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## Review

# Contribution of Clinical Neuroimaging to the Understanding of the Pharmacology of Methylphenidate

Luc Zimmer<sup>1,2,3,\*</sup>

**Methylphenidate (MPH) is currently the most widely used molecule in the pharmacologic treatment of attention-deficit hyperactivity disorder (ADHD). Although experience of its application now extends over several decades, its psychotropic nature, prolonged use in children, and chemical relation to amphetamines still raise doubts in the minds of prescribers and the families of the patients. Brain imaging has shed considerable light on the neuropharmacology of MPH. The two main *in vivo* neuroimaging techniques are positron-emission tomography (PET) and magnetic resonance imaging (MRI), and these can be applied in both animal models and humans. The present review seeks to show how human molecular and functional imaging has contributed to determining not only the molecular targets of MPH, and the action kinetics of the various pharmaceutical forms available, but also the connectivity and brain networks activated by treatment. We also discuss the perspectives opened up by new hybrid PET–MRI techniques that enable multimodal tracking of the impact of methylphenidate on neurotransmission.**

## The ADHD Syndrome

ADHD is a neuropsychiatric disorder involving difficulty in concentration, possibly associated with hyperactivity and/or impulsiveness. It has been described in the medical literature since the early 20th century: the earliest clinical description is attributed to the British pediatrician George Still, who in 1902 made the connection between motor agitation in children and difficulty in maintaining concentration [1]. In 1932, two neurologists from the Berlin Charité Hospital, Kramer and Pollnow, described comparable signs of what they called ‘hyperkinetic disease’ [2,3]. Some decades later, in the 1960s, the disorder was included in psychiatric classifications: the *International Classification of Diseases* (ICD) and the *Diagnostic and Statistical Manual of Mental Disorders* (DSM). In the same period, the clinical psychologist Keith Conners developed corresponding screening questionnaires aimed at parents and teachers [4].

The main clinical characteristics of ADHD are a behavioral triad of inattention, hyperactivity, and impulsiveness, with differences in their respective expression according to age, gender, and clinical context. Symptoms may thus be aggravated by situations involving sustained attention and intellectual effort, repetitive tasks, and fatigue, and may be temporarily relieved in situations of novelty or rewarded play [5].

## Trends

One of the main pharmacologic treatments for ADHD is MPH.

Although MPH is a psychostimulant studied in many clinical trials, many issues have remained unresolved regarding pharmacologic mechanisms, brain bioavailability, pharmacokinetic/pharmacodynamic relations, impacts on brain neurochemistry and connectivity, and positive and negative long-term effects.

The development of anatomic, isotopic, molecular, and functional brain imaging has made a considerable contribution to our knowledge of MPH.

In humans, PET imaging explores MPH brain bioavailability and molecular targeting of dopamine and norepinephrine transporters; MRI explores the neuroanatomic and functional effects of MPH.

New hybrid PET–MRI cameras enable multimodal exploration protocols generating simultaneous pharmacokinetic/pharmacodynamic modeling data for MPH.

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Diagnosis is often made at school age, where the prevalence of ADHD is 1–5%. However, the rate of diagnosis differs greatly between, for example, North America and Australia on the one hand and Europe on the other; this seems to be mainly because of differences in social and educational perception rather than in biological traits [6].

Although now listed in the DSM V, ADHD is not covered in the same way in psychiatric training in all countries. It has in fact been the subject of many controversies in recent decades, and different and sometimes opposing points of view have been put forward by child psychiatrists, neuropsychiatrists, psychologists, and psychoanalysts [7]. The relations between a child's hyperactivity on the one hand and family relationships, schooling and social attitudes have been hotly debated. Some opinion leaders have strongly challenged the very existence of any such syndrome, which they suspect of being invented by a society that seeks to control the personality of out-of-control children [8]. As will be seen below, the development of psychotropic treatments has only poured oil on the flames, especially as regards MPH, one of the main molecules employed to treat ADHD.

### Short History of MPH

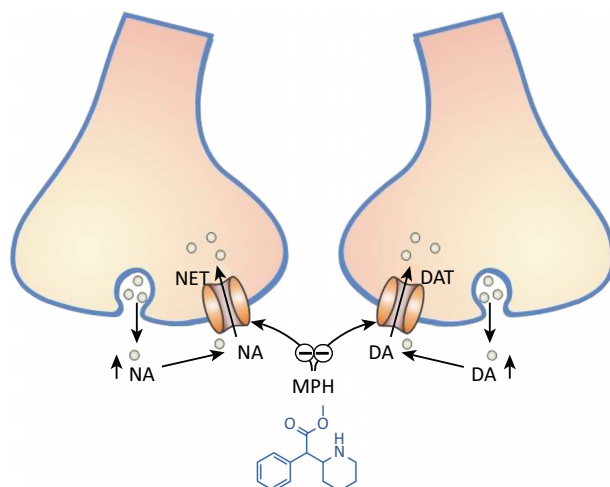
MPH was first synthesized by the medical chemist Leandro Panizzon in the Ciba laboratories in Basel in 1944 [9]. The story has it that it was his wife Marguerite, 'Rita' for short, who first noticed its cognitive effects on her tennis playing. The laboratory took out a patent in 1950, with indications for 'fatigue' and 'confusion'; soon after, in 1954, MPH was marketed under the trade-name Ritalin<sup>®</sup>, in acknowledgment of Rita [10]. The US FDA approved MPH in child psychiatry in 1961 and, a few years later, in one of the first controlled clinical trials, a team of pediatricians demonstrated that MPH improved everyday behavior in children with attention deficit [11]. This research bore out the original report by the American Charles Bradley of an amphetamine that paradoxically 'calmed' hyperactive children [12]. In 1977 the first results were published for a cohort of ADHD children receiving MPH [13], later followed by a large number of clinical studies. In 2016, more than 1500 clinical trials of MPH were referenced in PubMed ([www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed)).

Chemically MPH is a methyl-2-phenyl-2-(piperidin-2-yl)acetate: in other words it has a chemical structure that is partly similar to that of amphetamines [10], and thus has a partly similar action mechanism consisting of monoamine reuptake inhibition. *In vitro* and later in animal models, MPH was shown to be a dopamine (DA) and norepinephrine (noradrenaline, NA) reuptake inhibitor, progressively increasing the synaptic and extrasynaptic concentrations of these two neurotransmitters (Figure 1). Cognitively, these are involved in interconnected attention processes, with NA being particularly involved in concentration and DA in motivation [14]. While the first clinical studies showed and confirmed MPH effects in a majority of children treated, many issues were not addressed regarding pharmacologic mechanisms, brain bioavailability, pharmacokinetic/pharmacodynamic relations, neurochemical impact, impact on brain connectivity, and positive and negative long-term effects. Thus MPH would be classified as a psychostimulant in terms of its pharmacologic targets but, in therapeutic terms, 'calms hyperactive children', making it something of a psychopharmacological enigma [15].

