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Chapter 7

English summary, general discussion and conclusions

English summary and general discussion

Recent preclinical studies have highlighted the importance and urgent need to conduct clinical studies on the age-dependent, and possibly long-term, effects of psychotropic medication. This is particularly relevant as they are often prescribed to children and adolescents, whose brains are very plastic and, to a considerable extent, still undergo development. Moreover, in the past decades, these medications are prescribed in large numbers and increasingly so to children and adolescents suffering from Major Depressive Disorder (MDD) or Attention-Deficit/Hyperactivity Disorder (ADHD).

When drugs target a neurotransmitter system during its developmental phase, this may alter the normal developmental trajectory. As such, effects of a drug can outlast the drug's presence and induce long-lasting changes. This concept is known as *neurochemical imprinting* and predicts that neurotransmitter systems, like the dopamine (DA), gamma-aminobutyric acid (GABA) and serotonin (5HT) systems, may incorporate the effects of an early exposure to drugs targeting these systems. As such, drug exposure at an early age may lead to a different state or responsivity of the neurotransmitter system long after treatment has stopped, e.g. in adulthood[1].

The *neurochemical imprinting* theory has until now only been studied in preclinical studies, mainly rats, and it is thus so far unknown whether this phenomenon also occurs in the human brain. The main aim of this thesis was therefore to investigate whether the psychotropic medications methylphenidate (MPH) and selective serotonin reuptake inhibitors (SSRIs) induce age-dependent, and possibly long-lasting effects also in the developing human brain.

In this thesis, we focused on two commonly prescribed medications in children and adolescents: MPH for the treatment of ADHD (**Part I**) and SSRIs for the treatment of MDD and Anxiety Disorder (AD) (**Part II**). The main results of my thesis indicate that there is an age-dependent sensitivity of the human DA- and GABAergic system to MPH, but apparently not of the 5HTergic system to SSRI exposure. This final chapter summarizes the general results obtained in these two parts of this thesis, discusses the choices made in the designs, details limitations of our studies, and, based on relevant literature, provides future perspectives for further clinical research.

Part I – Attention-Deficit/Hyperactivity Disorder

Part I of this thesis addressed age-dependent, and possibly lasting, effects of MPH exposure on the brains of ADHD patients of different ages of first exposure. Even though the DAergic system has received the most attention as a major neurotransmitter system involved in ADHD, also the GABAergic system plays an important role in the pathophysiology of ADHD. Therefore, in **Chapter 2**, we investigated potential age-dependent effects of MPH exposure on the GABAergic system in three different groups that allowed us to study long-term effects of MPH exposure on the human GABA system. Patients were stratified into three groups: one group of patients whose first MPH exposure had occurred at an age at which the brain still undergoes considerable plasticity (i.e. during a period of ongoing brain development during childhood and adolescence[2,3]), one group of patients whose first MPH exposure had occurred at an age at which their brain has already further matured and can be considered mature (adult exposure), and one group of patients who were never exposed to MPH treatment.

In **Chapter 2**, we found that baseline GABA levels were lower in subjects first exposed to MPH during brain development, compared to subjects first exposed in adulthood. An acute challenge with MPH induced a significant increase in GABA levels only in subjects who were first exposed early in life, whereas this was not the case in the unexposed subjects or in the subjects first exposed at an adult age. From these results, we concluded that treatment with MPH during brain development alters the human GABAergic system and induces long-lasting alterations, i.e. a hyperactive GABA response to a DAergic stimulus.

Chapters 3 and **4** describe the results from a randomized clinical trial (RCT), entitled the 'Effects of Psychotropic drugs On the Developing brain' (ePOD) program. The aim of this study was to investigate the possible persisting, age-dependent effects of 16 weeks of MPH treatment on the DAergic system and function. In this RCT, children (10-12 years of age) and adults (23-40 years of age), all medication-naive patients diagnosed with ADHD, were randomly treated with either MPH or placebo for a period of 16 weeks. The participants underwent a neuropsychological test battery and an MRI scan session, including a functional MRI (fMRI) assessment of emotional dysregulation and attention, and they also participated in a sleep study. All measurements were done at three time-points during the trial: prior to treatment, during treatment and following a one-week washout after treatment end. In **Chapter 3**, the outcomes of the two fMRI tasks are reported. The first fMRI task measured emotion recognition, whereas the second

fMRI task measured response inhibition, two key cognitive processes that are impaired in ADHD.

