

The Role of Norepinephrine and Serotonin in ADHD

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1. Introduction:

The first actor in this chapter is norepinephrine (NE). NE belongs to the chemical group of the catecholamines and is also known outside the Americas as noradrenaline. The second actor is serotonin, an indole-amine that is better described chemically as 5-hydroxy-tryptamine (5-HT). Together with the catecholamines dopamine (DA) and epinephrine (adrenaline) they are known as the monoamines. These monoamines have an agent role in transmission between neurons – often in the synapse between neurons and their elements in apposition, sometimes between release and receptor sites that are further apart. Then the role is more reminiscent of hormonal

communication. Both roles are subsumed as 'neurotransmission'. These transmitters are located in well-characterized, similar neural pathways throughout the vertebrates.

This chapter is essentially concerned with the role of NE and 5-HT in the central nervous system (CNS) and how characteristics of 5-HT and NE transmission could contribute to the principle features of ADHD. This review starts with the basic aspects of monoamine biochemistry and neurochemical anatomy and proceeds over mechanisms of function (animal work) to investigations of their role in the neuropsychology, and nosology thought to underlie ADHD. However, throughout these considerations it should not be overlooked that

both 5-HT and NE pathways are widely distributed peripherally with functions additional to those considered here¹. Further it is also important to bear in mind in the ensuing discussion of NE and 5-HT function that many of the effects simply attributed to the activity of one or the other monoamine, are through multiple interactions, additionally dependent on another monoamine.

2. Biochemistry:

5-HT and NE synthesis depends on the availability of the amino-acids, tryptophan and phenylalanine, respectively. Tryptophan is hydroxylated in the rate-limiting step by tryptophan hydroxylase to the precursor 5-hydroxytryptophan (5-HTP) prior to conversion to 5-HT by decarboxylation. For NE synthesis, phenylalanine is hydroxylated to tyrosine prior to the rate-limiting hydroxylation to L-DOPA (**Fig. 1**). Decarboxylation then produces DA that can be dehydroxylated to NE. Many studies examining the effects of enhancing or depleting NE make use of the crucial role of tyrosine hydroxylase (TOH) and dopamine beta-hydroxylase (DBH). Studies of 5-HT depletion often use diets free of tryptophan for examining the effect of reducing 5-HT activity. Thus it is not surprising that dietary effects on the availability of factors needed for transmitter synthesis has been part of the agenda in some ADHD studies.

Breakdown (catabolism) occurs following post-synaptic uptake of the neurotransmitter, when the transmitter remains unused in the synapse, or after pre-synaptic re-uptake when not stored in vesicles. In detail the NE and 5-HT catabolic pathways can differ. Several enzymes are involved in both. But primary is the oxidation process (monoamine oxidase, MAO). For 5-HT this leads to 5-hydroxy-indoleacetic acid (5-HIAA: **Fig. 2**); for NE there are many intermediates resulting from the activities of several enzymes.

¹ For example, 5-HT has a prominent role in pulmonary and renal blood flow, as well as the enteric autonomic system (smooth muscle contraction): NE, released from post-ganglionic sympathetic neurons, also actively modulates vasoconstriction/dilation, especially heart and smooth muscle function (also the uterus, intestine, bronchi and iris). In addition NE modulates insulin secretion and several metabolic activities: (note also that NE is the precursor to epinephrine synthesis in the adrenal medulla).

Three trends emerge from metabolic studies that help the interpretation of clinical results. First, the primary products of stimulated central NE synthesis are mostly 3-methoxy- and dihydroxy-phenyl-glycol (MHPG, DHPG), while extra-neuronal products also include metanephrine and normetanephrine (MN, NMN: 1). As these latter metabolites along with vanillomandelic acid (VMA) do not cross the blood brain barrier peripheral measures of these metabolites likely reflect peripheral sources. Secondly these metabolites (e.g. NMN, VMA) often measured peripherally, can be excreted partially, after further metabolism, as homovanillic acid (HVA). This leads to some confusion over identifying the relative roles of NE and DA activity. Thirdly, NE and 5-HT are the preferred substrates for MAO type A, while tyramine, tryptamine and DA are the preferred substrates of MAO type B: but, the separation of function between these two isoenzymes is not tight. (e.g. selective inhibitors of both MAO-A [clorgyline] and MAO-B [selegiline] can reduce 5-HT catabolism.)

3. CNS Pathways:

3.1 NE

In the 1950s pioneer work demonstrated NE to be a chemical transmitter and to have its cells of origin in the brain stem (2, 3). The locus ceruleus (LC: A6) is located in the dorso-lateral pontine tegmentum just lateral to the fourth ventricle (4, 5: **Fig. 3**). It and the nearby A5, A7 nuclei (subcoeruleus) give rise to NE fibers innervating the forebrain (dorsal noradrenergic bundle), diencephalon, cerebellum and local brainstem nuclei. Some fibers also descend in the spinal cord (6). A more ventral bundle with fibers from the Nucleus tractus solitarius (A2) also innervates the diencephalon and a number of sub-cortical limbic regions (7). The LC in man is about 15 mm long and in adults contains some 40-60 thousand NE-containing cells. Of interest for animal models, there is much similarity between the LC in humans and that of the rat – even if the latter contains only 3% of the number of neurons in the human LC. Other transmitting agents such as neuropeptide Y, galanin and GABA may also be colocalized in these neurons.

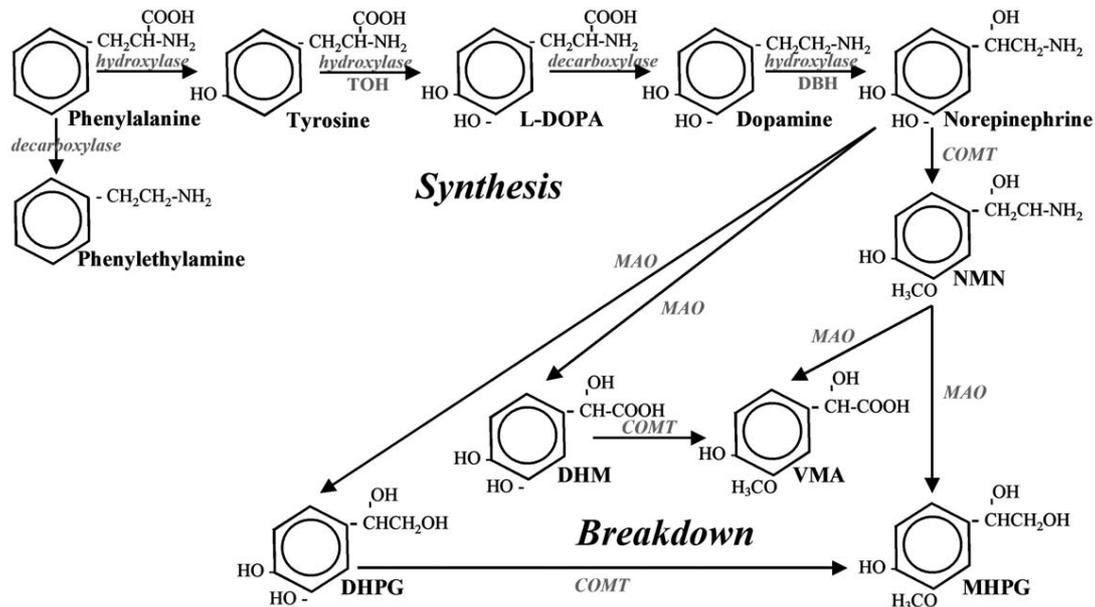


Figure 1

NE Metabolism: Biochemical pathways showing the synthesis and breakdown of NE.