In this context, the development of anatomic, isotopic, molecular and functional brain imaging (i.e., *in vivo* PET and MRI) imaging, has made a considerable contribution to our knowledge of this psychotropic molecule. The following sections show how imaging has been able to draw up and test pharmacologic, pharmacokinetic, and neurofunctional hypotheses involving restored balance between brain regions in ADHD children.

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Trends in Pharmacological Sciences

**Figure 1.** Probable Mechanism of Action of Methylphenidate (MPH) by Blocking Reuptake of Dopamine (DA) and Noradrenaline (NA) from the Extracellular Space. Abbreviations: DAT, dopamine transporter; NET, norepinephrine (noradrenaline) transporter.

## The Contribution of PET Neuroimaging to Understanding the Pharmacology of MPH

### MPH Targets and Drug Occupancy

PET imaging (Box 1) allows visualization of brain targets of interest if these have a specific radiopharmaceutical that has been validated in animal models and humans [16,17]. The dopaminergic system was initially the focus of much research given the development of PET radioligands targeting dopaminergic receptors, neuronal, and vesicular transporters and metabolism.

Imaging exploration of MPH neuropharmacology since the mid-1990s has thus been greatly dependent on the development of PET radioligands. One of the first human PET studies of the pharmacological effects of MPH was published by a team from the Brookhaven National Laboratory, in 2001 [18], showing that MPH indirectly increases extracellular brain DA concentrations, which in turn reduce the binding of [ $^{11}\text{C}$ ]-raclopride, a  $D_2$ -receptor radioligand sensitive to changes in DA levels [19]. The same team also used the radioligand [ $^{11}\text{C}$ ]-cocaine targeting the DA transporter (DAT) to assess the relation between binding and MPH dose in healthy volunteers [20,21]. Other radiotracers targeting DAT have also been used: [ $^{11}\text{C}$ ]-altropane [22,23] (Figure 2A) and [ $^{11}\text{C}$ ]-PE2I for modafinil exploration [24].

The principle underlying PET drug occupancy studies involves competition between the radiotracer binding to the target and the candidate drug targeting the same site. In the case of MPH, PET images of DATs are thus compared in a single subject before and after drug delivery, the difference between the two normalized images being expressed as percentage occupancy. This provides two types of information. First, it establishes indirectly that the drug actually penetrates the brain and binds to the DA receptor. This may not in itself seem highly original, because MPH targeting was already described in animals [25] in *in vitro* (binding) and invasive *in vivo* studies (intracerebral microdialysis), but only PET was able to demonstrate definitively such targeting of the DAT *in vivo* in humans. Second, a therapeutic dose could be correlated with a pharmacologically effective occupancy percentage in an integrated pharmacodynamic approach. Thus, at therapeutic doses, PET revealed that MPH blocks 60–70% of brain dopaminergic transporters [26].

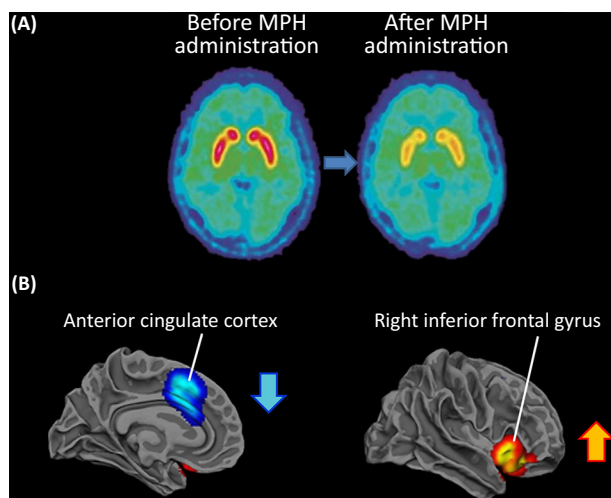
## Box 1. General Principles of PET

A PET protocol relies firstly on the production of positron-emitting radionuclides by a biomedical cyclotron, or particle accelerator. Particles (protons or deuterons) are accelerated and directed toward a target containing a specific gas or fluid. Collisions of particles with the target create positron-emitting isotopes such as carbon-11 ( $^{11}\text{C}$ ) and fluorine-18 ( $^{18}\text{F}$ ) with short half-lives (20 and 110 minutes, respectively). Once produced, the radionuclide must be rapidly incorporated into a molecule to produce a radiotracer (called a radiopharmaceutical when injected in humans). This radiolabeling step is performed in a dedicated radiochemistry lab and is followed by appropriate quality controls validated by a radiopharmacist before injection into the subject under the PET scanner.

After intravenous injection, the radioactivity emitted by the radiotracer diffusing in the subject and reaching its target provides an indirect measure of positron–electron annihilation leading to a pair of high-energy photons traveling apart at  $180^\circ$ . These two photons are simultaneously detected by two detectors linked in series that are placed on opposite sides of a ring of scintillation crystals encircling the subject within the PET apparatus. By collecting a statistically significant number of lines of response, mathematical algorithms can be used to reconstruct a 3D image that reflects the distribution of the positron-emitting molecules in the body. A modern PET scanner is capable of producing images of the human brain at a resolution of 3–6 mm. MicroPET cameras have been developed for rodent explorations with the same detection principle but with a reduced detector ring size, with a spatial resolution close to 1–1.5 mm.

The theory of PET relies on the ‘rule’ that the injected tracer mass will cause maximal target occupancy of 5%. This is because the tracer mass injected into the subject (‘tracer dose’) must be sufficiently small for the natural physiological state not to be affected. Numerous PET tracers have been designed to explore a variety of biological processes and molecular targets in the brain, ranging from cerebral perfusion and substrate metabolism to receptor targets and enzyme kinetics. PET shows exquisite sensitivity because PET tracers generally have high specific activity, defined as the ratio of the number of radioactive atoms to the total number of atoms in a given element (in a given chemical or physical form). Therefore, most PET images only require micrograms at most of the compound of interest, resulting in nanomolar or lower concentrations *in vivo*.

It should be borne in mind that the DAT is not the only pharmacologic target of MPH, and the drug also blocks the NA transporter (NET) with an affinity comparable to that for DAT [27]. This noradrenergic target was notably overlooked in many discussions of PET exploration of MPH because until recently no NET-specific PET radiopharmaceutical had been developed and validated in humans. Thus a whole aspect of MPH pharmacology was left to one side. In 2010 a team from Yale University filled this gap with [ $^{11}\text{C}$ ]-methylreboxetine [28], showing that, for any given dose, MPH blocked NET more strongly than DAT (0.14 mg/kg achieving 50% blocking of NET, and 0.25 mg/kg achieving 50% blocking for DAT), giving the noradrenergic effect a preponderant therapeutic role in the treatment of ADHD. Thus MPH at therapeutic doses blocks 70–80% of NETs, compared to 60–70% of DATs [28]. It should, however, be borne in



**Figure 2.** Brain Imaging of Methylphenidate (MPH) Action. (A) Positron emission tomography (PET) image of dopamine transporters in the striatum of human volunteers with a carbon-11 radiolabeled agent ( $^{11}\text{C}$ -altropine) before and after administration of an oral dose of methylphenidate (MPH). The percentage of drug occupation is obtained by comparing the two images. Adapted from [22]. (B) Functional magnetic resonance imaging (fMRI) meta-analysis results showing brain regions of decreased activation (blue) and increased activation (red/orange) after a single dose of MPH in children and adolescents with ADHD compared to placebo/off-medication. Adapted from [65].

mind that these MPH occupancy percentages are assessed in distinct brain regions: the striatal region for DAT, and the frontal lobes for NET.

