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Neuroimaging of Attention Deficit Hyperactivity Disorder: Can New Imaging Findings Be Integrated in Clinical Practice?

George Bush, MD[a,b,c,d,*]

[a]Department of Psychiatry, Harvard Medical School,
25 Shattuck Street, Boston, MA 02115, USA
[b]Psychiatric Neuroscience Division, Department of Psychiatry,
Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA
[c]MIT/HMS/MGH Athinoula A. Martinos Center for Functional
and Structural Biomedical Imaging (Massachusetts Institute of Technology,
Harvard Medical School and Massachusetts General Hospital), MGH–East,
CNY 2614, Building 149, Thirteenth Street, Charlestown, MA 02129, USA
[d]Clinical and Research Program in Pediatric Psychopharmacology,
Massachusetts General Hospital, Boston, MA 02114, USA

Neuroimaging research has provided a great deal of exciting new data on the neurobiology of attention deficit hyperactivity disorder (ADHD) and the neural effects of medications used to treat the disorder. Rapid technologic advances in neuroimaging, genetics, and neurochemical research techniques have converged with cognitive neuroscience and neuropsychologic

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findings to implicate dysfunction of frontostriatal structures (dorsal anterior midcingulate cortex, dorsolateral prefrontal cortex, caudate, and putamen) as likely contributing to the pathophysiology of ADHD, and several reports have helped elucidate the mechanism of action of stimulant medications. Although these developments are promising, they also create confusion over the possible usefulness of imaging techniques as an aid to clinical decision-making. Specifically they present challenges for the clinician as to how best to integrate this burgeoning literature and to determine when and how, if at all, to incorporate these new brain imaging capabilities into clinical practice.

Although currently there are no accepted uses for imaging in diagnosing ADHD (other than ruling out identifiable medical/neurologic conditions that may mimic ADHD), this article: (1) provides a context within which to understand the potential future role of imaging in clinical practice; (2) discusses the inter-relationship of clinical diagnostic controversies with imaging research; (3) briefly overviews the main imaging techniques used to study ADHD and highlights some major recent advances that exemplify the current state of imaging capabilities; (4) identifies issues and complexities facing psychiatric neuroimaging research in general and highlights disorder-specific challenges of ADHD research; and (5) suggests guidelines for possible future clinical uses of imaging in ADHD. It is not intended as a comprehensive review or meta-analysis of imaging of ADHD, which can be found elsewhere [1–12], but rather as a primer to aid clinicians and non-imaging ADHD researchers in understanding the relevant complexities of ADHD imaging and possible future applications to clinical practice.

> *Eight-year-old Johnny can't wait to climb into the sleek, space-age scanner. His mother—who has been concerned about his poor school performance, disruptive classroom behavior, and difficulties keeping friends—tries to give him one more kiss on the forehead, but he excitedly pulls away and hops up onto the scanner bed. The imaging tech gives mom a reassuring smile, slides Johnny into the large magnet, and shows Johnny how to work the video game buttons that keep him occupied during the scan. Twenty minutes later (all too short for Johnny), it's time for him to come out again. His child psychiatrist enters the adjoining consultation room and there explains to mom what the brightly-colored blobs on the incredibly realistic, seemingly 3-dimensional images of Johnny's brain mean. No, there are no tumors, but compared with the International Pediatric Brain Database (IPBD), Johnny's brain is 3.5% smaller than other boys his age, his cingulate cortex and caudate are smaller than normal, and his cortical attention network is underactive. Combining that with the PET scan results from earlier that morning, his diagnosis of ADHD, combined type, is confirmed, and the child psychiatrist explains to mom why a certain pattern of colored blobs on the scan indicates Johnny is more likely to respond to one medication than another. Reassured, mom smiles, and they all go back to the office to review the treatment plan.*

Of course, this scenario sounds wonderful, and (were it reality), represents an ideal situation for patient, parent, and clinician alike. Given the

frequent reports of new advances in brain imaging, many of which appear in the mainstream media, it even seems tantalizingly close to what might be possible in our current state-of-the-art facilities. The question is, though, just how far off is this vision from our current or near-future capabilities? This article provides a concise overview of ADHD imaging research potentially relevant to the clinical care of patients who have ADHD and offers guidance for the clinician to help determine when (at some time in the future) that imaging might finally be deemed acceptable and appropriate as an aid in clinical decision-making.

As detailed elsewhere in this issue, ADHD is a psychiatric disorder characterized by developmentally inappropriate symptoms of inattention, impulsivity, and motor restlessness [13]. Affecting approximately 5% of school-age children and frequently persisting into adulthood [14–16], ADHD is a source of great morbidity across the lifespan. Convergent data from neuroimaging, neuropsychologic, genetics, and neurochemical studies have implicated frontostriatal network abnormalities as the likely cause of ADHD [1–11,17,18], (see articles by Castellanos and Tannock, elsewhere in this issue). Although there is no currently accepted diagnostic imaging test for ADHD, can this rapidly growing database of information on the pathophysiology of ADHD and the biologic effects of medications used to treat it soon be translated into a protocol that would be useful in clinical practice?

Is the goal worthwhile?

Using brain imaging to study the pathophysiology of ADHD (which is already being done with multiple current imaging technologies) is intrinsically important, but it is another matter to attempt to translate that type of research (which can be done using group-averaged brain data) into the development of a clinically useful diagnostic imaging test for ADHD (which would require, by definition, the capability to reliably identify unique imaging biomarkers of ADHD in single subjects and would entail a large expenditure of time, effort, and money to properly validate). Before even beginning to discuss the technical challenges of such an endeavor, it is essential to ask whether pursuing the development of such a clinical imaging test is justified (ie, presupposing that such a diagnostic imaging test is eventually feasible—would it be worthwhile?). Imaging would entail a higher upfront cost that must be justified (shown to have added value above clinical diagnosis alone). To be clear, for the purposes of discussion, such a test would not be used as a screening test (as screening would be done much more quickly and cheaply by way of history and questionnaire). Such a test would instead be used in combination with clinical assessment, but with enough testing could conceivably one day be elevated to the status of gold standard (acceptable proof in and of itself that a patient has the disorder in question).