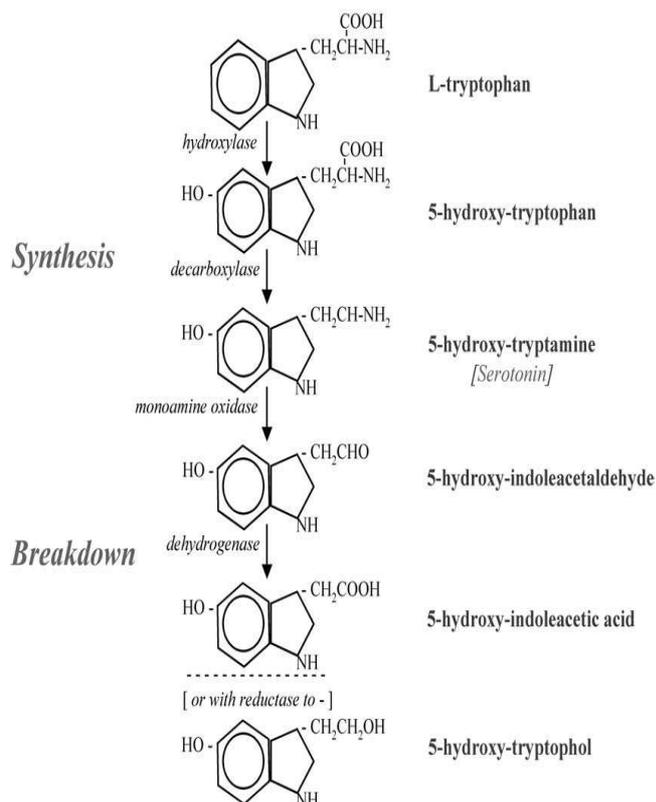


Figure 2

5-HT Metabolism: Biochemical pathways showing the synthesis and breakdown of 5-HT

To understand the function of the NE system it is important to appreciate that there is much dendrite branching locally within the LC and axonal branching between widely separate areas innervated by the same neuron (8). If one considers the vast areas of cortex innervated it may be that as few as 5% of transmitter

containing varicosities are located in conventional synapses (9). Most of the transmitter released has effect at a distance from the end of the axon. The densest input is to the laminae III and IV (review: 10). Alpha-1 and alpha-2 receptor types that can be pre- or post-synaptically located are distributed more across the superficial laminae, while beta sites may be found in most cortical laminae. ($\alpha 2a$ have a primarily frontal, $\alpha 2b$ a more thalamic and $\alpha 2c$ a brainstem distribution).

3.2 5-HT

5-HT was first demonstrated in the CNS of cats and dogs about 50 years ago (11, 12). The development of fluorescence histochemistry 10 years later led to the description of the basic components of the 5-HT projection system (13). In succeeding decades the development of antibodies, of immunohistochemical (13) and immunocytochemical methods led to the current understanding of the cell body origins and their heterogeneous termination patterns (14). For 5-HT there are 9 cell groups (B1-B9). B1-B5 are small cell groups located in the midline from the mid-pons to the caudal medulla (Fig. 4). They project locally and down the dorsal and ventral horns of the spinal cord. More significant for the current discussion are B6 and B7 (the dorsal raphe nuclei) that lie along the floor of the fourth ventricle near the LC, and ventrally the B8 group (the median raphe on the borders of the pons and midbrain.

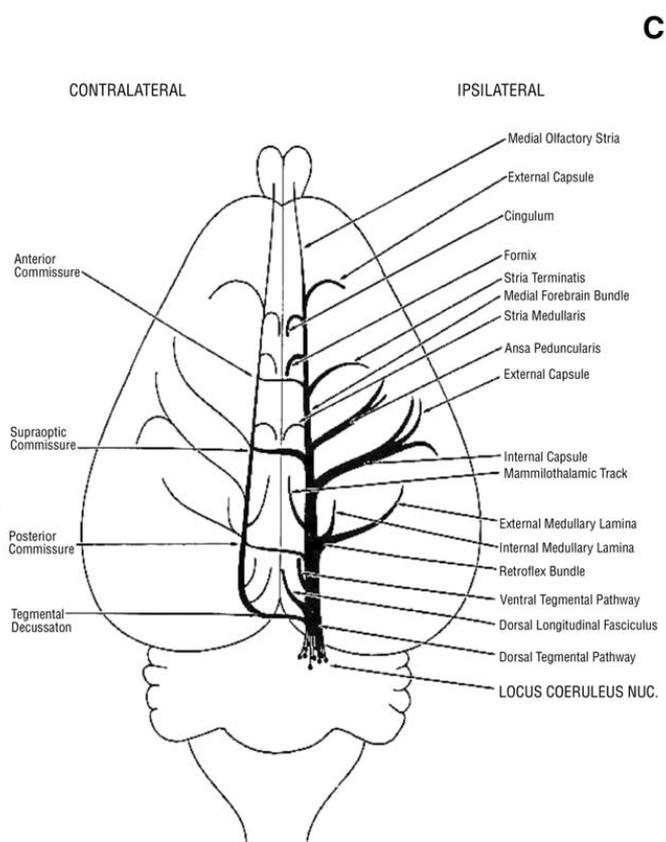
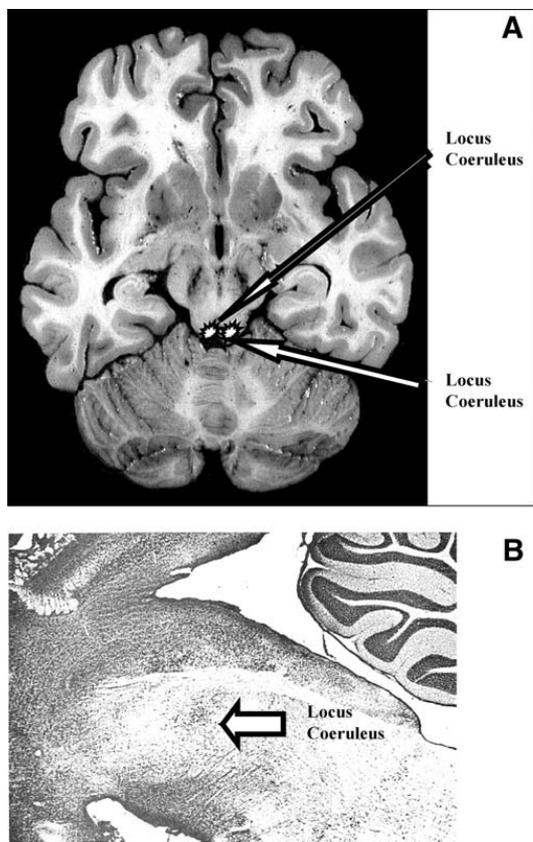


Figure 3
 Anatomical location of the locus coeruleus (LC) and the ascending pathways: a) bilateral brain stem locations of the LC in a horizontal section of the human brain (with the cerebellum just behind); b) sagittal view from the side through a rat brain with arrow pointing to the NE path deriving from the LC above; c) diagram of the ascending and commissural pathways arising from the LC in the rat brain (adapted from reference 4, and 5 with permission from Elsevier). [Innervation of the hippocampus proceeds via the fornix, while that of the medial and dorsal cortex passes through the cingulum with anterior cortex innervated by the rostral extension of the medial forebrain bundle.]

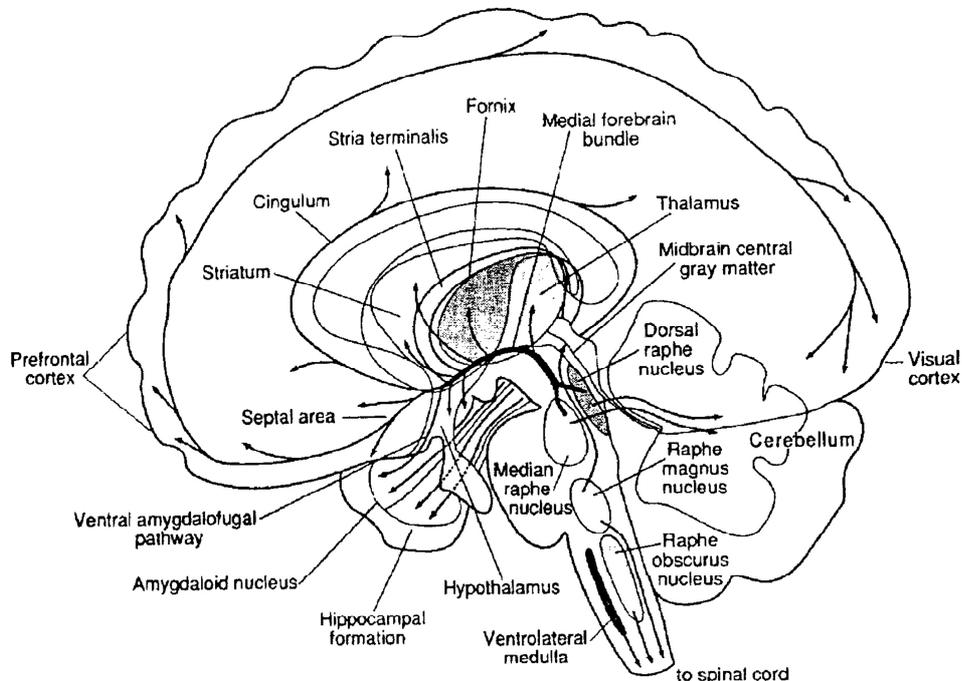


Figure 4
 Representation of the 5-HT projections ascending in the medial forebrain bundle from the dorsal and median raphe nuclei in the brain stem. Branching occurs above the thalamus to the limbic system, basal ganglia and cerebral cortices. 5-HT projections descend to the spinal cord from the raphe magnus and obscurus in the ventrolateral medulla. (Taken from reference 15 with permission of the NY Academy of Sciences).