The fact that two radiotracers, each targeting one of the main targets of MPH, are now available should enable the side effects of MPH to be explained in terms of poor tolerance of transporter occupancy: side effects related to DAT occupancy are dopaminergic and central, – sleep disorder, headache, anxiety, irritation, loss of appetite, and some rare cases of aggravated motor tics – whereas those related to NET occupancy are noradrenergic, with cardiovascular effects, such as tachycardia and moderate palpitation, and abdominal pain [29].

#### PET Drug Occupancy and Effect Duration

The biological half-life of MPH impacts on its action duration and thus on the dose and frequency of administration. Duration of action depends on biodistribution, brain kinetics, and metabolism and elimination time. PET DAT imaging enables target occupancy duration to be assessed based on the principle of competition, comparing PET images from naive subjects and after administration of a single dose of MPH, at various post-administration timepoints. It was thus shown that immediate-release MPH DAT occupancy duration strictly matches the 4 h duration of clinical efficacy [22]. This relatively short time of action, well correlated with the behavioral effects, requires administration of several doses per day, which is problematic for school-age children [30]. Several laboratories therefore developed extended-release forms of MPH (brand names Aptensio, Biphentin, Concerta, Equasym, Quasym, Quilivant, Medikinet, Metadate, Ritalin, and Rubifen). PET analysis revealed prolonged DAT occupancy, allowing a single drug dose to cover the whole period of daily activity [22]. It is noteworthy that the same PET approach was applied to NETs, but for the purpose of validating the NET radiotracer rather than with a view to developing a pharmaceutical extended-release form [28].

#### Change in DAT Density with Chronic Treatment

Long-course treatment with a psychotropic drug such as MPH in children raises the question of possible long-term, particularly neurochemical, effects. Does MPH alter the dopaminergic system? This is a legitimate question for physicians who renew their MPH prescriptions over successive years [31]. Long-course psychotropic therapy is never anodyne, especially in children and adolescents, whose brains are still developing. Moreover, experimental findings have raised many doubts in the medical community about putative links between ADHD and addiction [32] given the similarities and differences in action mechanism between MPH and cocaine [33]. Rodent studies showed that the amphetamine-like addictive effect of MPH implicates increased DAT expression [34].

These experimental findings and suggestions led researchers to monitor changes in DAT density, seen as an important factor in addiction, in subjects with ADHD [35]. A meta-analysis including nine studies showed decreased DAT density in non-treated children with ADHD [36]. What, then, is the impact of long-course MPH treatment? Is there a corrective effect that should be favorable? One team therefore used PET to monitor DAT density in young adults receiving long-course MPH (and other psychostimulants) [37]. Twelve months of MPH therapy in ADHD patients with ADHD led to a slight but significant increase in DAT density in the striatum (caudate, putamen, and ventral striatum) [37]. This was a preliminary study, requiring confirmation, but the results were in line with the strategy adopted by some physicians of introducing pauses in long-course MPH therapy, called ‘drug holidays’ [38]. Drug holidays were initially proposed to manage the side effects of MPH, particularly regarding growth retardation and possible drug tolerance. Several physicians observed a positive impact of breaks from medication on child growth, with an improvement of appetite and reduced insomnia [38]. It is, however, to be borne in mind that these observations were not correlated with imaging data and did not address any mechanism of action. This is clearly a therapeutic topic which could benefit from PET imaging.



### What Can PET Neuroimaging Tell Us about MPH and Addiction?

In the context of long-course psychotropic therapy in children, the amphetamine chemistry of MPH and its mechanism of action that mainly targets DATs raise questions for prescribers and concerns for families. If a molecule is blocking DA transport and thus increasing DA concentration, will it not overactivate the mesocorticolimbic dopaminergic pathway, also known as the pathway of pleasure and of addiction? [32,39]. An answer can be sought along two lines: psychopharmacologic and clinical/epidemiological.

Psychopharmacologically, MPH has properties that should make the addiction risk high. Addictive molecules primarily have a direct or indirect impact on dopaminergic neurotransmission, blocking the transporter or else activating opioid, cholinergic, nicotine, GABAergic, or cannabinoid receptors. The second precondition for addictiveness is speed of brain penetration and time to peak concentration. This is reflected in the typical self-administration routes preferred for addictive substances: intravenous injection, nasal inhalation, deep inhalation of smoke. The neurobiology of addiction explains how rapid intracerebral kinetics induce dopaminergic activity resulting in staccato electrophysiological signal bursts [40]. PET imaging sheds light on this mechanism. The PET microdosing technique consists of making a molecule (e.g., candidate drug) radioactive by means of carbon-11 radiolabeling, replacing a non-radioactive carbon-12 [41]. This technique was inspired by the microdose concept codified by medicinal drugs agencies for Phase 0 trials – delivering the candidate drug to healthy volunteers at unit doses lower than 100  $\mu\text{g}$  and at less than 1% of the predicted pharmacologic dose [42,43]. The PET imaging community drew an analogy with radioactive molecules used in humans, which, by definition, are always delivered at 'trace' levels of a few  $\mu\text{g}$ , in accordance with the PET microdosing concept [41]. The resulting radioactive molecule thus has exactly the same chemical structure as the molecule of therapeutic interest, with the same pharmacokinetic and pharmacodynamic properties, and can thus be administered orally and/or by injection such that brain penetration can be monitored in PET images and dynamic data. The radioactive drug may also accumulate in extracerebral regions, such as body organs, and this may be of interest for pharmacologic safety purposes. In MPH, carbon-11 radiolabeling demonstrated fast brain penetration, but with a plateau-shaped kinetics [44] that did not promote electrophysiological dopaminergic signal bursts. In parallel, on PET assessment, high-dose MPH binding to DATs did not correlate with experience of 'highs' in volunteer cocaine addicts [45]. These human PET data complement experimental pharmacology data, such as extracellular DA concentrations measured on intracellular dialysis in non-human primates [46], in which iterative extended release MPH administration did not increase craving for cocaine [47].

Finally, in point of fact and with the hindsight provided by decades of prescription to children, the figures published by epidemiological research teams and pharmacovigilance centers show relatively few reports of misadministration or abuse of MPH [48]. Misadministration (by sniffing or injecting) is rarely the responsibility of the young patient but rather of the people around him/her [49]. Moreover, recent studies reported that children receiving MPH for many years were not more liable to show addictive behavior in adulthood; some studies even reported that the treatment reduced the rate of addictive behaviors and protected ADHD children against risky behavior [50]. To sum up, MPH is associated with a low risk of addiction, but is nevertheless a psychotropic drug and treatment needs careful follow-up over the years.