There are many reasons that developing a diagnostic imaging test would be important. Such a test would simultaneously reduce overdiagnosis and underdiagnosis. Both goals are clinically important, because overdiagnosis leads to unnecessary exposure to medications and time-consuming behavioral treatment, with their additional costs, and underdiagnosis (and subsequent nontreatment) leads to increased functional, social, and occupational impairment and increased morbidity and mortality. Aside from the important alleviation of suffering and avoidance of unnecessary risk for individuals, ADHD's long-term economic burden to society is not trivial. If, as estimated, approximately 5% to 8% of children and 3% to 4% of adults have ADHD [15,19,20], and in light of the fact treatment lasts years and often decades, a diagnostic imaging test could be highly cost-effective, saving patients and society enormous amounts of money by eliminating unnecessary testing and treatment in some, and by targeting treatment appropriately in those who have ADHD. These long-term savings along with reducing indirect costs of ADHD by helping to decrease motor vehicle accidents and substance abuse [21,22] would more than justify the initial costs of imaging. An imaging-confirmed diagnosis would identify those who need treatment and possibly assist clinicians in monitoring treatment response and could help in refining treatment decisions based on subtyping of ADHD. Such a test would spare those patients ruled out for ADHD the risks and expense of unnecessary treatments and in these cases could help lead clinicians to identify other medical or neurologic disorders that mimic ADHD.

Furthermore, identification of an imaging biomarker for ADHD can improve treatment studies by refined case definition. Similarly, objective case definition can improve and facilitate cross-cultural studies, helping to address longstanding questions about differences in prevalence rates among different countries. Such a test would improve genetic studies by way of better case identification and by reducing variability/noise from analysis. An imaging-based diagnostic test would clearly benefit pharmaceutical development by potentially helping to identify new drug targets and by providing improved outcome measures. Imaging of ADHD could indirectly assist with the understanding of other disorders that also involve attention problems (schizophrenia, depression, and learning disorders) and could more directly improve the clinical treatment and research of patients who have complex cases involving comorbid conditions, such as ADHD in the presence of bipolar disorder or learning disabilities.

Finally, for the individual an imaging test could help with treatment compliance if the patient sees tangible evidence (on a brain scan) that he or she has ADHD and can be shown that the treatment selected is having an observable effect. Moreover, such an objective measure may reduce stigma if one can identify a neurobiologic causation and can therefore show that the patient is not simply "lazy" or unmotivated. For many reasons, the quest to develop a diagnostic imaging test is thus clearly justified.

Inter-relationship with clinical diagnostic issues

Before attempting to develop a diagnostic imaging test for ADHD, it is also essential to define what specifically such a test would be designed to identify, and herein lies one of the first major challenges. It must be recalled that at present the diagnosis ADHD is clinically defined, generally requiring the presence of developmentally inappropriate symptoms of inattention, impulsivity, and motor restlessness [13]. But as discussed elsewhere in this issue and in detail by McGough and Barkley [23], without an established neurobiologic pathophysiology, controversy remains as to how to best define ADHD clinically. In DSM-IV [13], three subtypes are recognized: inattentive, hyperactive–impulsive, and combined (reflecting a combination of the other two types). Symptoms must be observed early in life (before age 7 years), pervasive across situations, and chronic. The increasing identification of adults who have ADHD, however, and the inclusion of the diagnosis of ADHD, not otherwise specified, in DSM-IV [13] suggest that there may be multiple processes leading to ADHD, with different time courses and etiologies. This does not even involve consideration of alternate diagnostic schemes, such as the Wender Utah Criteria [24], which among other things emphasizes hyperactivity, thereby excluding the inattentive subtype of ADHD, and introduces potential confounds with the inclusion of "irritability" and "hot temper" as part of the formulation; or ICD-10 criteria [25], which are used more often in European studies. This review makes no value judgments on the validity of these and other diagnostic schemes, but merely raises these few points to illustrate that it must be recognized early on that there is a continual push–pull of clinical and neuroimaging findings that mutually influence one another and profoundly shape the conceptualization of what ADHD is and encompasses. The identification of the neural substrates underlying the proposed existence of ADHD subtypes is certainly a question that can be approached empirically using brain imaging, but the issue is complex. Similarly, data need to be viewed within a developmental context to determine if ADHD identified in youth is the same as that seen in adults, and consideration needs to be made for parsing out ADHD's overlap with comorbid conditions.

Brief review of different imaging modalities

In this section, the main functional imaging techniques currently used to study ADHD are introduced. This review does not discuss structural scanning techniques, such as morphometric (volumetric) studies, cortical thickness studies, or diffusion tensor (white matter tract tracing) techniques. Although these are all invaluable research tools, the small effect sizes typically observed in these studies make it highly unlikely that they could, on their own, become clinically useful (and even if they did, most of the same issues faced by functional imaging would apply to a structural imaging test).

Functional imaging studies (regardless of technique) can be broadly divided into studies of: (1) pathophysiology, (2) treatment effects, and (3) potential tests to aid clinical diagnosis. Generally, functional imaging studies have been designed using group-averaging statistical analytic techniques (ie, because of the usually limited power to detect reliable and robust results in individuals, analysis strategies have relied on reconstructing image data in a standardized anatomic space (eg, Talairach and Tournoux [26]) and comparing the results within a group-averaged sample of ADHD subjects with that of a healthy or psychiatrically-impaired control group). Such group-averaged designs can be useful in studying pathophysiology and medication effects, but are inadequate to assist in clinical diagnostic decision-making (which by definition requires the ability to reliably distinguish normal from abnormal at the individual subject level).

Radioactivity-based techniques

Single photon emission computed tomography (SPECT) and photon emission tomography (PET) were among the earliest functional imaging studies used. SPECT involves the injection or inhalation of radiopharmaceuticals (eg, xenon-133, iodine-123, or techitium-99m) that then distribute throughout the body and brain and emit single photon radiation (typically gamma rays) as they decay. More active brain areas receive greater blood flow, and thus greater amounts of the radioactive tracer, which is then detected with the SPECT camera. PET works similarly (ie, it also uses injected or inhaled radiopharmaceuticals, typically oxygen-15, carbon-11, or fluorine-18). As these decay they emit positrons, which are detected by the PET camera. Some PET methods are blood flow-dependent, whereas others measure cerebral metabolism rates. SPECT and PET have generally been supplanted by functional MRI (fMRI) for functional studies, because fMRI offers superior spatial and temporal resolution, and SPECT and PET's use of radiopharmaceuticals makes it ethically difficult to justify their use in healthy volunteers, especially children [27]. Both SPECT and PET, however, still have their important niche uses, because they can use radioligands for receptor characterization to measure dopamine transporter levels and to quantify extracellular dopamine [3,28–31], and it is possible that one day one of these types of uses could be translated into a clinically useful test.