The dorsal raphe is the larger group but along with the median raphe, both contain neurons using other transmitters (e.g. DA: 15).

There is a fairly broad overlap for the forebrain innervation from these two nuclei. The emphasis is on the neostriatum and frontal lobe for the dorsal raphe (with a decreasing gradient over the more caudal cortical regions), while the median raphe projects more to diencephalic and limbic structures. Output from the median raphe relays not just to the hippocampus, but extends to the cingulate and fairly evenly through the parietal and neighbouring cortices. The sensory and motor cortices show a mixed pattern, some with much 5-HT innervation (e.g. auditory and somatosensory cortex) some with less (e.g. motor cortex). Some areas receive high and low patches of input (visual cortex). There are morphologically two quite different forms of innervation, although their functional relevance remains obscure. The one with fine axons and small varicosities (inclusions), is found throughout cortical terminal regions, and is largely of dorsal raphe origin. The other is coarser with a large beaded form, is more sparsely distributed (mostly fronto-parietal and hippocampal regions) and mostly of median raphe origin (reviews: 15-17). 5-HT_{1a} binding sites are found as autoreceptors as well as postsynaptically on cholinergic neurons, and those using amino-acid transmission. It is noteworthy that 5-HT_{2a} sites are frequently found on DA and NE neurons (see reviews of the widespread distribution of the 5-HT₁ and 5-HT₂ classes of binding site in 18 and 19).

4. Interactions between monoamines:

4.1 5-HT – NE interactions

Many central effects of monoamines are modified by activity in pathways releasing other monoamines. Indeed, some of the autonomic effects of 5-HT of central origin are exerted via 5-HT_{2a} receptors on processes of the NE networks arising in the N. tractus solitarius (20). Interactions between the brainstem nuclei work both ways. NE can facilitate 5-HT release (e.g. via alpha-1 binding sites: 21, 22), while 5-HT can reduce NE activity (23, 24). This latter effect can occur in the brainstem via 5-HT_{1a} sites

potentiating local NE inhibitory feedback (25). However, in the cortices NE usually inhibits 5-HT release (via alpha-2 receptors: 26), while 5-HT can facilitate or reduce NE release (5-HT_{2a} [heteroreceptor] or 5-HT_{2c} binding sites [autoreceptors] depending on their pre-/postsynaptic loci: 27, 28).

4.2 5-HT - DA interactions

Many of the central effects of 5-HT arise via modulation of activity in DA paths. Often the levels of DA and 5-HT metabolites in samples of cerebrospinal fluid (CSF) drawn from healthy subjects are highly inter-correlated (29). Indeed, in ADHD children high levels of 5-HIAA and HVA decreased together in those responding to psychostimulant treatment (30). Thus it is not surprising to learn that increases of amphetamine-induced locomotion (31) and the associated induced release of DA (32) are modulated by 5-HT at 5-HT_{2a} receptors: both effects are suppressed by 5-HT_{2a} antagonists. Other ADHD-like features modeled in animals show DA/5-HT interactions. Shifts of attention and stimulus-reward learning, facilitated by methylphenidate, are impaired by reduced 5-HT synthesis (33). A separate psychostimulant action on reinforcement - the amphetamine-induced enhancement of response for conditioned reward - is suppressed by 5-HT stimulation (at mesolimbic 5-HT_{1b} sites: 34).

Reverse influences of DA on 5HT activity should not be overlooked. Neonatal damage to DA systems leads to large increases of 5-HT in the basal ganglia and cerebellum, though not in the cortex (35). There are potential consequences of such interactions in terms of treatment. Impulsivity in ADHD has a basis in the responsiveness of 5-HT neurons (36: 8.1 below) and the stimulation by 5-HT₂ agonists of premature responses in rats performing a choice task can be brought under control with DA antagonists (37).

A number of receptor sites underlie these mechanisms. Currently the 5-HT_{2a} /2c are among those that are better understood. 5-HT_{2a} sites are often located on neurons with projections ascending from the ventral tegmental area (38) and modulate active DA transmission, while 5-HT_{2c} sites affect tonic DA outflow (39). Agonism at these two sites

suppresses, while antagonism stimulates DA outflow. This action is better documented for mesocortical sites with 5-HT_{2a}, and for mesolimbic sites with 5-HT_{2c} sites (40-42). Effects of the 5-HT₁ receptor classes on DA release are less well understood² (26, 43).

4.3 NE - DA interactions

NE activity modulates the stimulation by amphetamine of DA release (46). But the mechanisms seem to differ between subcortical and cortical areas. In mesolimbic regions NE alpha-1 sites are needed for amphetamine to raise DA levels and elicit locomotion (e.g. 1b-knockout mice: 47). Alpha-2 agonists decrease mesolimbic DA levels, while alpha-2 antagonists are without effect (48). Mesolimbic DA release is also influenced by NE at beta-sites (49). But, in cortical regions alpha-1 sites can interfere with DA D₁ function (50) and blocking alpha-2 sites can raise DA levels like DA D₂ antagonists (51: cf. 9.2).

In cortical regions the interactions are complicated by an extra mechanism that has consequences for understanding ADHD treatment. Considerable extrasynaptic levels of DA are likely to interact with the numerous extrasynaptic DA receptors. But, this DA can also be taken up and cleared by NE transporters (52). So it is not surprising that chronic imipramine blockade of these sites leads to a down regulation of D₁ sites (53). Clearance of DA by both DA and NE transporters has been confirmed (54). But, further, a comparison of NE-innervated cortices with those receiving more or less DA innervation has shown that in both cases NE and DA levels can be reduced by alpha-2 agonists (e.g., clonidine) and increased by alpha-2 antagonists (e.g. idazoxan; 55). This demonstrates the co-release of DA from NE transporters. Thus, uptake and release of DA was recorded at NE uptake sites in the cortices (but not the basal ganglia). Inhibition of NE transporters influences both mesocortical NE- and DA- dependent function.

² Differences between reports likely reflect separate site-specific presynaptic roles on newly synthesized versus basal DA levels that in turn may vary between brain regions. For example, 5-HT_{1a} sites are mostly presynaptic in the brainstem, but postsynaptic in many projection areas. Thus, the presence of 5-HT_{1a} sites on dendrites in the VTA suggests a disinhibitory role (44), while 5-HT_{1b} mesolimbic sites facilitate DA release (45).

5. Development

5.1 NE

Catecholamine synthesis in the brainstem is in place in the middle of the second month of gestation. This matures up to around 13 weeks in parallel with the development of the ascending pathways (medial forebrain bundle) that penetrate the cortical plate at this time (56). Animal studies suggest the development lags behind that for DA at first, but overtakes it later (57).

Rodent and primate studies suggest that basal and stress-induced NE activity soars prepubertally, but falls back in adolescence, whereby changes in those reared away from their mother are less marked (58-60). Cortical alpha-2 receptors are evident before alpha-1 sites, but the latter expand postnatally while the alpha-2 concentration levels off. In puberty alpha-1 levels fall more than alpha-2 concentrations (61). Efficient control of NE function is mirrored by transporter mechanisms that also decline through puberty but rise again somewhat on attaining adulthood (62, 63). These developmental changes are reflected in 24h urine collections in human subjects (64). Compared to 8-12y-old children, in groups of younger and older teenagers NE levels fell by ca. 40% and its metabolite (MHPG) by two thirds (implying a halving of turnover activity). Yet by 20 years of age levels of both substances had again increased by a third.

It is not clear if there are gender differences in the development of the NE system. In contrast in the DA system a more marked overproduction of D₂- and D₁-like receptors between birth and puberty is reported for males. Indeed, in rodents mesolimbic D₁ binding appears to remain elevated in males (65)³.