### The Contribution of MRI Neuroimaging to Understanding MPH Pharmacology

MRI is a brain imaging technique (Boxes 2 and 3) that for a long time was considered to be useful for anatomic studies but not for functional and even less for molecular information. However, technological progress involving novel sequences of MRI acquisition has broadened the field of neuroimaging, and neuroanatomic techniques can now target white matter, fiber

### Box 2. General Principles of NMR

MRI and MRS rely on the NMR effect. This phenomenon is found only in nuclei possessing a non-zero magnetic moment (e.g., hydrogen) in the presence of an external static magnetic field which induces coherent precession of individual magnetic moments (spins) and gives rise to net magnetization. Detecting the NMR effect requires an additional time-varying magnetic field, applied perpendicularly to the static field, at the resonance condition or Larmor frequency, which is the frequency of the precession of the spins. For this, an additional radiofrequency coil is used. The perpendicular magnetic field pulses flip the longitudinal magnetization to an arbitrary angle with respect to the external static field. The transverse component of the flipped magnetization precesses around the static field at the Larmor frequency, inducing a time-varying voltage signal in the radiofrequency coil and constituting the source of the NMR signal.

pathways, and neurovascular coupling during brain activation [51]. The magnetic field intensity of clinical MRI scanners has also increased, from 1.5T to 3T, or even to 9.4T for dedicated human research cameras, increasing measurement sensitivity. Another advantage of MRI is that almost all protocols in neuroscience do not require injection of contrast medium, let alone of radioactive substances. This allows imaging in children and adolescents, which was scarcely conceivable for biomedical research using radioligands. Thus the majority of MRI studies of MPH effects could be performed in all subjects with ADHD, whereas previous PET studies were mainly performed in adults, with or without ADHD, but rarely in children or adolescents.

### MPH and the Development of the Cerebral Cortex

Because ADHD is mainly, but not only, a pediatric pathology, it soon became interesting to follow up its possible neurodevelopmental impact on brain growth [52,53]. Several teams pursued this by screening for surface abnormalities in prefrontal areas or abnormalities in thalamus volume [54]. For this, anatomic MRI sequences are highly informative, enabling measurements of cortical thickness, and repeated measurements allow longitudinal cohort follow-up. It was found that cortical thickness diminished more strongly during brain maturation in non-treated ADHD children than in controls [55], whereas ADHD children receiving MPH showed cortical growth (right motor cortex, inferior gyrus, parieto-occipital region) identical to that of non-ADHD controls. In their conclusions, the authors referred to a possible 'neuroprotective' effect of MPH in ADHD, but this conclusion seems hasty because this term implies (i) neuronal damage, and (ii) a pharmacological effect resulting in neuronal recovery, neither of which were observed. Another team more recently demonstrated 'normalization' of grey matter volume in subjects treated by MPH compared to non-treated ADHD controls; the regions of interest notably included the nucleus accumbens, a brain region involved in reward processing [56]. However, a more recent longitudinal study described that administration of MPH over a period of 1 year did not result in grey matter volume reduction versus placebo [57]. Other meta-analyses and longitudinal studies have not replicated the earlier findings of so-called 'normalization effects' and refuted a medication effect on brain volume [58]. Once again, it needs to be stressed that that interpretation of these imaging studies must be confirmed and refined before discussing the role of a possible anatomic 'normalization' of brain regions involved in attention and impulsiveness.

### MPH and White Matter Connectivity

Other studies have used diffusion tensor imaging (DTI), an MRI technique that measures brain connectivity by quantifying the *in vivo* diffusion of water molecules in brain tissue, thereby assessing the organization of the white matter and orientation of the main fiber tracts [59]. Water tends to spread parallel to myelinated fibers, and MRI images, usually expressed as fractional anisotropy and mean diffusivity, can thus reveal white matter disorganization. DTI studies that use tract-based spatial statistics have demonstrated microstructural abnormalities of white matter in patients with ADHD [60]. In addition to the fronto-striatal-cerebellar circuit, disturbances of white matter tracts have been measured in the bilateral hemispheres and posterior brain circuitries [60,61]. Several teams sought to assess long-term MPH effects on brain connectivity [61]. These tractographic studies, with at present few published results, showed no clear trends [62].



### Box 3. MRI Techniques

MRI is the main *in vivo* application of NMR. The generation of a stack of 2D images from individual signals from the same nuclei requires spatial coding by magnetic field gradients that vary linearly along the direction of the longitudinal magnetic field. After a slice has been selected, the signal must be spatially encoded in the two remaining dimensions. One of these directions is encoded by applying a phase-encoding gradient for a short period before signal acquisition. To achieve full spatial encoding, several experiments must be carried out with stepwise variation in the phase-encoding gradient. The third spatial dimension is encoded by applying a frequency-encoding gradient during data acquisition. Performing a 2D Fourier transform of the acquired signal yields the position of the contributing magnetization. Different patterns of pulses produced by the three orthogonal magnetic field gradients (MRI sequences) result in various image contrasts.

Contrast in MRI derives from the complex interplay of relaxation times, proton density, and instrument parameters. Anatomic images can be acquired as T1, T2, or T2\*-weighted images or as proton-density images. The spatial distribution of the relaxation rates can be mapped with quantitative measurements. Non-classical (but now routine) MRI techniques include, among others, (i) diffusion-weighted imaging (DWI) and diffusion-tensor imaging (DTI) that are sensitive to the diffusion of water protons, which, in disease, is affected by cytotoxic or vasogenic edema, or can be used to reconstruct brain neuronal fiber tracts; (ii) perfusion-weighted imaging (PWI) enables quantification of blood flow and blood volume, with or without injection of a contrast agent; (iii) magnetization transfer imaging (MTI) uses off-resonance pulses to saturate a pool of proton spins (e.g., macromolecular) and characterizes the exchange rate between this pool and another (e.g., surrounding water); (iv) fMRI relies on BOLD contrast, highlighting changes in brain activity during a task or stimulation. fMRI is especially used in pharmacological studies because it is able to measure both activated (positive BOLD effect) and inhibited (negative BOLD effect) regions after acute psychotropic drug administration; in this case it is referred to as pharmacoMRI.

Exploratory analyses of the impact of psychostimulants taken over the course of 1 year reported no significant difference in white matter microstructures. Attempts at meta-analysis likewise found no clearly reproducible trends [63]. Exploration of white matter connectivity seems relevant to follow-up of long-course psychotropic treatment, but two types of technical and methodological improvement will be necessary for this approach to be robust. First, MRI sensitivity needs to be enhanced by optimizing sequences and increasing the magnetic field because the structural effects sought are probably at present too subtle to be detected. Secondly, longitudinal studies are needed in which each subject is his or her own control following MPH treatment, and which include larger cohorts to enhance statistical power.