Functional MRI

The newest of the major functional imaging methods, fMRI presents several advantages for functional studies over SPECT and PET. It is noninvasive (no injections or inhalations are needed) and does not require subjects to be exposed to ionizing radiation. Subjects (including children) can thus be scanned repeatedly, facilitating longitudinal, developmental, and drug studies. This ability to repeatedly scan the same subject also permits progressive "functional dissections" within the same subject (ie, the same subject can be

scanned on different occasions using many different tasks, permitting researchers to probe different brain structures and networks). fMRI has superior spatial and temporal resolution, and tasks can be performed in either a blocked format or an event-related manner, allowing greater flexibility in task design. Also, newer arterial spin labeling techniques can and have been used to scan subjects in resting states and can provide absolute measures of regional cerebral blood flow [32–35]. Higher field strength magnets coupled with specialized cognitive activation tasks are able to produce reliable and robust results in individual subjects, which has enabled characterization of drug effects in single subjects and analyses of intersubject variability [36]. For these reasons, fMRI has become the dominant imaging modality used by cognitive neuroscientists and psychiatric functional imaging researchers and may potentially be able to be developed into a clinically useful imaging test.

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) is a noninvasive, MRI-based method for quantifying various neurochemicals, including putative markers for neuronal integrity, myelin breakdown, and others. In the small number of studies published to date [1,37–43], MRS has shown some early promise in identifying neurochemical abnormalities associated with ADHD. Although these MRS findings are preliminary, require group-averaging, often contain small samples with comorbidities, and are regionally limited, they do offer the promise of being able to noninvasively quantify biologically relevant neurometabolites. MRS is thus a technique deserving further exploration for usefulness in understanding ADHD pathophysiology and treatment and for a possible role in the clinical arena.

Electrophysiology studies

Electrophysiologic methods, including quantitative electroencephalograms (QEEG) and event-related potentials (ERPs) have been used in a large number of ADHD studies (for reviews, see [44–48]). QEEG generally involves computer-assisted spectral analysis of the EEG signal with relative and absolute quantification of alpha, beta, theta, and delta frequencies, and sometimes measures of coherence. The generators of these signals, however, are not localized to specific neural structures with any precision. Some proponents of QEEG have argued that it can distinguish patients who have ADHD from control subjects [45], with ADHD supposedly being characterized by ''theta excess'' and ''alpha slowing.'' The same review, however, later stated that ''theta excess'' or ''abnormal alpha'' is associated with dementia, schizophrenia, mood disorders, obsessive–compulsive disorder, specific developmental learning disorders, alcoholic intoxication, chronic alcoholism, mild to severe head injury, and postconcussion syndrome. Similarly, others have argued that a higher theta:beta ratio is associated with

ADHD, while simultaneously noting that this same pattern occurs in other psychiatric disorders [46]. Such nonspecificity renders QEEG clinically unproven.

ERPs are different from QEEG. They are measured using multielectrode arrays placed over the scalp and represent the averaged electrical response of the brain over many trials [49–51]. ERPs main problems are limited spatial resolution and the "inverse problem" (ie, there are no unique solutions when determining the position of sources within the head, making it extremely difficult to localize brain activity with certainty). ERPs do possess millisecond temporal resolution, however, and efforts to combine modalities (eg, using fMRI to spatially constrain source models and then ERPs to test the electrical activity within the identified nodes) may eventually be applied to ADHD research with success. There are no reports, however, that have successfully used ERPs to distinguish patients who have ADHD from healthy control subjects and other patients who have psychiatric disorders at the single subject level, so like the other imaging methods, ERPs are not deemed clinically useful at this time.

Functional imaging findings in attention deficit hyperactivity disorder

It is far beyond the purpose and scope of this article to provide even a cursory review of imaging results related to ADHD. For this the interested reader is referred to other reviews [1,4–9,12]. A couple of illustrative examples, however, help to show possible future avenues of research.

One such type of diagnostic test using fMRI might use cognitive activation task strategies. Examples of these might be the Multi-Source Interference Task (MSIT, [36,52,53]), Stroop and Stroop-like tasks [54–56], stop-signal or go/no-go tasks [57–59], or continuous performance tasks [60]. Akin to a cardiac stress test, these imaging tests use a cognitive/attention task or tasks to activate brain regions under conditions of engagement within the task to assess the functional integrity of the cortical structures supporting attention or response inhibition in neuropsychiatric disorders like ADHD. An ideal functional neuroimaging-based diagnostic test of this type should possess many of the following characteristics. (1) It must produce reliable and robust activation of the cortical regions of interest (ROI) within healthy individuals. (2) It should be hypothesis-driven (that is, pre-existing evidence should support a mechanism explaining why the task would be expected to recruit the ROI). (3) It should include the collection of concomitant imaging and performance data (reaction times and accuracy). (4) Testing procedures must be standardized. (5) The task instructions should be easy to learn and retain so that the task can be performed by subjects who have impaired attention or cognition (eg, ADHD or schizophrenia) and by subjects across a wide age spectrum (to enable developmental studies in children and studies of elderly subjects). (6) It should not

require an excessive time commitment, because children and elderly subjects tend to tire more easily than young adults. (7) It should not be language-specific (to allow cross-cultural studies). (8) Performance data should vary within a narrow range in healthy volunteers. (9) Imaging and performance data should be related. (10) Imaging and performance data should show temporal stability (ie, they should display sufficient test–retest reliability to permit longitudinal and treatment studies). (11) Imaging and performance data should be sensitive to changes with successful treatment. (12) Results should be disorder-specific.

Many of the currently used cognitive activation tasks (with currently available imaging methods) do not meet even the first cut for translation into a diagnostically useful task, because they do not reliably activate brain ROIs in single subjects. It is not fair to even expect this from them, however, for although they might produce reliable group-averaged data, they were designed to test groups, not to have the power to produce activation in single subjects. Although the MSIT may be more likely to one day meet the diagnostic test criteria, having been specifically designed with many of the ideal diagnostic features in mind [53] and having already demonstrated ability to activate the cingulo-frontal-parietal cognitive/attention network in approximately 95% of more than 100 subjects tested (Fig. 1) [52], the MSIT is far from being validated as a diagnostic test. Studies are underway using the MSIT to directly compare patients who have ADHD with healthy control subjects, and follow-up studies are planned to also include other disorders, such as schizophrenia and depression, but there is no prospective, large-scale study that would provide sufficient data for calculation of sensitivity, specificity, or other measures of diagnostic accuracy. At this point in time, there are no adequately validated functional diagnostic imaging tests.