For the emotion recognition paradigm, MPH increased amygdala reactivity in children one week after trial end compared to during treatment, whereas the reactivity decreased in the placebo treated children one week after trial end. However, MPH improved emotion dysregulation in the children during the trial. In contrast, in the adults, no effects were found of MPH over placebo on amygdala reactivity and emotion dysregulation. Furthermore, MPH treatment decreased amygdala-cortical functional connectivity in both age groups, but this connectivity was found to be stronger in adults than in children at trial end. For the response inhibition paradigm, MPH did not affect paracingulate reactivity, neither in children nor in adults, but did induce paracingulate-cortical hyperconnectivity in adults at trial end.

Our findings suggest that 16 weeks of treatment with MPH affected right amygdala activity, amygdala and paracingulate connectivity and emotion dysregulation in an age-dependent manner, as different results were reported for children compared to adults. The emotion recognition findings indicate that MPH treatment might result in a lasting increase in amygdala reactivity in children, which is a potential concern as heightened amygdala activation has been linked to emotion dysregulation[4]. Other clinical studies have shown an increased risk for anxiety or depression later in life after such heightened activity in adolescence[4-6]. However, these findings are in contrast with self-reported emotion dysregulation, which was positively influenced by MPH treatment during the trial in children (only). A possible explanation for this could be that depressive or anxiety symptomatology, as a possible result of heightened amygdala activity, was not observed until six years after MPH treatment in another large clinical trial[6]. Although we have not done a longer follow-up, we do show that on the short-term, there are no obvious negative effects of MPH on symptoms of emotion dysregulation, anxiety or depression, either in children or adults.

In **Chapter 4**, we investigated whether 16 weeks of MPH treatment affects sleep and whether these effects persisted after treatment cessation. Sleep was assessed using actigraphy, a sleep diary and questionnaires, at three time-points during the ePOD trial: baseline, during and post-treatment. Our results show that MPH treatment has a positive effect on sleep: sleep efficiency was significantly higher in the subjects treated with MPH compared to placebo treatment. Additional positive effects were found on sleep onset latency, total sleep time and mean wake bout time. No differences were found between MPH and placebo

treatment during the trial, indicating that MPH treatment did not negatively affect sleep during treatment. Furthermore, we did find a significant increase in sleep efficiency in the MPH treated subjects when baseline values were compared to post-treatment values. This was absent in the placebo treated subjects. We further investigated the effects of MPH on restless legs syndrome (RLS), but failed to find any effects of MPH on this readout. From these results, we conclude that there is a positive effect of MPH on sleep that outlasts the treatment period.

Collectively, the results in **Chapter 2** and **3** support the hypothesis that effects of chronic MPH exposure may depend on the age of first exposure, also in the human brain. When combining the results in **Chapter 2, 3** and **4,** the effects of MPH exposure also outlast the treatment period, consistent with the *neurochemical imprinting* hypothesis.

The persisting effects of early MPH exposure on later imbalances in the DAergic neurotransmitter system were not only investigated in this thesis, but have also been studied in the same subjects as studied here by Schrantee et al., who used a different technique to study imbalances in the DAergic system. ADHD patients that were first exposed early, but not later (i.e. in adulthood) showed lower cerebral blood flow (CBF) in DA innervated brain areas[7,8]. A 16 week MPH treatment induced age-dependent effects on the CBF response to MPH in the DA striatal-thalamic circuitry[8,9]. These age-dependent, lasting effects of ADHD medications on the DAergic neurotransmitter system are in line with the age-dependent effects of MPH on the emotional reactivity paradigm[10–12] we report in **Chapter 3**, and the persisting effects of MPH on sleep we report in **Chapter 4**, in which the DA system is also thought to play a role[13,14].

We furthermore report persisting changes in the GABAergic system in **Chapter 2**, in which we found lower GABA levels in those ADHD patients who were first exposed to MPH at a young age, but not in those for whom first drug treatment had started only later in life. The GABAergic system has been implicated in ADHD pathophysiology[15,16] and levels of behavioral impulsivity, i.e. a key feature of inhibitory control in ADHD, are found to be negatively correlated with brain GABA levels[17–19]. Indeed, it is thought that aberrations in the normal development of both the GABAergic and DAergic neurotransmitter systems might result in later imbalances and possible dysfunction of these systems, which could play a role in the behavioral symptomatology and persistence of ADHD[20,21].