### Impact of MPH on Brain Function

The neuroanatomic changes following MPH treatment suggest alterations in brain function, underlying the therapeutic effect. Such functional changes can be explored on functional MRI (fMRI). As discussed in Box 3, fMRI enables the measurement of increases and/or decreases in the BOLD (blood oxygen level-dependent) effect – local variation in oxygenated blood flow that indirectly tracks neuronal activity via neurovascular coupling. fMRI is known as pharmacoMRI when the pharmacologic effect of a molecule is being explored. Numerous studies have used pharmacoMRI to track the effects of MPH in human subjects [64]. Different experimental situations should be distinguished: acute versus chronic MPH therapy, healthy volunteers (particularly for acute administration) versus ADHD patients (chronic administration), and BOLD assessment in the resting state (without cognitive task) versus during standardized cognitive task performance.

Some protocols have involved acute MPH administration, generally comparing the BOLD activation pattern immediately following administration versus a non-treated ADHD control group [65] (Figure 2B). Specific activation was found: in the frontal cortex (inferior frontal cortex) during selective attention and response inhibition tasks [66,67]; in the parietal areas during tasks involving attention, error monitoring and interference inhibition; and in the striatum during tasks involving reward and response inhibition [68]. More recently, an fMRI study revealed that ADHD subjects chronically treated with MPH only displayed a cerebral blood flow effect after acute MPH challenge in children but not in adults [69]. In this paradigm the acute MPH

challenge was used a probe for DA function, and these results stressed the modulating effect of age on the response to MPH treatment.

Other protocols involved chronic MPH administration at usual dose. Comparing subjects under long-course MPH and subjects under treatment but in abstinence during the study period, isolated administration of an extra dose induced bilateral medial frontal activation [70] or basal cingulate region deactivation [71] during a task-interference test.

These studies, however, were methodologically heterogeneous (recruitment, dosage, cognitive tasks used during imaging) and thus showed a degree of heterogeneity in fMRI activation or deactivation patterns. Several meta-analyses sought to normalize the data to reveal common activation patterns indicating MPH effects during task performance [72]. Other studies explored activation patterns in ADHD children without specific cognitive tasks (i.e., resting-state fMRI) to reveal connections and synchronization between brain regions, especially for the striatal subregions involved in executive and emotional functions [73]. Long-course MPH was thus found to reduce resting-state functional connectivity between the inferior frontal cortex and the ventral striatum [74].

The challenge, however, is to understand the functional consequences of these brain region activation/deactivation patterns – in other words, what is the clinical expression of reduced activation in the anterior cingulate cortex and particular motor areas, and increased activation in the inferior frontal gyrus, following MPH treatment? Functional MRI can help to understand how MPH acts by modulating functional brain networks related to sustained attention [75], ideally in a longitudinal manner, from childhood to adulthood. It must be noted that the majority of imaging studies are based on group statistics and cannot therefore not make individual predictions. First attempts to use neuroimaging data to make individual diagnostic classifications of ADHD children based on automatic pattern recognition techniques are promising but still need to be validated [76].

#### MPH and Magnetic Resonance Spectroscopy (MRS) Studies

MRS (Box 4) is a nuclear magnetic resonance (NMR) technique that has *in vitro* chemical and biochemical applications but also enables a more analytic *in vivo* approach. Measuring the resonance spectrum *in vivo* allows several metabolites to be identified and quantified [77]. It has been used initially by some teams to compare spectra between ADHD rodent animal models before and after MPH treatment [78]. The hypothesis is that the functional changes induced by MPH are associated with neurochemical changes measurable on MRS, complementing the PET approaches described above.

In a preliminary study, it was suggested that glutamatergic metabolic abnormalities normalize under MPH. MRS data in adolescents receiving MPH showed a trend toward lower glutamate/myo-inositol and glutamine/myo-inositol ratios in the anterior cingulate cortex than in non-treated

#### Box 4. MRS Techniques

The basic principle of MRS is that the spin of a single isotope resonates at slightly different frequencies because of the molecular environment (i.e., chemical structure). This difference is called the chemical shift and is commonly expressed as a relative measurement in parts per million (ppm), rendering it independent of field strength. NMR spectroscopy measures chemical shifts from a defined volume within a sample (in chemistry and biochemistry) or an organ (in *in vivo* imaging). The NMR spectrum is the Fourier transform of the acquired NMR signal. Chemical shifts allow the identification of chemical substances, while the signal intensity is proportional to their concentration. MRS provides a non-invasive window into metabolism and can be viewed as an additional contrast to standard anatomical MRI examination. The concentration of several metabolites can be measured *in vivo* mainly through a proton spectrum of the brain (<sup>1</sup>H-MRS): creatine, choline, *N*-acetylaspartate, lactate, and myo-inositol. The relative levels of the corresponding spectral peaks can reflect cellular homeostasis or pathological states, and may be used as biomarkers.

ADHD subjects [79]. Other changes in metabolite ratios were found in adolescents in the prefrontal region [80] and amygdala [81]. More recently, a MRS study aimed to determine the relationship between changes in creatine, choline, and *N*-acetylaspartate levels in cortical brain regions of adults with ADHD following MPH treatment and the *SNAP25* gene polymorphism [82]: only subjects bearing the polymorphism showed an altered metabolite profile with MPH treatment. This biochemical application of MRI, however, still shows severe limitations in terms both of differences in data between reports and of the sensitivity of metabolite measurements, which is far lower than for PET. Moreover, these MRS studies are largely descriptive, with little pathophysiological interpretation [83]. Not many metabolites are analyzable on MR, and those that are detectable are not always neurotransmitters or biologically active.

#### Toward an MPH Response Biomarker?

Many teams pin their hopes on identifying and validating a biomarker to enable early assessment of good response to psychotropic treatment. This is crucial in psychiatry, where very many psychotropic drugs show low response rates: very often less than 50%, for example for antidepressants and antipsychotics. For MPH, the response rate is much better, nearly 70% [84], but being able to measure the response would make it possible to check, early during treatment initiation, whether MPH is proving effective [85], thus sparing non-responders from the adverse effects of no therapeutic benefit and allowing rapid access to an alternative, such as guanfacine or atomoxetine, depending on the market authorizations in the country in question.

A biomarker for early treatment response could in theory consist of a biological blood assay, a genetic test, a physiological parameter, or a questionnaire. However, the level of evidence required to validate a biomarker for treatment response is so high that many authors conclude that there are in psychiatry no validated biomarkers that can be used for research or for clinical purposes. It is hoped that imaging, and especially MRI, which is more easily accessible than PET, will identify biomarkers for neuropsychiatry (e.g., for depression [86]). PET has the disadvantage of requiring a radiochemical with pharmaceutical validation, and injecting a radiopharmaceutical requires radioactive dosimetry. MRI without contrast medium injection involves no ionizing radiation, making it easier to implement and suitable for wide-scale application, including in children.

A team has evaluated whether there are differences in striatal volume between good and poor responders to MPH, and reported that good responders had a higher concentration of grey matter in both caudate nuclei and right nucleus accumbens [87]. Other teams have applied the knowledge of functional connectivity gained in fMRI, detecting synchronized activation across brain regions [88], and then investigated whether these patterns differed between good and poor responders to MPH on attention performance criteria [74]. Patients under MPH with lower resting-state connectivity between striatal subregions and cortical regions were found to have better cognitive scores, suggesting better treatment response. Conversely, subjects with poorer scores in terms of inattention and impulsiveness despite MPH treatment showed greater connectivity between the same regions in the resting state. These results support hypotheses regarding the respective roles of different brain regions in cognitive tasks. They also open the way to identifying resting MRI patterns with sufficient sensitivity and specificity to predict treatment response. However, to our knowledge, no studies have shown a way to predict MPH response at an individual level.