Another promising but controversial avenue of investigation involves the quantification of striatal dopamine transporter (DAT). DAT is responsible for presynaptic reuptake of dopamine, and it has been shown that methylphenidate blocks DAT and increases extracellular dopamine [3,7,30,31,61]. Also, significant for a potentially useful diagnostic test, it has recently been shown that Altropane (a carbon-11 agent) and PET have demonstrated ability to image drug effects in single subjects (Fig. 2) [29]. Although initial reports found a large (up to 70%) increase in striatal DAT in patients who have ADHD [62], however, subsequent reports using different ligands and techniques have found lesser effect sizes, and in some cases, even lower DAT in patients who have ADHD [3,31]. The comparisons of ligands and techniques used in these different studies are far beyond the scope of this article, but suffice it to say that although the approach in general is extremely promising in helping understand the pathophysiology of ADHD and the mechanism of action of treatments for ADHD, the process of attempting to translate such exciting and pioneering work into a clinically useful diagnostic imaging task has only begun.
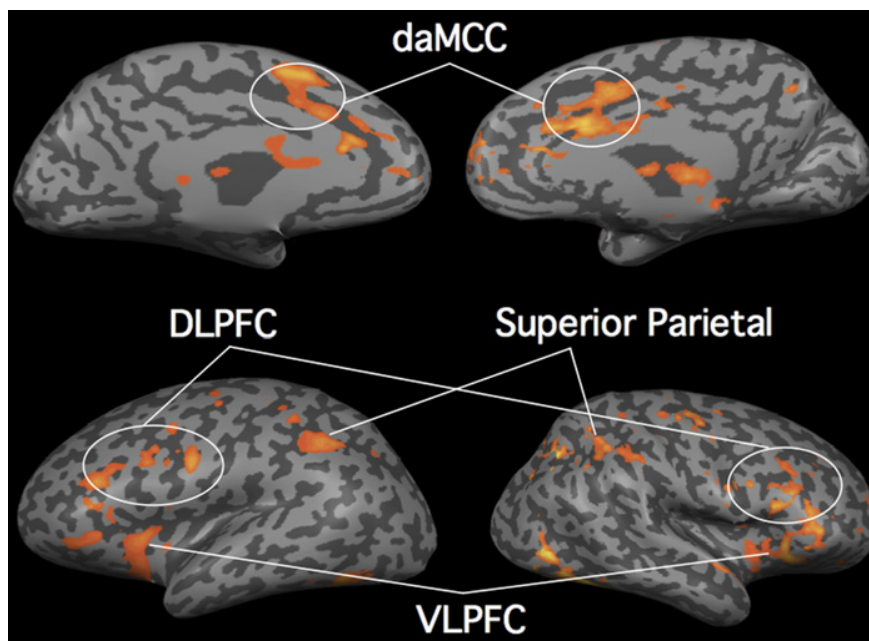
Fig. 1. Typical single subject fMRI response during MSIT. A typical single scan fMRI response during the MSIT is shown for an individual subject in the inflated view format (*light gray, gyri; dark gray, sulci*). Note the robust bilateral activation ($P < 10^{-4}$) in the cingulo-frontal-parietal cortical/attention network (daMCC, DLPFC, and superior parietal cortex). Additional activity is often seen, as here, in ventrolateral prefrontal cortex (VLPFC). The dorsal anterior midcingulate cortex (daMCC) lying on the medial surface of the frontal lobe maintains strong connections to dorsolateral prefrontal cortex (DLPFC), parietal cortex, and striatum. The daMCC is believed to play critical roles in complex and effortful cognitive processing, target detection, response selection and inhibition, error detection, performance monitoring, and motivation (see [74,75] for reviews). Particularly relevant to ADHD, it is believed to modulate reward-based decision-making [75,76]. Dysfunction of daMCC thus could lead to all of the cardinal signs of ADHD (inattention, impulsivity, and hyperactivity) and could explain the seeming paradoxic ability of patients who have ADHD to perform normally on some tasks (when motivated) but to show deficient performance when the task is not deemed salient. Numerous imaging studies have reported functional hypoactivity of daMCC [12,36], recent reports of structural and biochemical abnormalities of daMCC have been published [41,77–79], and methylphenidate has been shown to increase activity of daMCC [36]. That daMCC and the cingulo-frontal-parietal cortical/attention network can reliably be imaged in single subjects is promising, but much work needs to be done before using the MSIT as part of a clinical diagnostic imaging test for ADHD. (*Reproduced from* Bush G, Shin LM. The multi-source interference task: an fMRI task that reliably activates the cingulo-frontal-parietal cognitive/attention network in individual subjects. Nat Protoc 2006;1:308–13; with permission.)

Diagnostic imaging test issues

The next two sections discuss issues related to the development of diagnostic imaging tests. The first section addresses general concepts that apply to most proposed diagnostic imaging tests of psychiatric disorders. The second section highlights issues that may be more specific to the development of a possible ADHD diagnostic test.
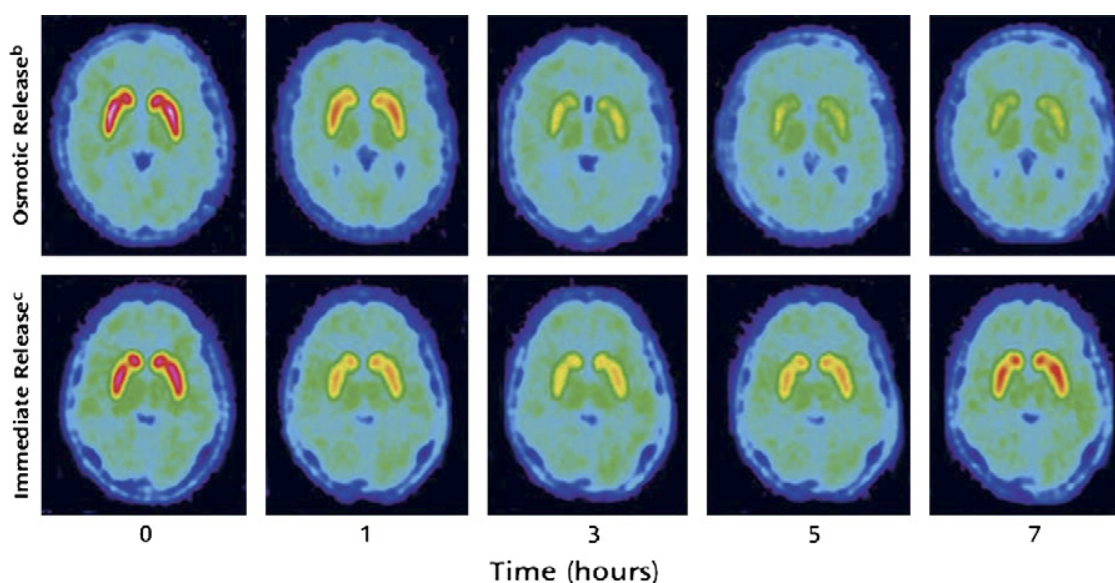
Fig. 2. Serial PET brain images showing striatal dopamine transporter receptor occupancy after receipt of a single dose of immediate-release or osmotic-release methylphenidate in two healthy subjects. (*A*) Dopamine transporter receptor occupancy in the striatum of two individual healthy adults was assessed by measuring binding of a carbon-11-labeled imaging agent (Altropane). Red color indicates greater binding of Altropane to presynaptic DAT; yellow indicates lesser binding by Altropane caused by displacement by methylphenidate. (*B*) Subject took long-acting osmotic release oral system methylphenidate. Significant drug effect is still observed at 7 h. (*C*) Subject took short-acting immediate release methylphenidate. Striatal DAT occupancy returns more quickly toward baseline level in hours 3 through 7. The technique exemplifies the capability to quantify drug effects with dynamic range within single subjects. (*Reproduced from* Spencer TJ, Biederman J, Ciccone PE, et al. PET study examining pharmacokinetics, detection and likeability, and dopamine transporter receptor occupancy of short- and long-acting oral methylphenidate. Am J Psychiatry 2006;163(3):387–95; with permission.)