5.2 5HT

Reports on the 5-HT system in animals show that the fine-axon system develops steadily from birth, with the fibers gradually concentrating in the first three layers of the cortex. The larger more beaded neurons

³ This difference may be further exaggerated by a leftward bias in males compared to a rightward bias of D₁ binding in females. However with maturation there is a decrease in the asymmetry in terms of DA and its metabolism (75).

develop later, but they also innervate the first three cortical layers and are forming pericellular innervation arrays by adolescence (66). 5-HT turnover remains relatively steady early in development while DA activity is rapidly increasing. However 5-HT activity, sensitive to stressors, may be depressed for example by rearing in isolation (67, 68). CSF measures taken from premature neonates to 6 month-old infants broadly confirm a large increase of DA metabolism while 5-HT turnover remains steady (69). Across this age-range the HVA/5-HIAA ratio doubled. This should not disguise, of course, that there is a large continuing prepubertal development of the 5-HT innervation of limbic and cortical areas in terms of binding sites and activity. However, the pace is moderate by comparison with the DA system (60, 70).

Human studies (platelet binding, postmortem reports) suggest that from the age of 10y, certainly from adolescence, 5-HT turnover and binding for 5-HT_{2a} and transporter sites decrease markedly (71-73). Indeed an associated down-regulation of 5-HT_{2a} sites has been monitored electrophysiologically (74). Concordant with this a drop of 50% or more was noted for 5-HT and its metabolite in urinary measures between 8-12 and 14-17 y-olds (64). This resulted in a halving of turnover rates that only partially recovered in young adults. In summary, the cortical innervation by 5-HT neurons is basically in place by birth, hyper-innervation is evident during childhood and this is cut back over puberty and adolescence. Details of the timing and localization of spurts and pauses are notable for numerous examples that are not in phase with DA developments. This provides many sensitive moments when environmental influences could disturb the balance of DA/5-HT interactions with largely unknown consequences.

6. Evidence for a monoaminergic contribution to ADHD -- Genetic studies

6.1 NE

Ten years on from Hechtman's review (76) studies are only starting to get under way to test her argument that genetic influences on NE will inform on ADHD. Genetic studies of features important to NE transmission and relevant to the ADHD condition have been few. They have

concentrated on the alpha-2a site for which NE has high affinity (where increased binding has been related to stress and frontal lobe cognition [77, 78]) and the re-uptake site, which if blocked (like the alpha-2a site) will lead to a decrease of neuronal firing (79). Metabolic enzymes (DBH, COMT and MAO: Fig. 1) have also received some attention. MAO activity, relevant for the breakdown of all the monoamines, has been related (inversely) to the expression of personality features thought to be relevant for groups or subgroups of ADHD subjects (e.g. impulsiveness, aggression and sensation-seeking: see discussion in 80).

A study using a so-called 'line-item' approach to the alpha-2a receptor (approximately the inverse of more conventional studies with single base-pair polymorphisms) found an allele associated with clusters of symptoms relevant to ADHD along with oppositional and conduct disorders (81). In contrast to this, another allele they examined related to anxiety and schizoid features. Studies focusing on this receptor seem promising. In contrast to the situation with DA, first reports on several polymorphisms relating to the NE transporter (NET1) have drawn a blank (82, 83). There is no evidence as yet that the NE transporter is relevant to the heritability of the ADHD phenotype.

Catechol-o-methyl transferase (COMT) activity is relevant to both DA and NE metabolism (Fig. 1). There is a low activity allele with methionine substitutions that is reported to be preferentially transferred in male Han-Chinese with ADHD, while the high activity form with valine substitutions was more common in the females (84). But while there is support for the transmission of the valine form in Israeli triads (85), in view of negative results from three other countries, the situation remains controversial.

Several polymorphisms have figured in studies of the genetic transmission of DBH (also for TOH), but there is little evidence for preferential transmission in ADHD (but see 86) and none for linkage (87). Consideration of MAO heritability also seems irrelevant to questions concerning the role of NE and 5-HT in ADHD. Associations were reported from a case control study of ADHD with comorbid externalizing problems (88) but earlier reports of

relationships to novelty-seeking have not been replicated (89).

6.2 5-HT

Little is known in relation to mental health about the genetic bases of the 22 or so subtypes of 5-HT binding sites currently known. Most studies have concentrated on

a) variants of the 5-HT1 class of receptors (especially 5-HT1b),

b) the 5-HT2 class (because of an association with DA release and motor activity (45), and an association of 5-HT2a blockade with reduced impulsivity in animals [90] and 'harm avoidance'⁴ in healthy adults [91]), and

c) the transporter (5-HTT). For the latter there are some features (alleles) that are transmitted and associated with a risk for ADHD (92, 93). Compared with a long form of the allele there is a short form with less efficient transcription efficiency and diminished 5-HT uptake.

Temperament contributes strongly to the normal response to novelty. The challenge of novel stimuli, as in the form of a stranger, naturally can lead to anxiety in the very young. This is important as temperamental or internalizing coping responses characterize ADHD children with very different comorbid problems. It is therefore of some interest that more anxiety was recorded to strangers in infants homozygous for the short form of the 5-HTT-linked promoter region length polymorphism (5HTT LPR), but less anxiety was observed in those with genotypes including one or more copies of the long form (94). Auerbach et al (95) also reported that infants homozygous for the short form were less easily distressed, and tended to be more withdrawn needing a longer latency to smile. Yet, it may emerge that the absence of the short form characterizes vulnerability for a heritable form of ADHD (96), for if it is associated with higher thresholds for provoking anxiety, it may coincide with the ease of risk-taking evident in many ADHD subjects. One awaits the results from prospective infant studies with interest.

⁴ Harm avoidance is one of three personality dimensions on the Cloninger scales. The other two dimensions, novelty seeking and reward dependence, were not related to 5-HT2a binding in this study.

With respect to 5-HT1b receptors, a recent report on 115 ADHD families using the transmission disequilibrium test for a particular polymorphism (G861C) showed a tendency for parental transmission of this allele, and in particular for paternal transmission to the child that was affected (97, 98). Quist et al. (99) had already pointed out a linkage disequilibrium of the 5-HT2a receptor (polymorphism His 452Tyr allele) with ADHD in these families indicating a preferential transmission of the 452Tyr allele to the affected offspring. While this was not confirmed by Hawi et al. (97), data from a symptomatic adult group also suggest that the gene for 5-HT2a sites played a role in the ADHD pathology recorded (100). Clearly one is still too close to the onset of such studies to be able to draw firm conclusions.

7. Methods:

Invasive methods for measuring transmitter activity in the CNS in vivo are available in animals (e.g. dialysis probes, electrochemistry) and adult persons (e.g. PET studies of ligand binding) but are not justified from an ethical standpoint in children. Measures must be conducted peripherally. There are 3 possible points of access along the route of elimination of excess monoamines and their metabolic products. These are the cerebrospinal fluid (CSF), the blood (including plasma and platelets) and the urine. Opinions differ widely on the extent to which these peripheral measures can reflect CNS function. Somatic sources of 5HT are particularly high. As there is no reason to suspect that in otherwise somatically healthy ADHD children that central systems are differentially impaired with respect to peripheral systems, crude indicators may be sought in the comparison of baseline measures between groups. The effects of challenges with monoaminergic drugs or environmental conditions on biochemical measures represent a good method for testing the functionality of NE and 5-HT pathways.

The extracerebral release of transmitters does not interfere with CNS transmission as there is a blood brain barrier with a powerful pump that transports them from brain to blood. What can cross the blood-brain barrier out of the brain and influence concentrations measured peripherally? Basically all the monoamines can pass with varying degrees of

ease passively or actively out of CNS tissue (review, 101), although as acid metabolites do not equilibrate across the blood-brain membranes, they are sensitive to active transport mechanisms (101). These mechanisms of active clearance may contribute to differences reported between blood or plasma and CSF measures. (Regions where the blood brain barrier does not so function include the circumventricular and sub-fornical organs, the choroid plexus and area postrema of the medulla.) However, measures derived from venous blood and urine, often reflect challenges to the system, at least at a qualitative level. Peripheral and central monoamine activities are often correlated: if the correlations are not good, they are still strong enough to be relevant to the study of behavior (103).

Some limits and influences on the study of monoamine activity from peripheral sources should also be recognized. In most cases changes in a peripheral catchment cannot not be attributed to over or under activity in any particular part of the CNS⁵. Further, it should not be overlooked that just as the processes of synthesis, release and uptake of transmitters change with age, so do the characteristics of the blood-brain barrier (104). These are poorly documented. The integrity of the blood brain membranes may receive insult from illness and their properties may be influenced by drug treatment. For example, it has been suggested that neuroleptic treatment can increase permeability (105).