#### Concluding Remarks

MPH is presently the main molecule used in the pharmacologic treatment of ADHD. Despite several decades of experience and hundreds of clinical studies, prescribers and families continue to have concerns about its psychotropic nature, prolonged use in children, and

chemical relation to amphetamines. Moreover, while its mechanism of action is held to consist mainly of blocking DATs and NETs, its theoretical psychostimulant action does not account for its clinical benefit in terms of controlling impulsiveness and increasing concentration [89]. In this context, brain imaging on PET and MRI has greatly contributed to our understanding of the neuropharmacology of MPH. Both techniques are highly translational, being applicable in both animal models and humans.

PET has shed light on the molecular targets of MPH in humans, visualizing and quantifying both DATs and NETs by means of radiotracers labeled with carbon-11 or fluorine-18. These studies also measured drug occupancy time in healthy volunteers, enabling adjustment of daily dosage and also extended-release forms of MPH. Finally, direct radiolabeling of MPH has shed light on its brain-penetration kinetics, providing further evidence of low addictiveness. The main limitations of these studies, with the radiochemical and radiopharmacological requirements inherent to the PET technique, were, first, that they were very largely conducted in healthy volunteers rather than in ADHD subjects; it would be unethical to expose children or adolescents to the radiation doses involved in injecting a radiopharmaceutical. Second, there have been very few human PET studies of NAT: the appropriate radiopharmaceutical was only very recently developed and, to our knowledge, has yet to be used to explore NAT as a target under MPH treatment (see Outstanding Questions). It would be especially interesting to set up a study protocol to explore DAT and NAT in a single subject under MPH by successive injection of the respective radiotracers, [ $^{11}\text{C}$ ]PE2I and [ $^{11}\text{C}$ ]methylreboxetine, on the same day, which is now technically feasible. It would also be interesting to monitor and compare these two MPH targets during long-course MPH treatment in adults.

MRI, taking advantage of multiple sequences, has also allowed exploration of the morphologic and functional impact of long-course MPH. It has been especially useful to be able to include children and adolescents in these non-ionizing MRI protocols so as to explore actual patients. Functional MRI has been of prime interest in exploring the brain regions that are activated and deactivated under MPH. The pharmacMRI approach opens the way to real *in vivo* pharmacology in patients, but functional connectivity analyses will need improving because they are often of borderline statistical significance owing to small sample sizes and heterogeneous study designs, hindering meta-analysis of common patterns. MRS protocols are attractive because they can assess brain neurochemistry without using radiopharmaceuticals, but presently lack sufficient sensitivity to explore molecules of biological interest in the brain.

Great hope is now placed on the recently developed PET–MRI technology: hybrid cameras now enable simultaneous measurements using PET imaging with radiopharmaceutical injection and MRI with the same morphologic, functional, and spectroscopic modalities as a classic 3T MRI camera. A simultaneous multimodal study, instead of taking a PET image on one camera and then an MR image on another, holds promise for elegant experimental designs to explore MPH neuropharmacology. One can envisage PET analysis of MPH drug occupancy of DAT or NAT with simultaneous measurement of functional activation in the affected brain regions. This would, for the first time, enable true pharmacokinetic/pharmacodynamic exploration of MPH.

## References

1. Still, G.F. (1902) The Goulstonian lectures on some abnormal psychological conditions in children. *Lancet* 159, 1008–1013
2. Kramer, F. and Pollnow, H. (1932) Über eine hyperkinetische Erkrankung im Kindesalter. *Monatsschr. Psychiatr. Neurol.* 82, 21–40
3. Martinez-Badía, J. and Martínez-Raga, J. (2015) Who says this is a modern disorder? The early history of attention deficit hyperactivity disorder. *World J. Psychiatry* 5, 379–386
4. Conners, C.K. and Barkley, R.A. (1985) Rating scales and checklists for child psychopharmacology. *Psychopharmacol. Bull.* 21, 809–843
5. Green, M. et al. (1999) *Diagnosis of Attention-Deficit/Hyperactivity Disorder*, US Agency for Health Care Policy and Research
6. Baweja, R. et al. (2015) Impact of attention-deficit hyperactivity disorder on school performance: what are the effects of medication? *Paediatr. Drugs* 17, 459–477

## Outstanding Questions

Can brain imaging enable exploration of the pharmacokinetics/pharmacodynamics of MPH?

What can PET imaging reveal about NAT as a target for MPH treatment?

Can brain imaging determine the addiction risk of MPH?

Does long-course MPH alter brain structures?

Does long-course MPH alter brain neurochemistry?

Can brain imaging enable early identification of MPH responders/non-responders?