General issues

As stated, there is currently no accepted role for functional imaging in guiding clinical diagnosis or therapeutic decision-making. Despite the exciting preliminary advances that have been made in understanding pathophysiology and drug treatment mechanisms, none of the imaging modalities has been fully validated in the peer-reviewed literature as a proven method for reliably distinguishing patients who have ADHD from normal control subjects, distinguishing patients who have ADHD from other subjects who have other psychiatric or neurologic comorbidities, identifying subtypes of ADHD, or predicting treatment response at the level of the individual subject. To achieve full validation, what are some of the main benchmarks that would need to be met?

First, of paramount importance, it must be recognized that the exciting potential for brain imaging and the complexity of the technology underlying it must in large part be ignored when evaluating whether a test based on an imaging technique would be worthwhile diagnostically. The only real

questions to be answered are the exact same ones that must be asked of any type of diagnostic laboratory test:

1. How well does the test identify the condition of interest (here, ADHD)?
2. How well does the test distinguish the condition from other similar disorders?
3. Is the test feasible and cost-effective?

More formally, any proper validation of a proposed diagnostic imaging test would have to include peer-reviewed published data (collected in dedicated testing performed in large samples of carefully characterized subjects) that quantifies and meets or exceeds the appropriate and acceptable benchmarks. As shown, such validation of any proposed diagnostic imaging test for ADHD has to go beyond simple documentation of sensitivity and specificity, and ADHD evaluations present many complicating factors that need to be addressed.

Briefly, diagnostic testing validation requires at the least quantification of sensitivity and specificity. To calculate these measures, the proposed test results are compared in binary fashion (ie, test result positive versus negative) to the gold standard (the procedure or test that unambiguously defines the pathology, such as a biopsy or direct surgical inspection in the case of tumor). Herein lies the first problem—as seen, there is no gold standard for ADHD, which, in the absence of defined pathophysiology, remains a clinical diagnosis whose criteria are fluid and still a matter of debate. For the sake of further discussion, here one could choose to accept the latest DSM-IV diagnosis as a proxy gold standard, but admittedly must recall in the end that this is not a true gold standard. Sensitivity of the diagnostic test indicates the proportion of true positives the test identifies (as compared with the gold standard), whereas specificity refers to the proportion of true negatives correctly identified. Although both of these values are important to know, they do not provide sufficient information about the diagnostic accuracy of the test, because in clinical practice, one is more interested in approaching the problem from the other direction (ie, one would be given the scan result of "normal" or "abnormal" scan, and would therefore need to know how well that scan result reflected the presence or absence of ADHD).

This information is expressed in the form of predictive values [63]. The positive predictive value of an imaging test is the proportion of patients who have positive scans that are correctly diagnosed, whereas the negative predictive value of an imaging test is the proportion of patients who have negative scans that are correctly diagnosed. These predictive values are not absolute but relative, and their estimates can be heavily influenced by the prevalence of the abnormality. Another important characteristic to know is the likelihood ratio (sensitivity/1-specificity), which reflects the certainty about a positive diagnosis [63,64].

Another complicating factor is that the preceding evaluations are performed on discrete (yes/no or positive/negative) data. In reality, imaging

data are most likely going to be continuous data (eg, percent fMRI signal change or dopamine transporter binding). In these cases, it is highly unlikely that two completely separable distributions of data exist (one for ADHD, one for healthy subjects), but rather that the two sample distributions overlap and therefore a cutoff needs to be set for distinguishing a positive from a negative result. In these types of cases, receiver operating characteristic (ROC) plots (which plot sensitivities versus the inverse of specificities) can be useful in selecting an appropriate cutoff value and comparing one or more measurements [65]. Similarly, although some forms of diagnostic imaging tests may be amenable to "expert interpretation," for the most part this approach should be minimized, and even in these cases, empiric data with strict and explicit criteria must be provided and independently replicable. Also, "expert interpretation" methodologies still provide quantitative diagnostic accuracy information and test–retest reliability measures and are subject to rigorous standardization. There should be no acceptance of a claim that is pinned solely on "specialized knowledge" without the support of fully independent replication. Certainly specialized training and clinical imaging expertise are required for the interpretation of any diagnostic imaging test (because this is the current model for much of neuroradiology)—but any such claims should not be accepted until there is consensus agreement that such claims are adequately supported by proof of technique from unbiased, independent replications.

This list is far from exhaustive—there are many more general issues that are important to address before being able to fully evaluate diagnostic accuracy. What is the effect size (ie, the magnitude of the mean differences between the ADHD and healthy populations, taking into account the degree of variance in the samples)? What is the diagnostic specificity with respect to other disorders that may produce test results that overlap with ADHD? What is the test–retest reliability within a particular imaging center or the variability between readers or between imaging centers? For fuller discussion of these and other issues beyond the scope of this article, the interested reader is referred to a concise statement of standards for reporting studies of diagnostic accuracy [66], but the short list of general issues alone provided here should already provide pause for consideration before accepting claims that a diagnostic test for ADHD has been developed.

Attention deficit hyperactivity disorder-specific issues and technologic challenges

Beyond the more general issues discussed, there are myriad complicating factors that face psychiatric neuroimagers in general, and more specifically, related to the imaging of ADHD. A brief sampling of such factors is offered here:

1. Any tests using specific tasks (cognitive interference tasks, target detection, vigilance tasks, response inhibition, working memory tasks) may

be useful, but it must be recalled that each tests only a specific cognitive domain and does not provide a comprehensive picture of patients who have ADHD. It may be that a battery of tests could and will be used, but this approach is complex and requires cooperative patients, which may be difficult in ADHD populations, especially in young children who have ADHD.

2. Some resting state or dopaminergic tests may be confounded, because controversy exists surrounding the definition of whether or not the healthy brain has a "default resting state" or how to determine in a simple way if a subject is "mentally resting" (and this is then compounded by the likelihood that patients who have ADHD may have increased activity or variability at "rest") [67,68].