An alternative approach is with the use of models that represent the specific feature of interest rather than the whole system. Relevant choices here include selection of the platelet fraction from blood to examine receptor function: thus, the binding characteristics of platelet 5-HT transporters model precisely those of the central transporter (106). A rather different type of model involves study of a particular breed of animal whose CNS

⁵ Usually blood samples for plasma or platelet analyses are collected from the arm. However, a series of studies compared veno-arterial gradients from the left/right jugular, hepatosplanchnic, forearm and cardiac vessels and showed that it is possible to separate the contributions from various somatic organs, as well as cortical vs. sub-cortical contributions (e.g. 101, 107-109).

responsivity resembles in certain ways that of children with ADHD (next section).

8. Animal models:

8.1 Rodent

Two widely cited models come to mind. The one proposes to 'model' hyperactivity with chemical lesion of DA pathways with 6-hydroxydopamine (usually using desipramine to protect NE terminals). The other compares some symptom dimensions shown by spontaneous hypertensive rats (SHR) in comparison to their genetic controls, the Wistar-Kyoto strain (WKY). In this second example, while largely peripheral NE systems contribute to the dominant feature of hypertension, the changes do not leave central NE systems unaffected. Further, 5-HT systems are also partly involved in the control of blood pressure⁶. The strength of the 'lesion-model' lies in the reliable stimulation of increased locomotion. However there is an overriding weakness. While the lesion renders the system hypofunctional in one sense, DA receptors become supersensitive to DA stimulation to produce the activity. This form of DA hyperactivity is not the basis for motor hyperactivity in ADHD subjects where there is much evidence for a (relatively) hypo-DA function. Nonetheless, as both psychostimulants and agents acting on other monoaminergic systems can calm ADHD patients (section 10), it is important that not only methylphenidate antagonizes hyperlocomotion in lesioned rats, but antagonists of 5-HT and NE transporters also reduce the locomotion elicited from lesioned rats (110). Indeed, the 5-HT modulation is not limited to the transporter and DA D2 mechanisms. 5-HT2 antagonists (e.g. ritanserin) also prevent D1 stimulation of hyperlocomotion arising from a lesion-induced supersensitive neostriatum (111). Clearly this most dopaminergic of symptoms, motor activity, can also be modulated by activity of the other monoamines, one way in psychopathology and in another way perhaps with successful treatment.

⁶ Regulation of blood-pressure by the Nucleus of the Tractus Solitarius is upregulated by increased 5-HT turnover in the SHR (118): hypertension may reflect an increase of sensitivity to stimulation of 5-HT2 receptors (119, 120).

What features pertinent to ADHD does the SHR model that may also be influenced by NE and 5-HT? The SHR explores more (112), though activity can be context dependent (113), reminiscent of situational rather than pervasive hyperkinetic children. SHRs may learn Hebb-mazes, active-avoidance tasks and multiple reversals faster than controls (114, 115), yet this sometimes reflects poor WKY performance (113). Sometimes the SHR has difficulty with passive avoidance, water-mazes extinctions, longer-term working memory and delayed response learning (e.g. temporarily withholding response for gratification: 116, 117). To a degree these difficulties, especially the last one, do mirror some of the features of ADHD.

Unfortunately neither quantitative relationships of NE and 5-HT activity to SHR behavioral function nor their responses to pharmacological challenge have been much studied. A few reports suggest that NE and 5-HT systems function differently, but even here the locus of control is poorly understood. Basal release of NE in slices of prefrontal cortex does not differ between SHR and WKY rats (121). The vesicular stores are not depleted. But brainstem, cortical (122) and even CSF levels (123) of NE are higher than normal. These levels are managed better after treatment with alpha-2 agonists that specifically reduce NE release (121, see guanfacine in section 9.1). Thus, autoreceptor-mediated control of NE release seems to be poorly regulated in the prefrontal cortex of SHRs (124) even though synaptosomal NE uptake is also reported to be higher in SHRs vs. WKY controls (125). What about the 5-HT system? An analysis of amines and metabolites in the prefrontal cortex and parts of the brainstem containing the LC and raphe (122) showed a significant decrease of 5-HT turnover in the brain stem (and a non-significantly lower turnover in the cortex). While this may simply reflect the bases for hypertension, one should recognise the influence this could have on mesocortical NE and DA activity (section 4). Further, considering the difficulty that SHRs (and ADHD children) have in withholding response on interval schedules, data consistent with the SHR neurochemistry just described comes from a study of blockade of NE and 5-HT uptake on differential responding at low rates of response (a 72 sec schedule:126). This study

reported that a range of NE uptake inhibitors enhanced, while a range of 5-HT uptake inhibitors impaired the efficiency of withholding responses appropriate to the delays of the schedule. One may conclude that the rodent model provides evidence for the "potential" for NE and 5-HT control of higher (dys)functions relevant to ADHD.

In looking to the future, it is appropriate to introduce a potentially useful model based on a new genetic variant of mouse, the Coloboma strain. Hyperactivity in this animal appears to result from a reduction of SNAP-25, a protein that regulates presynaptic exocytotic catecholamine release (127). Unexpectedly, while DA utilization is low, calcium-dependent NE concentrations are high. Also unexpected is that use of a neurotoxin specific to NE terminals (DSP-4) not only reduces NE but also hyperactivity. This suggests a link between NE transmission and motor activity, and prompts the search for other potentially relevant mouse models that are suitable to study with genetic knockout techniques. One concerns neurexin proteins involved in exocytotic mechanisms and in the binding to postsynaptic neuroligins (128). This promotes the coupling of impulse-related transmitter release to efficient post-synaptic docking. Arguably this mechanism in its (in)efficiency could make an important contribution to aspects of the ADHD condition.

Lastly, in the absence of an established model of developmental processes leading to ADHD, a brief mention is made of the potential for further study of the role of perinatal anoxia/hypoxia. The model involves placing rat pups in a nitrogen atmosphere for about 25 minutes. After 3-9 weeks DA and 5-HT metabolism is unusually high in the hippocampus and neostriatum (129), a feature that leads animals to make many errors on tests of sustained attention (130). The stages of a rat's development are difficult to equate with that of a child, but considering that major (differential) changes of 5-HT activity were noted during development (section 5) closer study could prove valuable. Another effect of anoxia is to alter CNS and peripheral levels of neuropeptide Y (NPY: 131). NPY is commonly localized in NE neurons, and raised NPY levels have been reported in many ADHD children (132), as would be expected from raised NE

levels. Work with the SHR shows increased NPY binding, that NPY enhances the effects of alpha-2 receptor agonism (e.g. vasoconstriction) and that while NPY administration decreases motor activity in normotensive animals, it increases it in the SHR (133, 134). Clearly there are several leads in the developmental hypoxia model and the SHR that should be followed up.

8.2 Primate

Recent reviews on the contribution of transmitter systems to ADHD give prominence to NE alongside DA, to the neglect of 5-HT and other candidates (135). These views are predicated on the undisputed role of impaired frontal activity in ADHD performance where delayed reinforcement (136), response inhibition, error- (137) and change-detection were studied (138). But the weight of the argument lies on a series of studies demonstrating that stimulation of NE activity in monkeys, when catecholamines are depleted, enhances working memory (WM) task performance: too little impairs, facilitated by alpha-2 stimulation; too much impairs, reflecting alpha-1 stimulation (where the low affinity of alpha-1 sites for NE means that they are active at high NE concentrations: 77, 139). Yet, the evidence for WM dysfunction rather than impairments of other executive functions in ADHD remains equivocal. A few studies have reported impairments of digit/arithmetic (140, 141) and visuo-spatial span (142-144). But the impairments are often small (c.1 standard deviation, 145), more of a problem for those with comorbid reading/learning difficulties (146) or are found only where the task loads on attentional capacity (147). Indeed, many of the differences disappear after covarying for IQ (148) and with increasing age (149-151). It is doubtful if impaired WM performance is a salient part of the neuropsychological profile of ADHD (152) or contributes significantly to other executive functions such as planning (153, 154).

It is therefore important to define the role of NE in tasks pertinent to ADHD. NE activity relates to vigilance, signal-detection abilities and attention-related processes. NE activity can alter (tune) the signal to noise ratio improving attention to relevant stimulation (review 10, 155). A series of studies has shown that fluctuations of neuronal discharge in the LC of monkeys correlate with performance on a

continuous performance test (CPT) of sustained attention (156). These authors have shown that while phasic LC firing is associated with good performance, elevated tonic discharge rates are associated with errors of commission, decreased sensitivity (d-prime), increased criterion levels for stimulus identification (beta decreased). The latter situation was improved by clonidine. Although clonidine does not seem to help ADHD children on the CPT (the sedative action seems to dominate), guanfacine can improve performance (157). Nonetheless, while the monkey-model provides some insight as to what could be happening in ADHD, it is not surprising that this complex relationship is not mirrored in a simple relationship between MHPG and CPT performance. Neither urinary nor plasma nor CSF levels of MHPG were related to CPT errors of omission or commission (158-160). However, the latter study (160) did mention a trend for a negative relationship between the HVA/MHPG ratio with d-prime. This suggests there is a potentially important imbalance between the two main catecholamine actors in ADHD in the determination of 'currently' relevant stimulation. The question remains open whether action at the alpha-2 receptor is the best way to 'tune' the NE role in tuning in ADHD cognition.

