med 323(20): 1361-6.