3. It is well known that dopaminergic firing can change rapidly (on a trial-by-trial basis), and that dopaminergic cells show tonic firing (longer term stable) and phasic firing (which changes on a second-by-second basis). It may be that these temporal effects could produce disparate findings in some dopaminergic studies.

4. There is the high likelihood that ADHD may represent a syndrome that can be caused by or associated with multiple causes (one group may have dopaminergic, noradrenergic, serotonergic, or cholinergic abnormalities), whereas there may be others who have genetic-based structural abnormalities, and still others with disordered cortico-cortical connections), all of which may have differing imaging profiles.

5. A similar accounting must be made for phenomenologic subtypes of ADHD, because it is likely that inattentive, hyperactive, and combined subtypes have distinct features when imaged.

6. Studies involving task performance must take into account the effects of variable performance on imaging data on mean differences between groups and on trial-to-trial variability within individual runs for a subject [68,69].

7. Related to performance and as discussed at length previously [1], error detection systems in the brain can have a profound impact on brain imaging results and must be accounted for using sophisticated data analysis techniques.

8. ADHD imaging in particular must address developmental issues. How does normal development affect age-defined norms? Is ADHD a unitary concept that remains the same throughout one's lifespan, or are there age-related adjustments?

9. Anatomic variability of brain structures makes region definition complex. Further complicating this fact are suggestions that ADHD brains show greater degrees of anatomic variability than healthy brains [36]. Such variability needs to be quantified and accounted for.

10. Laterality effects must be addressed. Most brain structures are bilateral, but what implications does this have for a test result? How are data to be interpreted if effects are normal range on one side and abnormal on

the other? Are only right- or left-sided results clinically meaningful? Should data be averaged to provide one single data point for bilateral structures? Does handedness affect results?

11. There are many potential confounds (anxiety, substance abuse, effects of other medications, caffeine, IQ) that can be controlled in a research study but that may affect results and interpretation in the real world.

12. There are likely to be potential confounds from differential effects of motivational status. Reward and decision-making systems involve many of the same structures implicated in ADHD (cingulate, dorsolateral prefrontal cortex, striatum), and it is hard to imagine that this effect is easily addressed for all subjects.

13. Medication status is another issue that must be paid attention. Although wash-out procedures adequate to produce nearly complete elimination of the medications can validly be used in some studies, the long-term effects of medications are not yet known and represent a potential confound [70]. Medication-naive normative data are needed, and the effects of varying wash-out periods for medications commonly used in patients who have ADHD need to be considered.

14. Finally, all this assumes that the clinician is dealing with an honest patient who has a lack of malfeasance or potential for secondary gain. In the real world, competitive forces and attempts at secondary gains (test accommodations, disability payments, desire to obtain legal source of amphetamines) are certain to lead some patients to attempt to affect test results in some way, and procedures must be in place to guard against this.

Guidelines for considering imaging for clinical purposes

In just a few short decades, functional imaging has made great strides in helping to elucidate the pathophysiology of ADHD and the mechanism of action of the stimulant medications that are the mainstay of ADHD treatment. The veritable explosion of cognitive neuroscience work on the brain's attention, affective, motor, and motivation systems, combined with the rapid pace of technologic advances, has promised to make the next few decades exciting times for ADHD researchers. As has been shown, however, there remains a huge amount of work to be done to translate these early successes into a clinically useful diagnostic imaging test. Although the requirements may seem daunting, they can likely eventually be met with perseverance, patience, and time. That said, there can be no short cuts, and imaging researchers and clinicians alike need to ensure that sufficient proof of diagnostic accuracy and reliability are proven before accepting a proposed methodology.

Until a proposed diagnostic test is fully validated (ie, has satisfactorily met the criteria listed, including publication and independent replication in peer-reviewed journals and widespread acceptance in the field), there

can be no ethical use of functional imaging outside of the research realm—especially any type of invasive research or technique that exposes children to ionizing radiation—because there is no accepted, identified benefit, only unnecessary risk and unjustified additional cost. Any such imaging must only be performed in a research context with the oversight of an established human subjects committee or investigational review board. Any other use at this time would be unethical.

The eventual development of a diagnostic imaging test for ADHD would be a wonderful advance, but it would not be a panacea, nor should it replace clinical judgment. Clinicians would be advised as always to first take a careful history, perform a physical examination, obtain relevant blood tests, and then consider whether imaging would tangibly guide diagnostic decision-making or treatment. The differential diagnosis for ADHD is large [71], and although it is agreed that functional imaging is not currently useful for confirming a diagnosis of ADHD [72,73], in certain cases with an index of suspicion (eg, atypical presentation, abnormal neurologic findings, abrupt change in behavior/personality), structural MRI can presently be recommended to rule out disorders mimicking ADHD.

It is hoped that one day, just as a chest radiograph can be useful in guiding treatment decisions by distinguishing between pneumonia and bronchitis, a validated ADHD imaging test could be used as an adjunct to a comprehensive clinical evaluation of ADHD. Until then it must be remembered that colorful brain images can be dramatic, and this fact (when combined with brain imaging's highly technical nature) can unfortunately lead to a situation with potential for misinterpretation or worse—outright misuse and deliberate exploitation. Efforts to push forward the technology need to be matched with equal vigor in protecting patients from unproven methods. In particular, we must clearly define for ourselves and our patients the acceptable uses of imaging and ensure that these techniques are properly validated and integrated with clinical evaluation.

Acknowledgments

References

[1] Bush G, Valera EM, Seidman LJ. Functional neuroimaging of attention-deficit/hyperactivity disorder: a review and suggested future directions. Biol Psychiatry 2005;57(11):1273–84.

[2] Seidman LJ, Valera EM, Bush G. Brain function and structure in adults with attention-deficit/hyperactivity disorder. Psychiatr Clin North Am 2004;27(2):323–47.

[3] Spencer TJ, Biederman J, Madras BK, et al. In vivo neuroreceptor imaging in attention-deficit/hyperactivity disorder: a focus on the dopamine transporter. Biol Psychiatry 2005; 57(11):1293–300.

[4] Durston S. A review of the biological bases of ADHD: what have we learned from imaging studies? Ment Retard Dev Disabil Res Rev 2003;9(3):184–95.

[5] Giedd JN, Blumenthal J, Molloy E, et al. Brain imaging of attention deficit/hyperactivity disorder. Ann N Y Acad Sci 2001;931:33–49.

[6] Dickstein SG, Bannon K, Xavier Castellanos F, et al. The neural correlates of attention deficit hyperactivity disorder: an ALE meta-analysis. J Child Psychol Psychiatry 2006;47(10): 1051–62.