9. Evidence for monoamine contributions to ADHD -- NE and 5-HT activity

9.1 Evidence from group comparisons

Does the metabolism of NE and 5-HT differ between children with ADHD and those without a psychiatric or medical diagnosis? The question is based on the assumptions that

- a) pathological-developmental factors affecting transmitters in the body will affect peripheral and central metabolism similarly, and
- b) transmitter metabolism underlies the expression of the behavioral and cognitive measures typical of ADHD.

To a degree both assumptions are equivocal. The main limit to interpretation of the answer (apart from the caveat over the sample's source) lies with the knowledge that there are many other factors involved in the efficient coupling of nervous activity to the appropriate post-synaptic response that have not been

studied, and may not necessarily influence the metabolic parameters as currently measured.

Analyses of CSF, blood compartments and urine (**Table 1 below**) indicate that in the ADHD condition MHPG levels (NE metabolite) are usually lower than normal: less clearly NE levels may be increased. Overall this suggests a decreased turnover. There is a hint that other catabolic pathways may be differentially affected (cf. NMN levels). The severity of the core symptoms does not influence the results (161, 162). But, over the 4-5 years from pre- to post-puberty when a number of symptoms regress, MHPG levels have been noted to increase or normalize (163). Further, some studies that deliberately contrasted sub-groups find that several comorbidities (independent of their nature) appear to counteract the metabolic decrease: e.g. in those with a reading disorder (159), and in 15 subjects with high levels of anxiety (not in Table: 103).

The results for the 5-HT system are more limited reflecting in part the methodological issues (section 7). However, if one brings the separate findings together, there is an indication of an increase of 5-HT turnover largely reflecting decreases in 5-HT levels (**Table 1**). Nonetheless, as with NE, it must be recognized that there will be sub-groups, however defined, for whom the effects associated with the core symptoms will be masked by other features. One such example is shown by the contrast between ADHD boys brought up in families with or without alcoholic fathers (164). Those with this experience showed a larger cortisol response to a challenge dose of fenfluramine than those without an alcoholic father. This was interpreted as reflecting increased 5-HT receptor sensitivity.

Another example of the influences of comorbidity on 5-HT activity concerns impulsivity. Impulsive aggression (oppositional behaviors; 30, 165) has been associated with low plasma and CSF 5-HIAA and synaptic availability of 5-HT. This contrasts with the generalization noted in the preceding paragraph. Intriguingly, Oades et al. (36) compared the binding characteristics of the platelet 5-HT transporter with clinical ratings (impulsivity/ distractibility, externalizing/ aggression) and the (in)ability to withhold responses on the Stop-Signal task (cognitive

impulsivity). Decreased affinity correlated with poor response inhibition (cognitive impulsiveness) but not clinical ratings, even though the cognitive and clinical indices of impulsivity were related. In contrast aggressive behavior related to increased 5-HT transporter affinity⁷. (See section 6.2: genetic control of 5-HT availability by the transporter [HTTLPR]).

Cognitive impulsivity might be expected to reveal itself on the CPT test of sustained attention in the form of an increased rate of false alarms. However, as yet, both high (blood: 166) and low levels of 5-HT (tryptophan depletion: 167) have been related to more errors of commission. But, d-prime, reflecting target sensitivity, was reported to decrease as the excretion of the 5-HT metabolite increased (160), which supports interpretations of the platelet study, above.

Lastly another indication that there may be 2 ADHD sub-groups differing in the sensitivity of the 5-HT system comes from neurophysiological study of the augmenting-reducing response using event-related potentials. The N1/P2 component may increase (augment) or decrease (reduce) in response to increases of salience (loudness of sounds). An augmenting response is a feature of sensation-seeking (168), and ADHD subjects who respond to amphetamine (169). Increasing stimulus intensity-dependence relates to decreasing 5-HT activity (and vice versa, cf. effects of alcohol and lithium: 170). Among ADHD subjects who do not respond to amphetamine, a reducing response to auditory stimuli is typical (169). It remains unclear how closely coupled 5-HT activity is with the augmenting-reducing phenomenon. But, it would be worthwhile combining biochemical measures in ADHD subjects with/without the conduct problems that are influenced by 5-HT activity with this paradigm

9.2 Evidence from pharmacological treatments:

The question addressed here is whether there is evidence that treatments that exert a good effect on the ADHD condition also exert a minor or major effect by way of the NE or 5-HT

⁷ Reductions of binding site affinity should normally be off-set by increased receptor capacity. If this does not occur then more 5-HT remains available in the synapse.

systems? As noted 20 years ago, “The large number of efficacious drugs does not support

any single neurotransmitter defect hypothesis” (171a). Here, we ask if there is convincing

TABLE I

Comparisons of components of NE and 5-HT metabolism in urine, blood, and CSF samples from ADHD and controls.

Source	hyperactives	controls	metabolite	monoamine	change vs. controls	reference
Urine	9	6	MHPG		none	231
Urine	7	12	MHPG & NMN		decrease & increase	232
Urine	13	14	MHPG	NE	none & increase	233
Urine	15	13	MHPG		decrease	234
Urine	10	10	MHPG		increase	235
Urine	9	9	MHPG		decrease	236
Urine	73	51	MHPG		decrease	237
Urine	28	23	MHPG		decrease	238
Urine (2h)	20	22	MHPG NMN, MN, VMA, NMN/NE	NE	none & none all increase *	103
Urine (1h)	15	16	DOPEG	NE	decrease & none	239
Urine	13	13	MHPG/NE, MHPG, HVA/MHPG	NE	decrease none & none trend increase	240
Urine	14	9	MHPG/NE, MHPG,	NE	trend decrease none & increase	132
Urine	15(37)	21	MHPG		none	179
Urine	31	26	MN, NMN & NMN/NE		none	185
Urine	x (severe)	y (mild)	VMA	NE	none & none	162
serum	35	19		NE	none	241
serum	49	11		NE	none	242
plasma	12	11		NE	trend increase	243
plasma	8 (+RD)	14 (-RD)	MHPG		decrease (if no RD)	159
plasma	14	9		NE	trend increase	132
plasma	35 (many vs. few symptoms)			NE	none	161
CSF	29 (vs.20 conduct disorder)		MHPG		trend increase	158
Urine	13	13	5-HIAA/5-HT, 5-HIAA, HVA/5-HIAA	5-HT	none, increase, & decrease decrease	240
Urine	14	9	5-HIAA/5-HT, 5-HIAA,	5-HT	trend increase, increase, & decrease	132
Urine	15(37)	21	5-HIAA		none	179
Blood	25	vs. norm		5-HT	decrease	244
Blood	49	11		5-HT	none	242
Blood	70	vs. norm		5-HT	decrease	245

Serum	11	11		5-HT	decrease	246
Platelet	17	75	5-HIAA		none	241
Platelet	55	38		5-HT	none	247
plasma	35 (many vs. few symptoms)			5-HT	decrease	161
CSF	24	6	5-HIAA		none	248
CSF	6	16	5-HIAA		none	249
CSF	29 (vs.20 conduct disorder)		5-HIAA		none	158