When combining the results from Schrantee et al.[8,9] and those reported in this thesis, together with the growing body of evidence from preclinical studies, we conclude that treatment with MPH during brain development seems to induce age-

dependent and lasting effects on the DAergic and GABAergic neurotransmitter systems. The next step would be to focus on the consequences of these medication-induced neurobiological changes on cognition and behavior, and to investigate e.g. whether the lasting effects remain present when the time since treatment cessation increases. A meta-regression analysis that focused on response inhibition, working memory and sustained attention in ADHD patients investigated whether MPH had an age-dependent effect on these cognitive measures[22]. However, they did not observe age-dependent effects of MPH on executive functions in children and adults with ADHD, but concluded that medication-naive adolescent studies are needed before this conclusion can be confirmed. Moreover, they suggest that it is of interest to determine how these cognitive effects relate to behavioral impairment, but studies done so far on this topic used only small sample sizes. Hopefully, future studies with increased sample sizes and inclusion of medication-naive adolescent subjects will provide more insight in the age-dependent effects of MPH on cognitive and behavioral measures.

Part II – Major Depressive Disorder

Part II of this thesis focused on the 5HT system, particularly on age-dependent, possibly lasting effects of antidepressant (mainly SSRI) exposure on the (developing) brain of patients with MDD and/or AD. In **Chapter 5**, we first assessed whether phMRI can detect dose-related hemodynamic responses to SSRIs and whether this technique can be compared to single photon emission computer tomography (SPECT) in terms of its ability to detect with sufficient sensitivity serotonin transport (SERT) occupancy in a group of healthy female volunteers. Our results show that (after an acute oral dosage of citalopram) phMRI can indeed detect differential effects of SERT occupancy in a dose-dependent manner. This suggests that phMRI can serve as a (non-ionizing) alternative for SPECT or Positron Emission Tomography (PET) for studies that aim to measure the 5HT system in vivo.

In **Chapter 6**, phMRI was used as a proxy measure for 5HT function and applied to a clinical study. Here, the age-dependent effect of SSRI exposure on the 5HT system was investigated in patients with a life-time MDD and/or AD diagnosis. Again, comparable to **Chapter 2**, three patient groups were investigated: subjects first exposed to SSRI treatment before the age of 23 (i.e. during a period of ongoing brain development), patients first exposed after the age of 23 years (i.e. after their brain had matured and are considered adult), and patients that were never exposed to SSRIs. In addition, a group of healthy female subjects was

included to compare the functioning of the 5HT system between healthy subjects, and subjects with a life-time MDD and/or AD diagnosis. We found an overall decline in the CBF of the amygdala, hippocampus and orbitofrontal cortex (OFC), in response to an acute 5HT challenge with citalopram, regardless of group.

The lack of group differences in this human study was in contrast with earlier preclinical phMRI studies, showing that e.g. rats that were exposed to SSRIs during development, displayed an altered response to an acute SSRI challenge, compared to rats that were first exposed to SSRIs only in adulthood[23]. However, despite the current lack of difference in our human study, it is still too early to conclude that SSRIs do not affect the development of the human 5HTergic system. One reason would be that either the young/adolescent human brain is differently sensitive to SSRIs, e.g. due to differences in metabolism or blood-brain-barrier function between rodents and humans, such that the changes reported in rodents do not happen in humans at all (although this may be different for MPH, see **Part I**). Alternatively, our current study design (i.e. its retrospective nature and a heterogeneous study population) may not have allowed to properly study these aspects. We studied four regions of interest (ROIs) in **Chapter 6;** i.e. the OFC, hippocampus, amygdala and thalamus, but possibly a whole brain analysis might have uncovered CBF changes in different brain regions. Furthermore, we included both healthy controls subjects and subjects with a life-time MDD diagnosis, whereas the preclinical studies only included healthy animals, which could have contributed to differences in results. To address these aspects, future, longitudinal studies with a more homogeneous (patient)group are needed. In sum, based on the work reported in **Part II** of this thesis, we conclude that although phMRI is a reliable technique to investigate SSRI-induced changes of the human 5HTergic system, longitudinal studies are needed to fully understand the age-dependent effects of SSRIs on the 5HTergic system.

