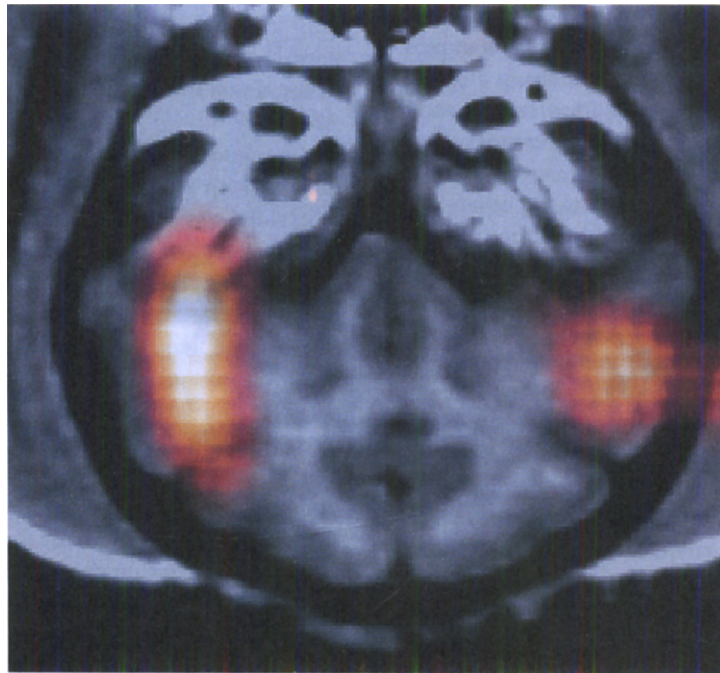


## Genetics of Childhood Disorders: XVIII. ADHD, Part 2: Norepinephrine Has a Critical Modulatory Influence on Prefrontal Cortical Function

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Dysfunction of the prefrontal cortex (PFC) is a fundamental component of attention-deficit/hyperactivity disorder (ADHD). The PFC uses working memory to intelligently guide behavior, inhibiting inappropriate impulses or distractions and allowing us to plan and organize effectively (see pre-

vious column). Individuals with ADHD are consistently impaired on tests of frontal lobe function, and both structural and functional imaging studies have shown evidence of altered PFC function in individuals with ADHD. In particular, the right PFC has been shown to be consistently smaller in



**Fig. 1** Effects of guanfacine on regional cerebral blood flow (rCBF) in the prefrontal cortex of a monkey performing a spatial working memory task. rCBF was measured using technetium-99m ECD–single photon emission computed tomography (SPECT). Monkeys received guanfacine (0.7 mg/kg) or saline 2 hours before cognitive testing.  $^{99m}\text{Tc}$  ECD was infused while the animal was performing the working memory task. After testing, the monkey was anesthetized and brought to the SPECT camera for imaging. The figure represents a magnetic resonance imaging (MRI) coronal section of a monkey brain at the level of the prefrontal cortex with the difference between the guanfacine and saline SPECT scans overlaid on the MRI. Guanfacine improved performance of the task and significantly increased rCBF in the dorsolateral prefrontal cortex surrounding the principal sulcus, the brain region most critical for spatial working memory performance. In contrast, guanfacine had no effect on rCBF in a control region unrelated to the task, the auditory association cortex. Reprinted by permission of Elsevier Science from “The alpha-2A adrenoceptor agonist, guanfacine, increases regional cerebral blood flow in dorsolateral prefrontal cortex and improves accuracy in monkeys performing a spatial working memory task” by Avery R, Franowicz JCS, Studholme C, van Dyck CH, Arnsten AFT, *Neuropsychopharmacology* 23:240–249, copyright 2000 by the American College of Neuropsychopharmacology.

ADHD subjects than in age-matched controls, and the inability to suppress responses to salient but irrelevant stimuli correlates with reduced volume of the right PFC (Casey et al., 1997).