[7] Swanson JM, Kinsbourne M, Nigg J, et al. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. Neuropsychol Rev 2007;17(1):39–59.

[8] Casey BJ, Nigg JT, Durston S. New potential leads in the biology and treatment of attention deficit-hyperactivity disorder. Curr Opin Neurol 2007;20(2):119–24.

[9] Nigg JT, Casey BJ. An integrative theory of attention-deficit/ hyperactivity disorder based on the cognitive and affective neurosciences. Dev Psychopathol 2005;17(3): 785–806.

[10] Seidman LJ, Valera EM, Makris N. Structural brain imaging of attention-deficit/hyperactivity disorder. Biol Psychiatry 2005;57(11):1263–72.

[11] Valera EM, Faraone SV, Murray KE, et al. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. Biol Psychiatry 2007;61(12):1361–9.

[12] Bush G. Dorsal anterior midcingulate cortex: roles in normal cognition and disruption in attention-deficit/hyperactivity disorder. In: Vogt BA, editor. Cingulate neurobiology and disease. Oxford, UK: Oxford University Press; in press.

[13] Association AP. Diagnostic and statistical manual of mental disorders. 4th edition. Washington, DC: American Psychiatric Press; 1994.

[14] Biederman J. Attention-deficit/hyperactivity disorder: a life-span perspective. J Clin Psychiatry 1998;59(Suppl 7):4–16.

[15] Faraone SV, Biederman J, Mick E. The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychol Med 2006;36(2):159–65.

[16] Faraone SV, Biederman J, Spencer T, et al. Attention-deficit/hyperactivity disorder in adults: an overview. Biol Psychiatry 2000;48(1):9–20.

[17] Arnsten AF. Stimulants: therapeutic actions in ADHD. Neuropsychopharmacology 2006; 31(11):2376–83.

[18] Biederman J, Faraone SV. Current concepts on the neurobiology of attention-deficit/hyperactivity disorder. J Atten Disord 2002;6(Suppl 1):S7–16.

[19] Faraone SV, Sergeant J, Gillberg C, et al. The worldwide prevalence of ADHD: is it an American condition? World Psychiatry 2003;2(2):104–13.

[20] Faraone SV, Biederman J. What is the prevalence of adult ADHD? Results of a population screen of 966 adults. J Atten Disord 2005;9(2):384–91.

[21] Biederman J. Impact of comorbidity in adults with attention-deficit/hyperactivity disorder. J Clin Psychiatry 2004;65(Suppl 3):3–7.

[22] Wilens TE, Faraone SV, Biederman J. Attention-deficit/hyperactivity disorder in adults. JAMA 2004;292(5):619–23.

[23] McGough JJ, Barkley RA. Diagnostic controversies in adult attention deficit hyperactivity disorder. Am J Psychiatry 2004;161(11):1948–56.

[24] Wender PH. Attention-deficit hyperactivity disorder in adults. New York: Oxford University Press; 1995.

[25] Organization WH. ICD-10 Classification of mental and behavioural disorders: diagnostic research criteria. Geneva (Switzerland): World Health Organization; 1992.

[26] Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain. New York: Thieme Medical Publishers; 1988.

[27] Castellanos FX. Proceed, with caution: SPECT cerebral blood flow studies of children and adolescents with attention deficit hyperactivity disorder. J Nucl Med 2002;43(12): 1630–3.

[28] Madras BK, Xie Z, Lin Z, et al. Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. J Pharmacol Exp Ther 2006;319:561–9.

[29] Spencer TJ, Biederman J, Ciccone PE, et al. PET study examining pharmacokinetics, detection and likeability, and dopamine transporter receptor occupancy of short- and long-acting oral methylphenidate. Am J Psychiatry 2006;163(3):387–95.

[30] Volkow ND, Wang GJ, Fowler JS, et al. Imaging the effects of methylphenidate on brain dopamine: new model on its therapeutic actions for attention-deficit/hyperactivity disorder. Biol Psychiatry 2005;57(11):1410–5.

[31] Volkow ND, Wang GJ, Newcorn J, et al. Brain dopamine transporter levels in treatment and drug naive adults with ADHD. Neuroimage 2007;34(3):1182–90.

[32] Detre JA, Wang J. Technical aspects and utility of fMRI using BOLD and ASL. Clin Neurophysiol 2002;113(5):621–34.

[33] Aguirre GK, Detre JA, Wang J. Perfusion fMRI for functional neuroimaging. Int Rev Neurobiol 2005;66:213–36.

[34] Kim J, Whyte J, Wang J, et al. Continuous ASL perfusion fMRI investigation of higher cognition: quantification of tonic CBF changes during sustained attention and working memory tasks. Neuroimage 2006;31(1):376–85.

[35] Wang Z, Wang J, Connick TJ, et al. Continuous ASL (CASL) perfusion MRI with an array coil and parallel imaging at 3T. Magn Reson Med 2005;54(3):732–7.

[36] Bush G, Spencer TJ, Holmes J, et al. Functional magnetic resonance imaging of methylphenidate and placebo in attention-deficit/hyperactivity disorder during the Multi-Source Interference Task. Arch Gen Psychiatry, in press.

[37] Carrey N, MacMaster FP, Fogel J, et al. Metabolite changes resulting from treatment in children with ADHD: a 1H-MRS study. Clin Neuropharmacol 2003;26(4):218–21.

[38] Courvoisie H, Hooper SR, Fine C, et al. Neurometabolic functioning and neuropsychological correlates in children with ADHD-H: preliminary findings. J Neuropsychiatry Clin Neurosci 2004;16(1):63–9.

[39] Jin Z, Zang YF, Zeng YW, et al. Striatal neuronal loss or dysfunction and choline rise in children with attention-deficit hyperactivity disorder: a 1H-magnetic resonance spectroscopy study. Neurosci Lett 2001;315(1–2):45–8.

[40] MacMaster FP, Carrey N, Sparkes S, et al. Proton spectroscopy in medication-free pediatric attention-deficit/hyperactivity disorder. Biol Psychiatry 2003;53(2):184–7.

[41] Perlov E, Philipsen A, Hesslinger B, et al. Reduced cingulate glutamate/glutamine-to-creatine ratios in adult patients with attention deficit/hyperactivity disorder—a magnet resonance spectroscopy study. J Psychiatr Res 2007;41:934–41.

[42] Sun L, Jin Z, Zang YF, et al. Differences between attention-deficit disorder with and without hyperactivity: a 1H-magnetic resonance spectroscopy study. Brain Dev 2005;27(5):340–4.

[43] Yeo RA, Hill DE, Campbell RA, et al. Proton magnetic resonance spectroscopy investigation of the right frontal lobe in children with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 2003;42(3):303–10.