Methodological considerations

Measurement techniques

There are several limitations worth mentioning with respect to the techniques and designs we used here. In **Chapter 2**, proton magnetic resonance spectroscopy (1H-MRS) was used to quantify GABA levels in the prefrontal cortex. Although MRS is currently the only available technique to reliably measure human brain metabolites *in vivo*, a large voxel is needed in order to obtain a sufficiently high signal-to-noise ratio (SNR) as well as to reliably fit and estimate GABA levels.

Direct quantification of metabolite concentrations using MRS is very complicated and therefore ratios are used to express relative metabolite concentrations to a reference metabolite. We have chosen the unsuppressed water peak as a reference signal, as it has a high SNR and clear spectral separation from the GABA signals. Also creatine could be used as a reference metabolite, and is indeed often chosen for that reason by others, due to its relative stability and the lack of a chemical shift with respect to GABA[24]. Nonetheless, creatine has a lower SNR compared to water, and the form of the creatine signal could in principle differ between the ON and OFF scans in the MEGA-PRESS sequence we used here. This would be due to changes in the underlying GABA signal that has a shared location of origin with creatine. For future studies, possible improvements would be to make use of the recent advancements in 7T protocols (resulting in increased SNR and better chemical shift dispersion[25]), or to use chemical shift imaging for an increased coverage, instead of single voxel spectroscopy[26].

In **Chapter 3**, fMRI was used as a technique to assess effects of MPH exposure on amygdala activity and connectivity during an emotion recognition paradigm. FMRI measures the blood-oxygen-level dependent (BOLD) signal that is based on the principle that changes in brain activity are associated with changes in blood flow. However, the BOLD signal can be further affected by several factors, including medication, which can e.g. via cardiovascular actions, affect blood flow in the brain directly, hereby altering the BOLD signal. Furthermore, the BOLD signal is a sum of both inhibitory and excitatory input to a neuron or neuronal networks, which could cancel each other out when they receive both inputs at the same time.

For our sleep measurements in **Chapter 4**, we used actigraphy. The actigraph is a small unit, worn for several nights in order to measure the human rest and activity cycles during day and night, and uses movement as a proxy to measure the sleep pattern of the subject. Another technique that more directly measures sleep is polysomnography. With polysomnography, the subject stays in the lab overnight to record the bio-physiological changes that occur during sleep. However, the use of polysomnography is very costly, impractical and it does not represent the sleeping pattern of the subject, a child in our case, in its natural environment. In our study, actigraphy was therefore preferred over polysomnography. Moreover, a recent study compared actigraphy and polysomnography in ADHD patients and healthy controls. They reported that for some sleep variables, like sleep onset latency and sleep duration, an acceptable correlation between polysomnography and actigraphy was present in ADHD patients treated with MPH or placebo. However, in placebo-treated ADHD

patients, actigraphy tended to overestimate sleep efficiency with 9.3% compared to polysomnography, which was estimated to be 8.5% in MPH-treated ADHD patients, and 5.6% in healthy control subjects. As our trial only included ADHD patients and no control subjects, this overestimation should not raise a concern about the use of actigraphy in our study, according to their conclusions[27]. In view of our study design in this particular age group and setting, we choose to use actigraphy in the ePOD trial, also for practical reasons. As we also compared both groups the same way, we consider it unlikely that major differences have been introduced between the groups as a result of our use of actigraphy for measuring sleep variables.

In **Chapter 5** and **6**, phMRI was used. PhMRI measures brain hemodynamics after administration of a drug or pharmacological challenge, and allows to measure the activity and responsivity of a given neurotransmitter system, in this case the 5HT system. PET and SPECT are two widely applied techniques that can be used to measure the 5HTergic system as well, but due to their use of radio ligands, they are not suitable for use in children, nor do they allow to measure changes in the 5HT system repeatedly, or over a longer period of time. In our studies in **Chapter 5**, we therefore included an additional SPECT measurement, which allowed for a comparison of the phMRI response with the 5HT transporter occupancy in the same subject, as measured with SPECT. From the results, we conclude that phMRI can detect dose-dependent changes in baseline CBF. The phMRI signal, however, did not correlate with changes in SPECT measurements, possibly due to the fact that SPECT measures transporter binding directly, although both techniques did correlate with SSRI plasma levels separately. PhMRI is an indirect measure of the SERT, as it namely measures the hemodynamic response in the whole synapse, which includes both presynaptic transporters and pre- and postsynaptic receptors, in addition to neurotransmitter release[28]. Our results in **Chapter 6** differ from studies that used BOLD or arterial spin labeling (ASL) based phMRI, and that measured age-dependent changes after an SSRI challenge[29–31]. As we discussed in **Chapter 6**, these differences are likely explained by the fact that both measurements are influenced by different direct and indirect processes following an SSRI challenge, in addition to the heterogeneous sample. This can involve direct 5HT release, but also (de)activation of 5HT receptors. Although phMRI is a promising technique that allows to non-invasively measure biological variations and abnormalities in the 5HT system, e.g. in response to SSRI treatment, future studies would benefit from additional measures that can distinguish between neuronal activation and vascular effects per se. One possibility would be to use a dual-echo ASL sequence that obtains both