The cognitive functioning of the PFC is modulated in a critical manner by the neuromodulators norepinephrine (NE) and dopamine (DA). Intriguingly, a recent positron emission tomography study showed reduced fluorodopa binding in the PFC of adults with ADHD, indicative of altered NE and/or DA transmission in the PFC in this disorder (Ernst et al., 1998). Although most previous research has focused on DA mechanisms, NE influences on PFC function are just as powerful. Indeed, as recent research suggests that low doses of the ADHD medication methylphenidate (Ritalin®) preferentially release NE in rat brain, NE mechanisms may be particularly relevant to our understanding of ADHD. This column will review the evidence that NE has an essential beneficial influence on the working memory and attention functions of the PFC through actions at postsynaptic,  $\alpha_{2A}$ -noradrenergic receptors, while very high levels of NE release appear to engage  $\alpha_1$ -noradrenergic receptors and impair PFC functions.

NE has been associated with attention regulation for many years. NE cells of the locus ceruleus increase their firing in response to behaviorally relevant stimuli. Selective depletion of NE in the forebrain makes animals more distractible. At least some of these behavioral changes are likely due to altered NE in the PFC. Either global depletion of catecholamines or depletion restricted to the PFC impairs working memory and attention regulation, while having little effect on basic visual discrimination and associative abilities.

Anatomical studies have documented the NE innervation of the PFC in rodents, monkeys, and humans. NE axons from cells of the locus ceruleus terminate throughout the PFC with moderate density. The  $\alpha$ - and  $\beta$ -noradrenergic receptor subtypes have been observed in the PFC, and the  $\alpha_{2A}$ -noradrenergic receptor has been localized both presynaptically and postsynaptically in the primate PFC. Although previous research focused on presynaptic  $\alpha_2$ -receptors ("autoreceptors" that decrease NE release), it is now appreciated that the vast majority of  $\alpha_2$ -receptors in the brain are localized postsynaptic to NE cells. In the monkey PFC,  $\alpha_{2A}$ -receptor immunoreactivity has been documented over the postsynaptic thickening of dendritic spines of pyramidal cells, demonstrating an anatomical substrate for postsynaptic actions.

$\alpha_2$ -Noradrenergic agonists such as clonidine, guanfacine, and medetomidine have been shown to improve a variety of cognitive functions subserved by the PFC in rodents, monkeys, and humans (Jakala et al., 1999). Systemic administration of these compounds can enhance performance of working memory tasks, response inhibition, and planning, particularly under distracting conditions. These improvements are blocked by cotreatment with  $\alpha_2$ -antagonists, consistent with actions at  $\alpha_2$ -receptors. In contrast,  $\alpha_2$ -agonists have little effect or actually

impair performance of tasks that depend on posterior cortices or subcortical structures, indicating functional specificity.  $\alpha_2$ -Agonists are particularly potent in enhancing PFC functions in subjects with catecholamine depletion due to either experimental manipulations (e.g., the neurotoxin 6-OHDA or reserpine) or natural conditions (e.g., aging, vitamin B deficiency in Korsakoff amnesia). The finding that  $\alpha_2$ -agonists become more, rather than less, efficacious in subjects with catecholamine depletion is consistent with drug actions at postsynaptic  $\alpha_2$ -receptors.

Three  $\alpha_2$ -receptor subtypes have been identified in humans: the A, B, and C subtypes. Pharmacological profiles suggest that the  $\alpha_{2A}$ -subtype underlies the PFC-enhancing effects of  $\alpha_2$ -agonists. Thus agonists such as guanfacine, which are relatively selective for the  $\alpha_{2A}$ -subtype, are able to improve PFC function with fewer side effects than nonselective agonists such as clonidine (Arnsten, 1998). Recent studies in mice with a mutation of the  $\alpha_{2A}$ -receptor (a "functional knockout") support this hypothesis: guanfacine improves the working memory performance of wild type mice but has no effect in mice with a mutation of the  $\alpha_{2A}$ -receptor (reviewed by Arnsten, 2000). In contrast,  $\alpha_2$ -agonists remain effective in mice with a knockout of the  $\alpha_{2C}$ -subtype. Work with the  $\alpha_{2B}$ -knockout remains to be done. Thus, studies to date have focused on the importance of the  $\alpha_{2A}$ -receptor for PFC function.