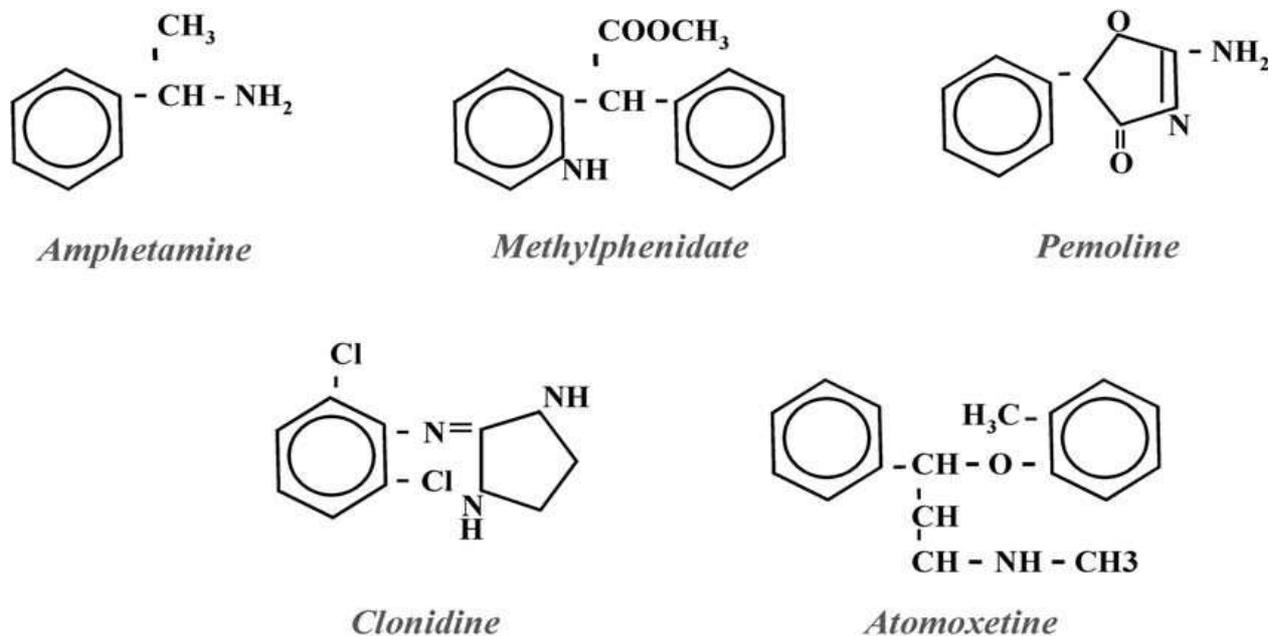


Figure 5

The biochemical structure of 5 agents with therapeutic effects on ADHD. Amphetamine, methylphenidate and pemoline are psychostimulants that block the pre-synaptic re-uptake of monoamines: clonidine is an alpha-2 agonist at NE binding sites (including somatic autoreceptors): atomoxetine relatively specifically blocks NE re-uptake

evidence that NE and 5-HT should be ruled in, rather than out of any potentially explanatory model for ADHD? Let us first consider the agents that have proved most efficacious in the treatment of ADHD, the **psychostimulants** methylphenidate, amphetamine (and pemoline: **Fig. 5**). Below, evidence from other agents with significant effects on the NE and 5-HT systems that result in more modest but significant clinical effects in patients with ADHD are considered.

The dominant effect of **methylphenidate** is to block re-uptake of impulse-released DA at the DA transporter resulting in increased extracellular availability of DA (the oral dose to block 50% of sites is about 0.25 mg/kg; 171b). But it also binds to the NE transporter strongly and the 5-HT transporter very weakly. **Amphetamine** binds with each monoamine transporter and can raise extracellular levels by stimulating the release of extra-vesicular newly-synthesized transmitter and blocking re-uptake. The former mechanism is usually emphasized as

treatments that block catecholamine synthesis inhibit the effects of amphetamine more than methylphenidate. (Caveat: the mechanism of stimulating the transporter to release transmitter, or block the re-uptake varies with dose, and specific data vary with measures made in vitro or in vivo: 172). A modest degree of MAO inhibition has also been reported. Pemoline (caveat: liver toxicity) will not be further discussed: its effects are specific to the release and uptake of DA (173).

In preclinical studies in rodents, methylphenidate (0.75-3.0 mg/kg, iv) does not increase motor activity or mesolimbic levels of DA, but it does increase extracellular levels of NE (e.g. in the limbic system: 174). Similar doses of amphetamine (sc) increase limbic and frontal levels of NE to a greater extent (and release DA: 172). While higher doses (e.g. 20 mg/kg) of methylphenidate still release NE they do not increase levels of 5-HT. Nonetheless such pharmacological doses have been reported to enhance 5-HT metabolite levels in fronto-striatal regions (175). In contrast 2.5/3.0 mg/kg amphetamine can raise 5-HT levels 3-fold and increase its metabolism (e.g. neostriatum: 176). Sub-chronic amphetamine treatment has been reported to sensitize brainstem 5-HT_{1a}, but not 5-HT_{2a} sites (177).

Do the biochemical responses to the psychostimulants reflect expectations from the preclinical results? First, care must be taken with the interpretation of results as the variability between reports, whether from different or the same authors can be marked for measures taken from the CSF, plasma or urine. Secondly, HVA levels, as noted above can reflect peripheral NE metabolism⁸, also tend to decrease/normalize after methylphenidate treatment, whether or not the patients responded clinically (urine, 179; CSF, 30). Both studies noted that although 5-HT metabolism was not necessarily high, levels tended to decrease with treatment following corrections of high levels DA metabolism and symptom improvement..

The only clear result for NE, 5-HT and their metabolites is that urinary MHPG levels decrease after amphetamine (7/7 studies) but

not after methylphenidate treatment (3/3 studies: Table 14.1 in 180). VMA levels were also reduced after amphetamine in 3/3 studies. For other metabolites increases and decreases have been reported and no clear pattern emerges. It is surprising that unequivocal changes of NE levels are not usually recorded after methylphenidate treatment. At first sight it is enigmatic that the frequently reported low turnover for NE in ADHD patients should be further lowered in those who respond to psychostimulant treatment (181). A possible explanation derives from electrophysiological recordings in primates (182). A parallel is drawn between an overly tonic firing mode for the LC during poor CPT performance and the sustained attention problems in ADHD. Low activity facilitates interactions with many stimuli rather than focused attention. Stimulants decrease the tonic activity and facilitate a transition to a phasic firing mode. This counteracts the 'hypo-arousal' in the system. The coupling of information transfer is improved, even though the overall NE turnover rate decreases further.

Raising the issue of arousal encourages mention of the biochemical support for the concept of hypo-arousal in ADHD from measures of adrenaline and phenylethylamine (PEA). Adrenaline levels tend to be low in urine samples from ADHD children and the adrenergic (and cortisol) response to stress is reduced (183-185⁹). Adrenaline levels rise with methylphenidate or amphetamine treatment (186-188). This is consistent with the simple concept of low levels of arousal becoming partially normalized by stimulant treatment. PEA is a naturally occurring amphetamine-like derivative that results from decarboxylation of phenylalanine, a precursor to normal catecholamine synthesis (Fig.1). Levels are frequently found to be raised in a range of psychiatric, excited conditions (e.g. acute schizophrenia, bipolar disorder, some obsessive compulsive and psychopathic conditions) but reduced in depression (189-192). They are lower

⁸ Peripheral NA metabolites were reported to be high in the ADHD urinary samples (178).

⁹ However, in highly anxious children, usually with internalizing problems, urinary adrenalin levels can indeed be high with respect to patients without prominent anxiety (180). Slightly higher levels of plasma adrenaline reported in ADHD children (132) may likewise have reflected the cognitive testing that occurred around the same time.

in ADHD, even if PEA levels are not significantly correlated with symptom severity itself (179, 193). Psychostimulant treatments raise PEA levels (194, 195). PEA levels may reflect endogenous homeostatic mechanisms for promoting catecholamine activity (e.g., like amphetamine, PEA increases CSF levels of NE and DA in non-human primates [196]). In summary, while both psychostimulants lead to an increase of extracellular catecholamines, they differ a) on the mechanism at the transporter, b) on its relation to impulse flow and c) at clinically relevant doses only amphetamine significantly influences 5-HT activity; yet it is clear that specific effects of methylphenidate (and atomoxetine) at the NE transporter can bring about significant changes in the activity of both catecholamines, especially in mesocortical regions.

The relatively recent introduction of **atomoxetine**, a selective NE transport inhibitor, as an efficacious form of ADHD treatment merits attention; however, independent studies of the nature of the improvement and biochemical effects remain sparse. In rodents it raises mesocortical NE and DA levels three-fold. Like methylphenidate it is without influence on the 5-HT system, but in contrast it is without effect on nigro-striatal or mesolimbic catecholamines (197). [Note that methylphenidate also raises mesocortical NE and DA levels to a similar degree.] The focus of attention on the mechanisms underlying its efficacy returns to the role of cortical NE transporters on the availability of both catecholamines (section 4.3). Atomoxetine improves each of the diagnostically important symptom clusters (inattention, impulsivity and activity: 198), but results of more specific tests of attentional abilities or of cognitive impulsivity remain unclear.