7. Tallow-Golden, M. *et al.* (2016) What do general practitioners know about ADHD? Attitudes and knowledge among first-contact gatekeepers: systematic narrative review. *BMC Fam. Pract.* 17, 129
8. Kiely, B. and Adesman, A. (2015) What we do not know about ADHD . . . yet. *Curr. Opin. Pediatr.* 27, 395–404
9. Panizzon, L. (1944) La preparazione di piridil- e piperidil-arilacetontitili e di alcuni prodotti di trasformazione. *Helvetica Chim. Acta* 27, 1748–1756
10. Wenthur, C.J. (2016) Classics in chemical neuroscience: methylphenidate. *ACS Chem. Neurosci.* 7, 1030–1040
11. Conners, C.K. and Eisenberg, I. (1963) The effects of methylphenidate on symptomatology and learning in disturbed children. *Am. J. Psychiatry* 120, 458–464
12. Bradley, C. (1937) The behavior of children receiving benzedrine. *Am. J. Psychiatry* 94, 577–585
13. Barkley, R.A. (1977) The effects of methylphenidate on various types of activity level and attention in hyperkinetic children. *J. Abnorm. Child Psychol.* 5, 351–369
14. Schomaker, J. and Meeter, M. (2015) Short- and long-lasting consequences of novelty, deviance and surprise on brain and cognition. *Neurosci. Biobehav. Rev.* 55, 268–279
15. Advokat, C. and Scheithauer, M. (2013) Attention-deficit hyperactivity disorder (ADHD) stimulant medications as cognitive enhancers. *Front. Neurosci.* 29, 82
16. Lancelot, S. and Zimmer, L. (2010) Small-animal positron emission tomography as a tool for neuropharmacology. *Trends Pharmacol. Sci.* 31, 411–417
17. Zimmer, L. and Luxen, A. (2012) PET radiotracers for molecular imaging in the brain: past, present and future. *Neuroimage* 61, 363–370
18. Volkow, N.D. *et al.* (2001) Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J. Neurosci.* 21, RC121
19. Wang, G.J. *et al.* (1999) Reproducibility of repeated measures of endogenous dopamine competition with [<sup>11</sup>C]raclopride in the human brain in response to methylphenidate. *J. Nucl. Med.* 40, 1285–1291
20. Volkow, N.D. *et al.* (1999) Methylphenidate and cocaine have a similar *in vivo* potency to block dopamine transporters in the human brain. *Life Sci.* 65, PL7–12
21. Volkow, N.D. *et al.* (2002) Relationship between blockade of dopamine transporters by oral methylphenidate and the increases in extracellular dopamine: therapeutic implications. *Synapse* 43, 181–187
22. Spencer, T.J. *et al.* (2006) PET study examining pharmacokinetics, detection and likeability, and dopamine transporter receptor occupancy of short- and long-acting oral methylphenidate. *Am. J. Psychiatry* 163, 387–395
23. Spencer, T.J. *et al.* (2010) A PET study examining pharmacokinetics and dopamine transporter occupancy of two long-acting formulations of methylphenidate in adults. *Int. J. Mol. Med.* 25, 261–265
24. Kim, W. *et al.* (2014) *In vivo* activity of modafinil on dopamine transporter measured with positron emission tomography and [<sup>18</sup>F]FE-PE2I. *Int. J. Neuropsychopharmacol.* 17, 697–703
25. Heal, D.J. *et al.* (2009) The neuropharmacology of ADHD drugs *in vivo*: insights on efficacy and safety. *Neuropharmacology* 57, 608–618
26. Volkow, N.D. *et al.* (1998) Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. *Am. J. Psychiatry* 155, 1325–1331
27. Gatley, S.J. (1996) Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sci.* 58, 231–239
28. Hannestad, J. (2010) Clinically relevant doses of methylphenidate significantly occupy norepinephrine transporters in humans *in vivo*. *Biol. Psychiatry* 68, 854–860
29. Golmirzaei, J. *et al.* (2016) Psychopharmacology of attention-deficit hyperactivity disorder: effects and side effects. *Curr. Pharm. Des.* 22, 590–594
30. Chan, E. *et al.* (2016) Treatment of attention-deficit/hyperactivity disorder in adolescents: a systematic review. *JAMA* 315, 1997–2008
31. Barbaresi, W.J. *et al.* (2014) Long-term stimulant medication treatment of attention-deficit/hyperactivity disorder: results from a population-based study. *J. Dev. Behav. Pediatr.* 35, 448–457
32. Robbins, T.W. (2002) ADHD and addiction. *Nat. Med.* 8, 24–25
33. Volkow, N.D. *et al.* (1996) Cocaine addiction: hypothesis derived from imaging studies with PET. *J. Addict. Dis.* 15, 55–71
34. Calipari, E.S. *et al.* (2013) Methylphenidate amplifies the potency and reinforcing effects of amphetamines by increasing dopamine transporter expression. *Nat. Commun.* 4, 2720
35. Volkow, N.D. *et al.* (2015) Recovery of dopamine transporters with methamphetamine detoxification is not linked to changes in dopamine release. *Neuroimage* 121, 20–28
36. Fusar-Poli, P. *et al.* (2011) Striatal dopamine transporter alterations in ADHD: pathophysiology or adaptation to psychostimulants? A meta-analysis. *Am. J. Psychiatry* 169, 264–272
37. Wang, G.J. *et al.* (2013) Long-term stimulant treatment affects brain dopamine transporter level in patients with attention deficit hyperactive disorder. *PLoS One* 8, e63023
38. Ibrahim, K. and Donyai, P. (2015) Drug holidays from ADHD medication: international experience over the past four decades. *J. Atten. Disord.* 19, 551–568
39. Bisagno, V. *et al.* (2016) Cognitive enhancers versus addictive psychostimulants: the good and bad side of dopamine on prefrontal cortical circuits. *Pharmacol. Res.* 109, 108–118
40. Samaha, A.N. and Robinson, T.E. (2005) Why does the rapid delivery of drugs to the brain promote addiction? *Trends Pharmacol. Sci.* 26, 82–87
41. Bergström, M. *et al.* (2003) Positron emission tomography microdosing: a new concept with application in tracer and early clinical drug development. *Eur. J. Clin. Pharmacol.* 59, 357–366
42. Wilding, I.R. and Bell, J.A. (2005) Improved early clinical development through human microdosing studies. *Drug Discov. Today* 10, 890–894
43. Burt, T. *et al.* (2016) Microdosing and other Phase 0 clinical trials: facilitating translation in drug development. *Clin. Transl. Sci.* 9, 74–88
44. Swanson, J.M. and Volkow, N.D. (2002) Pharmacokinetic and pharmacodynamic properties of stimulants: implications for the design of new treatments for ADHD. *Behav. Brain Res.* 130, 73–78
45. Volkow, N.D. *et al.* (1999) Blockade of striatal dopamine transporters by intravenous methylphenidate is not sufficient to induce self-reports of “high”. *J. Pharmacol. Exp. Ther.* 288, 14–20
46. Schiffer, W.K. *et al.* (2006) Therapeutic doses of amphetamine or methylphenidate differentially increase synaptic and extracellular dopamine. *Synapse* 59, 243–251
47. Gill, K.E. *et al.* (2012) Chronic treatment with extended release methylphenidate does not alter dopamine systems or increase vulnerability for cocaine self-administration: a study in nonhuman primates. *Neuropsychopharmacology* 37, 2555–2565
48. Weyandt, L.L. *et al.* (2016) Prescription stimulant medication misuse: where are we and where do we go from here? *Exp. Clin. Psychopharmacol.* 24, 400–414
49. Clemow, D.B. and Walker, D.J. (2014) The potential for misuse and abuse of medications in ADHD: a review. *Postgrad. Med.* 126, 64–81
50. Fredriksen, M. and Peleikis, D.E. (2016) Long-term pharmacotherapy of adults with attention deficit hyperactivity disorder: a literature review and clinical study. *Basic Clin. Pharmacol. Toxicol.* 118, 23–31
51. Ugurbil, K. (2016) What is feasible with imaging human brain function and connectivity using functional magnetic resonance imaging. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371, 1705
52. Shaw, P. *et al.* (2007) Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proc. Natl. Acad. Sci. U. S. A.* 104, 19649–19654
53. Rogers, J.C. and De Brito, S.A. (2016) Cortical and subcortical gray matter volume in youths with conduct problems: a meta-analysis. *JAMA Psychiatry* 73, 64–72