Evidence suggests that  $\alpha_2$ -agonists act directly in the PFC to enhance working memory function. The beneficial effects of  $\alpha_2$ -agonists disappear in subjects with PFC ablations, suggesting that the PFC is the substrate for drug actions. Consistent with this idea, systemic administration of guanfacine or clonidine has been shown to enhance regional cerebral blood flow in the PFC of both human and nonhuman primates performing PFC tasks. For example, Figure 1 shows the areas of enhanced regional cerebral blood flow in the dorsolateral PFC of a monkey performing a spatial working memory task. Lesions of this same area markedly impair performance of this task.

More direct evidence for PFC actions comes from animal studies in which the drug is infused directly into the PFC. Infusions of guanfacine into the monkey dorsolateral PFC produce a delay-related improvement in working memory performance. Similarly, infusion of medetomidine into the PFC of aged rats improves working memory as tested by the delayed alternation task. Conversely, infusion of the  $\alpha_2$ -antagonist, yohimbine, into the PFC of monkeys produces a marked, delay-related impairment in working memory performance. These results indicate that endogenous NE stimulation of  $\alpha_2$ -receptors in the PFC has a critical influence on behavioral regulation. Infusions of  $\alpha_1$ - or  $\beta$ -adrenergic antagonists were without effect on performance, highlighting the importance of  $\alpha_2$ -receptors to PFC function. Recent electrophysiological studies have shown that iontophoretic application of yohimbine onto PFC cells in monkeys performing a working memory task reduces delay-related firing of PFC neurons, the cellular measure of working memory (Li et al., 1999). Conversely,

either iontophoretic or systemic administration of an  $\alpha_2$ -agonist enhances delay-related activity. Thus,  $\alpha_2$ -receptor stimulation is critical for PFC function at both the cellular and behavioral level.

In contrast to the marked beneficial effects of  $\alpha_2$ -receptor stimulation on PFC functions, high levels of  $\alpha_1$ -noradrenergic receptor stimulation impair PFC function (reviewed by Arnsten, 2000). These detrimental actions appear to come into play under conditions of very high NE release, e.g., during uncontrollable stress. In this regard, it is of interest that NE has much lower affinity for  $\alpha_1$ -receptors than for  $\alpha_2$ -receptors, suggesting that high levels of NE release may be needed to significantly engage detrimental  $\alpha_1$  mechanisms. Administration of  $\alpha_2$ -agonists can restore PFC cognitive function in stressed subjects with very high levels of catecholamine release, suggesting that both pre- and postsynaptic  $\alpha_2$ -receptors can contribute to beneficial effects depending upon the state of the subject. Current research is exploring the second-messenger mechanisms underlying the detrimental effects of  $\alpha_1$ -receptor stimulation. Evidence to date suggests that  $\alpha_1$ -receptor stimulation impairs PFC function through activation of the phosphatidylinositol/protein kinase C intracellular signaling pathway. It is intriguing that overactivity of this intracellular pathway has been linked to mania, a disorder that shares some similarities to the symptoms of ADHD.