[44] Loo SK, Barkley RA. Clinical utility of EEG in attention deficit hyperactivity disorder. Appl Neuropsychol 2005;12(2):64–76.

[45] Hughes JR, John ER. Conventional and quantitative electroencephalography in psychiatry. J Neuropsychiatry Clin Neurosci 1999;11:190–208.

[46] Snyder SM, Hall JR. A meta-analysis of quantitative EEG power associated with attention-deficit hyperactivity disorder. J Clin Neurophysiol 2006;23(5):440–55.

[47] Chabot RJ, di Michele F, Prichep L. The role of quantitative electroencephalography in child and adolescent psychiatric disorders. Child Adolesc Psychiatr Clin N Am 2005;14(1):21–53, v–vi.

[48] Barry RJ, Clarke AR, Johnstone SJ. A review of electrophysiology in attention-deficit/hyperactivity disorder: I. Qualitative and quantitative electroencephalography. Clin Neurophysiol 2003;114(2):171–83.

[49] Barry RJ, Johnstone SJ, Clarke AR. A review of electrophysiology in attention-deficit/hyperactivity disorder: II. Event-related potentials. Clin Neurophysiol 2003;114(2):184–98.

[50] Tannock R. Attention deficit hyperactivity disorder: advances in cognitive, neurobiological, and genetic research. J Child Psychol Psychiatry 1998;39(1):65–99.

[51] Liotti M, Pliszka SR, Perez R, et al. Abnormal brain activity related to performance monitoring and error detection in children with ADHD. Cortex 2005;41(3):377–88.

[52] Bush G, Shin LM. The multi-source interference task: an fMRI task that reliably activates the cingulo-frontal-parietal cognitive/attention network in individual subjects. Nat Protoc 2006;1:308–13.

[53] Bush G, Shin LM, Holmes J, et al. The multi-source interference task: validation study with fMRI in individual subjects. Mol Psychiatry 2003;8:60–70.

[54] Bush G, Frazier JA, Rauch SL, et al. Anterior cingulate cortex dysfunction in attention-deficit/hyperactivity disorder revealed by fMRI and the counting Stroop. Biol Psychiatry 1999;45(12):1542–52.

[55] Smith AB, Taylor E, Brammer M, et al. Task-specific hypoactivation in prefrontal and temporoparietal brain regions during motor inhibition and task switching in medication-naive children and adolescents with attention deficit hyperactivity disorder. Am J Psychiatry 2006;163(6):1044–51.

[56] Zang YF, Jin Z, Weng XC, et al. Functional MRI in attention-deficit hyperactivity disorder: evidence for hypofrontality. Brain Dev 2005;27(8):544–50.

[57] Aron AR, Dowson JH, Sahakian BJ, et al. Methylphenidate improves response inhibition in adults with attention-deficit/hyperactivity disorder. Biol Psychiatry 2003;54(12):1465–8.

[58] Vaidya CJ, Austin G, Kirkorian G, et al. Selective effects of methylphenidate in attention deficit hyperactivity disorder: a functional magnetic resonance study. Proc Natl Acad Sci U S A 1998;95(24):14494–9.

[59] Durston S, Tottenham NT, Thomas KM, et al. Differential patterns of striatal activation in young children with and without ADHD. Biol Psychiatry 2003;53(10):871–8.

[60] Valera EM, Faraone SV, Biederman J, et al. Functional neuroanatomy of working memory in adults with attention-deficit/hyperactivity disorder. Biol Psychiatry 2005;57(5):439–47.

[61] Volkow ND, Fowler JS, Wang G, et al. Mechanism of action of methylphenidate: insights from PET imaging studies. J Atten Disord 2002;6(Suppl 1):S31–43.

[62] Dougherty DD, Bonab AA, Spencer TJ, et al. Dopamine transporter density in patients with attention deficit hyperactivity disorder. Lancet 1999;354(9196):2132–3.

[63] Altman DG, Bland JM. Diagnostic tests 2: predictive values. BMJ 1994;309(6947):102.

[64] Deeks JJ, Altman DG. Diagnostic tests 4: likelihood ratios. BMJ 2004;329(7458):168–9.

[65] Altman DG, Bland JM. Diagnostic tests 3: receiver operating characteristic plots. BMJ 1994;309(6948):188.

[66] Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. BMJ 2003;326(7379):41–4.

[67] Raichle ME, MacLeod AM, Snyder AZ, et al. A default mode of brain function. Proc Natl Acad Sci U S A 2001;98(2):676–82.

[68] Sonuga-Barke EJ, Castellanos FX. Spontaneous attentional fluctuations in impaired states and pathological conditions: a neurobiological hypothesis. Neurosci Biobehav Rev 2007;31:977–86.

[69] Castellanos FX, Sonuga-Barke EJ, Scheres A, et al. Varieties of attention-deficit/hyperactivity disorder-related intra-individual variability. Biol Psychiatry 2005;57(11):1416–23.

[70] Langleben DD, Acton PD, Austin G, et al. Effects of methylphenidate discontinuation on cerebral blood flow in prepubescent boys with attention deficit hyperactivity disorder. J Nucl Med 2002;43(12):1624–9.

[71] Pearl PL, Weiss RE, Stein MA. Medical mimics. Medical and neurological conditions simulating ADHD. Ann N Y Acad Sci 2001;931:97–112.

[72] Flaherty LT, Arroyo W, Chatoor I, et al. Brain imaging and child and adolescent psychiatry with special emphasis on SPECT. 2005. p. 1–11.

[73] Zametkin A, Schroth E, Faden D. The role of brain imaging in the diagnosis and management of ADHD. ADHD Report 2005;13(5):11–4.

[74] Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 2000;4(6):215–22.

[75] Bush G, Vogt BA, Holmes J, et al. Dorsal anterior cingulate cortex: a role in reward-based decision making. Proc Natl Acad Sci U S A 2002;99(1):523–8.

[76] Williams ZM, Bush G, Rauch SL, et al. Human anterior cingulate neurons and the integration of monetary reward with motor responses. Nat Neurosci 2004;7(12):1370–5.

[77] Makris N, Biederman J, Valera EM, et al. Cortical thinning of the attention and executive function networks in adults with attention-deficit/hyperactivity disorder. Cereb Cortex 2007;17(6):1364–75.

[78] Seidman LJ, Valera EM, Makris N, et al. Dorsolateral prefrontal and anterior cingulate cortex volumetric abnormalities in adults with attention-deficit/hyperactivity disorder identified by magnetic resonance imaging. Biol Psychiatry 2006;60:1071–80.

[79] Shaw P, Lerch J, Greenstein D, et al. Longitudinal mapping of cortical thickness and clinical outcome in children and adolescents with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 2006;63(5):540–9.