54. Batty, M.J. *et al.* (2015) Morphological abnormalities in prefrontal surface area and thalamic volume in attention deficit/hyperactivity disorder. *Psychiatry Res.* 233, 225–232
55. Shaw, P. *et al.* (2009) Psychostimulant treatment and the developing cortex in attention deficit hyperactivity disorder. *Am. J. Psychiatry* 166, 58–63
56. Villemonteix, T. *et al.* (2015) Grey matter volumes in treatment naïve vs. chronically treated children with attention deficit/hyperactivity disorder: a combined approach. *Eur. Neuropsychopharmacol.* 25, 1118–1127
57. Tebartz van Elst, T. (2016) The effect of methylphenidate intake on brain structure in adults with ADHD in a placebo-controlled randomized trial. *J. Psychiatry Neurosci.* 41, 422–430
58. Hoogman, M. (2017) Subcortical brain volume differences in participants with attention deficit hyperactivity disorder in children and adults: a cross-sectional mega-analysis. *Lancet Psychiatry* 4, 310–319
59. Vorona, G.A. and Berman, J.I. (2015) Review of diffusion tensor imaging and its application in children. *Pediatr. Radiol.* 45, S375–381
60. Chen, L. *et al.* (2016) A systematic review and meta-analysis of tract-based spatial statistics studies regarding attention-deficit/hyperactivity disorder. *Neurosci. Biobehav. Rev.* 68, 838–847
61. de Zeeuw, P. *et al.* (2012) Decreased frontostriatal microstructural organization in attention deficit/hyperactivity disorder. *Hum. Brain Mapp.* 33, 1941–1951
62. Schweren, L.J. *et al.* (2013) MR imaging of the effects of methylphenidate on brain structure and function in attention-deficit/hyperactivity disorder. *Eur. Neuropsychopharmacol.* 23, 1151–1164
63. de Luis-García, R. (2015) Attention deficit/hyperactivity disorder and medication with stimulants in young children: a DTI study. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 57, 176–184
64. Anderson, I.M. *et al.* (2008) Assessing human 5-HT function *in vivo* with pharmacofMRI. *Neuropharmacology* 55, 1029–1037
65. Rubia, K. *et al.* (2014) Effects of stimulants on brain function in attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. *Biol. Psychiatry* 76, 616–628
66. Rubia, K. *et al.* (2011) Methylphenidate normalizes fronto-striatal underactivation during interference inhibition in medication-naïve boys with attention-deficit hyperactivity disorder. *Neuropsychopharmacology* 36, 1575–1586
67. Rubia, K. *et al.* (2011) Methylphenidate normalizes frontocingulate underactivation during error processing in attention-deficit/hyperactivity disorder. *Biol. Psychiatry* 70, 255–262
68. Rubia, K. *et al.* (2009) Methylphenidate normalises activation and functional connectivity deficits in attention and motivation networks in medication-naïve children with ADHD during a rewarded continuous performance task. *Neuropharmacology* 57, 640–652
69. Schranter, A. *et al.* (2016) Age-dependent effects of methylphenidate on the human dopaminergic system in young vs adult patients with attention-deficit/hyperactivity. A randomized clinical trial. *JAMA Psychiatr.* 73, 955–962
70. Posner, J. *et al.* (2011) The attenuation of dysfunctional emotional processing with stimulant medication: an fMRI study of adolescents with ADHD. *Psychiatry Res.* 193, 151–160
71. Peterson, B.S. *et al.* (2009) An fMRI study of the effects of psychostimulants on default-mode processing during Stroop task performance in youths with ADHD. *Am. J. Psychiatry* 166, 1286–1294
72. Rubia, K. *et al.* (2014) Brain abnormalities in attention-deficit hyperactivity disorder: a review. *Rev. Neurol.* 58, S3–S16
73. Posner, J. *et al.* (2013) Dissociable attentional and affective circuits in medication-naïve children with attention-deficit/hyperactivity disorder. *Psychiatry Res.* 213, 24–30
74. Hong, S.B. *et al.* (2015) Functional dysconnectivity of corticostriatal circuitry and differential response to methylphenidate in youth with attention-deficit/hyperactivity disorder. *J. Psychiatry Neurosci.* 40, 46–57
75. Rosenberg, M.D. *et al.* (2016) Methylphenidate modulates functional network connectivity to enhance attention. *J. Neurosci.* 36, 9547–9557
76. Marquand, A.F. *et al.* (2011) Pattern classification of working memory networks reveals differential effects of methylphenidate, atomoxetine, and placebo in healthy volunteers. *Neuropsychopharmacology* 6, 1237–1247
77. McKay, J. and Tkáč, I. (2016) Quantitative *in vivo* neurochemical profiling in humans: where are we now? *Int. J. Epidemiol.* 45, 1339–1350
78. Adriani, W. *et al.* (2007) <sup>1</sup>H MRS-detectable metabolic brain changes and reduced impulsive behavior in adult rats exposed to methylphenidate during adolescence. *Neurotoxicol. Teratol.* 29, 116–125
79. Hammerness, P. *et al.* (2012) Brain biochemical effects of methylphenidate treatment using proton magnetic spectroscopy in youth with attention-deficit hyperactivity disorder: a controlled pilot study. *CNS Neurosci. Ther.* 18, 34–40
80. Wiguna, T. *et al.* (2012) Effect of 12-week administration of 20-mg long-acting methylphenidate on Glu/Cr, NAA/Cr, Cho/Cr, and ml/Cr ratios in the prefrontal cortices of school-age children in Indonesia: a study using <sup>1</sup>H magnetic resonance spectroscopy (MRS). *Clin. Neuropharmacol.* 35, 81–85
81. Wiguna, T. *et al.* (2014) The amygdala's neurochemical ratios after 12 weeks administration of 20 mg long-acting methylphenidate in children with attention deficit and hyperactivity disorder: a pilot study using <sup>1</sup>H magnetic resonance spectroscopy. *Clin. Psychopharmacol. Neurosci.* 12, 137–141
82. Ünal, G.A. *et al.* (2016) Relationship between the SNAP-25 gene and the effects of methylphenidate on the anterior cingulate cortex of patients with adult attention deficit hyperactivity disorder: a magnetic resonance spectroscopy study. *Eur. Rev. Med. Pharmacol. Sci.* 20, 2443–2449
83. Endres, D. *et al.* (2015) Normal neurochemistry in the prefrontal and cerebellar brain of adults with attention-deficit hyperactivity disorder. *Front. Behav. Neurosci.* 9, 242
84. Hanwella, R. *et al.* (2011) Comparative efficacy and acceptability of methylphenidate and atomoxetine in treatment of attention deficit hyperactivity disorder in children and adolescents: a meta-analysis. *BMC Psychiatry* 11, 176
85. Faraone, S.V. *et al.* (2014) Biomarkers in the diagnosis of ADHD – promising directions. *Curr. Psychiatry Rep.* 16, 497
86. Chi, K.F. *et al.* (2015) Imaging predictors of remission to anti-depressant medications in major depressive disorder. *J. Affect. Disord.* 186, 134–144
87. Moreno, A. *et al.* (2014) Striatal volume deficits in children with ADHD who present a poor response to methylphenidate. *Eur. Child Adolesc. Psychiatry* 23, 805–812
88. Rosenberg, M.D. *et al.* (2016) A neuromarker of sustained attention from whole-brain functional connectivity. *Nat. Neurosci.* 19, 165–171
89. Millard, W.J. and Standish, L.J. (1982) The paradoxical effect of central nervous system stimulants on hyperactivity: a paradox unexplained by the rate-dependent effect. *J. Nerv. Ment. Dis.* 170, 499