In summary, research in animals demonstrates that either too little  $\alpha_2$ -receptor stimulation or too much  $\alpha_1$ -receptor stimulation can impair PFC cognitive function. These findings suggest that altered NE transmission could contribute to symptoms of ADHD. For example, mutations in the synthetic enzymes for NE or in  $\alpha_{2A}$ -receptors could lead to insufficient  $\alpha_{2A}$ -receptor actions and impaired PFC function. Mutations of proteins such as the NE transporter would lead to higher levels of NE in the synapse and excessive stimulation of  $\alpha_1$ -receptors that would also impair PFC function. It will be interesting to observe whether genetic studies find associations between these proteins and ADHD symptoms, as suggested by some preliminary studies. Genetic studies have already found a consistent association between DA  $D_4$  receptor polymorphisms and ADHD symptoms, particularly in adults. In this regard it is important to remember that NE has very high affinity for  $D_4$  receptors; indeed it has higher affinity for  $D_4$  than for noradrenergic  $\alpha$ - or  $\beta$ -receptors. However, we do not currently understand how either DA or NE may act at  $D_4$  receptors to alter PFC function.

The critical importance of NE to PFC function may explain why selective noradrenergic agents have been successful in treating ADHD. The nonselective  $\alpha_2$ -agonist, clonidine, has had modest success in treating ADHD symptoms. More recently, the selective  $\alpha_{2A}$ -agonist, guanfacine, has also reduced symptoms of ADHD and improved performance of PFC tasks in both open-label and controlled trials (reviewed in Arnsten, 2000). Guanfacine likely reduces impulsivity and enhances attention regulation by strengthening PFC control of behavior.

Similarly, the nonselective NE reuptake blocker, desipramine, and the new, selective NE reuptake blocker, tomoxetine, have been shown to ameliorate ADHD symptoms. Noradrenergic therapeutics will be the topic of the next column in this series.

The data presented here suggest possible common mechanisms for how these compounds may be helpful in treating ADHD. Both  $\alpha_2$ -agonists and NE reuptake blockers may serve to normalize NE transmission in the PFC and thus enhance PFC function. In subjects with underactivity of the NE system, these compounds could increase postsynaptic  $\alpha_2$ -receptor stimulation in the PFC through either direct stimulation of these receptors (guanfacine) or by increasing available NE levels in the synapse (NE reuptake inhibitors). Conversely, in subjects with overactivity of the NE system, actions at presynaptic receptors might predominate to reduce NE tone. However, it is important to remember that agents such as guanfacine can improve PFC performance in normal subjects or in individuals with altered DA activity. Thus, PFC cognitive enhancement with  $\alpha_2$ -agonists does not necessarily signify altered NE activity. Instead, it is likely that a variety of insults to PFC circuits can result in ADHD symptoms, and enhanced noradrenergic  $\alpha_2$ -receptor stimulation in PFC may help to overcome these problems irrespective of their cause.

#### WEB SITES OF INTEREST

<http://www.sciam.com/askexpert/medicine/medicine9.html>  
<http://www.sciam.com/1998/0998issue/0998barkley.html>  
<http://www.chadd.org/fact6.htm>  
<http://www.add.org/main/abc/hallowell.htm>

#### ADDITIONAL READINGS

- Arnsten AFT (1998), Catecholamine modulation of prefrontal cortical cognitive function. *Trends Cogn Sci* 11:436–447  
 Arnsten AFT (2000), Through the looking glass: differential noradrenergic modulation of prefrontal cortical function. *Neural Plast* 7:133–146  
 Casey BJ, Castellanos FX, Giedd JN et al. (1997), Implication of right frontostriatal circuitry in response inhibition and attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 36:374–383  
 Ernst M, Zamerkin AJ, Matochik JA, Jons PH, Cohen RM (1998), DOPA decarboxylase activity in attention deficit disorder adults: a [fluorine-18] fluorodopa positron emission tomographic study. *J Neurosci* 18:5901–5907  
 Jakala P, Riekkinen M, Sirvio J et al. (1999), Guanfacine, but not clonidine, improves planning and working memory performance in humans. *Neuropsychopharmacology* 20:460–470  
 Li B-M, Mao Z-M, Wang M, Mei Z-T (1999), Alpha-2 adrenergic modulation of prefrontal cortical neuronal activity related to spatial working memory in monkeys. *Neuropsychopharmacology* 21:601–610

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