A range of well-known antidepressants can also positively influence ADHD symptoms (e.g. MAO inhibitors, desipramine, review 199). In 7 trials **desipramine** (DMI), known for its blockade of NE uptake, is reported to modestly improve hyperactivity, impulsivity, distractibility and some limited aspects of learning (paired associates) and recall (match to familiar figures: 200, 201). Yet it has no apparent effect on the CPT measure of sustained attention (201, 202). Cardiac side-effects discourage the use of DMI,

but, as with other “helpful” treatments, DMI can decrease NE excretion, along with its central and peripheral metabolites (203). DMI may not so much alter basal levels of NE but increase those arising from stimulus-coupled release of NE, a parallel to methylphenidate’s action (10). Unfortunately there is little information on dose-dependent biochemical effects or correlations with the reported behavioral improvements.

Less well-documented are effects of DMI on the 5-HT system. This surprises as tertiary antidepressants like imipramine with an effect on the 5-HT transporter exert modest improvements like the secondary antidepressants (e.g. DMI: 204). Recently Overtoom and colleagues (205) reported on a left-right discrimination test in ADHD children treated with either DMI, methylphenidate, L-dopa or placebo. The discrimination became a stop-signal test with a no-go tone rapidly following some of the discriminanda. Methylphenidate treatment speeded reaction times and decreased omissions and discrimination errors. That L-dopa (promoting post-synaptic DA levels) had no effect does not show that DA had no effect, as the synaptic mechanisms differ from the other agents investigated. But it promotes speculation that methylphenidate was at least in part influencing the NE system. More intriguing still is that inhibition on the stop-task improved only after DMI treatment. Fortunately the authors recorded prolactin responses to treatment. These decreased as expected after the two “DA” treatments, but increased after DMI. The supposition that this was a 5-HT effect was confirmed by their finding that serum 5-HIAA levels decreased. This seems to confirm the proposition (section 9.1) that changes in the 5-HT system may relate to cognitive impulsivity, while other attentional effects may reflect NE / catecholaminergic activity.

Clonidine is not a treatment of first choice. This reflects its side effects (blood-pressure, sedation, dizziness) and that its efficacy is largely restricted to oppositional problems (e.g. aggression¹⁰, frustration tolerance, co-

¹⁰ Relevant to the study of NE’s role in comorbid aspects of ADHD is that beta-NE blockers also yield positive results in treating problems related to aggression, despite potential cardiac problems with this form of treatment (214).

operation: 206). However, some improvements in hyperactivity and impulsivity have been reported (meta-analysis, 207), especially when co-administered with methylphenidate (208, 209). Further, performance on some specific tests of frontal executive functions can be enhanced (210), and response speed and errors on tests of sustained attention improved with clonidine treatment (211). Two reports (212, 213) confirmed the inhibitory effects of clonidine expected from preclinical studies by showing that MHPG levels decreased in ADHD children and young adults. (The literature on hypertension also shows falling NE concentrations.) It is therefore of interest to look at clonidine's agonist activity at alpha-2 NE receptors.

A direct action at alpha sites was assumed to underlie the enhanced growth hormone response to a pharmacological challenge with clonidine (213). While an increased receptor sensitivity may be consistent with simple interpretations of clonidine's inhibitory influence, and perhaps that of guanfacine, the implication from platelet alpha-2 receptor binding is different (215). This group used the platelet model of binding to predict stimulant response. They found a generally low level of binding: ADHD children with relatively normal binding responded to treatment, and those with low levels were non-responders. However, other interpretations of clonidine's action are possible, and some expectations can be generated from animal studies. Using systemic doses in the range of 0.1-1.0 mg/kg (and local treatments), clonidine not only reduces NE release (in the brainstem and cortices) but reduces brainstem and cortical 5-HT release (21, 216). These studies show that alpha-1 and alpha-2 sites exert opposite facilitatory and inhibitory influences on 5-HT release. Further, of interest for the interpretation of the roles of mesocortical DA and NE (section 4.3), the stimulation of increases of cortical DA and NE (e.g. by clozapine treatment) can be prevented by quite moderate doses of clonidine (0.015 mg/kg: 217).

In summary, clinical and preclinical work with clonidine show *a*) limited but significant improvements in some areas of function relevant to ADHD; *b*) reduced 5-HT release could underlie the modulation by clonidine of

aggressive and impulsive behavior (15); *c*) a mechanism for reduced NE release to incur reduced DA release, which could have both helpful (hyperactivity) and less helpful consequences (appreciation of reinforcement). In a similar vein, lowering NE release may enhance sustained attention performance (211), and it may raise the degree to which alpha-2 rather than alpha-1 receptors (with a lower NE affinity) might assist cortical function (e.g. working memory and related executive functions, 218). Nonetheless hard evidence for binding differences in ADHD children is lacking, and treatments aimed at the alpha-2 receptor could be counter productive in the appropriate control of responses to stress.

Two of three open trials of **Guanfacine**, an agonist at alpha-2 NE sites, found a modest improvement of ratings of attention and impulsivity, with one demonstrating fewer errors on the CPT (157, 219, 220). Controlled trials (vs. amphetamine) in adult ADHD patients showed comparable reductions of symptoms and even an improvement of the Stroop color-word naming, so often impaired in childhood ADHD (221). In children with ADHD and comorbid tics teacher ratings improved in half the patients, who also performed a CPT more accurately (222). Thus, a modest degree of success for Arnsten's alpha-2 NE hypothesis (139) appears to be realized, although with a certain risk of lethargy, bradycardia and hypotension the agent should perhaps be held in reserve for psychostimulant non-responders.

Despite indications that some treatments may achieve therapeutic effects (e.g. impulsivity) by an action on 5-HT systems, direct attempts using agents with unequivocal effects on 5-HT metabolism have been largely without success (e.g. the precursor amino acid tryptophan, [223] fenfluramine that facilitates 5-HT release, [186]; an agonist at 5-HT_{1a} sites, buspirone [review: 224]). It is sobering and important to note that while a particular agent may reduce symptoms and alter monoaminergic metabolism, it is not known that the metabolic changes are related to the psychopathological changes. The report of Donnelly and colleagues (186) is salutary. Fenfluramine treatment (0.6-2.0 mg/d) had no significant therapeutic effect on ADHD boys aged 6-12y. However, urinary NE, MHPG, VMA and epinephrine all decreased

significantly, as did plasma MHPG and platelet 5-HT levels. Yet, for those with impulsive aggression, and delinquency there is a clear relationship with low 5-HT activity, be it expressed as reduced platelet binding of imipramine (e.g. 225), plasma 5-HIAA (165) or prolactin response to fenfluramine challenge (226).

10. General:

Early proposals that NE could have a causal role in ADHD and hyperkinetic behavior in particular (227) were based on the effect of amphetamine to reduce NE activity during arousal. Now there is a widespread belief that children with ADHD are under- rather than over-aroused: yet there is an increasing consensus that NE function has something to do with the symptoms (204). Evidence in this chapter shows that NE activity undoubtedly modulates attentional mechanisms both directly (tuning signal to noise ratios) and indirectly (via the control of mesocorticolimbic DA release). NE may influence other relevant behaviors depending on their dependence on cognitive mechanisms (e.g. environmental stimulation facilitating hyperkinesis) and the nature of the mechanisms underlying comorbid conditions. Crucial mechanisms include the control of catecholamine availability in the cortex (via the transporter) and phasic firing modes in the LC. Both of these should be targets for treatment.

Common to a consideration of the relative role of NE and 5-HT in ADHD is the increasing appreciation of a crucial role for the transporter in determining the availability of monoamines. Thus, cortical NE transporters can release DA (55), the DA transporter is regulated by a variety of substrates including 5-HT (228) and the NE transporter (cf. knockout mice) modulates the perception of reinforcement (229). This latter finding has implications for understanding the aversion to accepting delays between response and reinforcement gradients associated with the SHR and with ADHD subjects (230).

5-HT mechanisms are also relevant to the expression of features of ADHD by direct (transporter mediated reuptake mechanisms) and indirect mechanisms (modulation of DA activity, especially in the initiation of behavioral responses). These have been under-researched in view of more clearly established relations of

5-HT activity to the expression of externalizing responses more frequent in comorbid conditions. Now it is appreciated that 5-HT activity has a role in information processing (modulating gain) and cognitive impulsivity. The appreciation of these roles and the *interactions* of the three monoamines should make it easier to tailor treatment to the particular individual (im)balance of the pattern of cognitive, motivational and motor bases to be found in a given patient.

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