changes in CBF and BOLD, which should improve phMRI signal sensitivity considerably[32].

For the phMRI and fMRI studies reported in this thesis, we chose to perform ROI-based analyses instead of whole-brain analyses for our primary outcomes. This decision was based on the fact that we wanted to directly translate preclinical work on neurochemical imprinting to human research, with a priori selected ROIs based on the available (preclinical) literature. In addition, whole brain analyses suffer from the multiple comparison problem, thereby lowering the statistical power to detect small effects. As we could only include relatively small samples, ROI-based analyses were preferred due to increased statistical power. Nevertheless, ROI analysis averages over multiple voxels, which implies that if different voxels respond differently to the stimulus, this effect is averaged out. Furthermore, it is possible that our findings in preclinical models do not directly translate to the same regions in humans, and therefore future studies with more statistical power should consider other brain areas as well.

Study designs

In **Chapter 2, 5** and **6**, we used a retrospective, cross-sectional approach to study effects of psychotropic medication on the brain at different ages of treatment onset. The potential limitations of this design are well known and include recall bias, a.o., and the fact that the directions of relationships are difficult to determine, since all conditions are measured simultaneously. In an attempt to overcome at least some of these limitations, we organized a randomized controlled trial, as reported on in **Chapter 3** and **4**. This ePOD RCT was unique in multiple aspects: it was the first clinical trial that directly compared *medication-naive* children and adults with ADHD while using the same imaging and cognitive measures in both groups. Furthermore, a placebo group was included, that allowed us to study the specific effects of MPH treatment in patients with ADHD, with no other differences between our groups. However, as only children were included in **Chapter 4**, we cannot report on any specific, age-dependent effects of MPH on sleep and it is thus not known whether similar changes would occur in adults.

The ePOD trial included only boys and men of a narrow and specific age range (boys 10-12 years, men 23-40 years). This makes it difficult to extrapolate our results from this trial to the general population (including women, very young children or elderly). The current age ranges were chosen since a) the peak prevalence of ADHD is around 10 years of age[33], and b) the brain is considered to

be fully matured after the age of 23[34]. Furthermore, due to ethical considerations, we were only allowed to study the age-dependent effects of MPH treatment on the brain for up to four months. As the waiting list to see a psychiatrist in the Netherlands took this long at the start of our study, this allowed a maximum treatment duration of four months without causing a delay in treatment for the patients.

Ideally, one would like to compare MPH treatment with placebo treatment for a longer period of time. Another research group also performed an RCT[35], and reported that 14 months of pharmacological treatment did not influence outcome six to eight years later[6]. However, this study did not include a placebo group in their design, but only compared different therapies (medication management, behavioral treatment, combination of the former two and routine community care)[35]. Inclusion of a placebo group in their study, or implementing a longer treatment period in our RCT was however, due to medical ethical considerations, not possible. Because of this, a retrospective, cross-sectional study design was used in **Chapter 2** for MPH and in **Chapter 6** for citalopram. Despite the shortcomings of this retrospective design mentioned before, this did allow us to investigate the age-dependent effects of MPH, or of SSRI exposure, over a much longer time period (on average four to seven years after last medication exposure). Furthermore, a naturalistic follow-up study of the ePOD trial has recently started: ePOD 2.0, in which we will investigate effects of MPH on brain development three years after trial exposure. Nearly all subjects started medication treatment for their ADHD diagnosis, and we will therefore use a cumulative dosage to study the effects of exposure to different dosages of MPH on the development of the brain. With the results from ePOD 2.0, we expect to gain more insight into the structural and functional consequences of the effects we so far reported in the original ePOD RCT[8,9].

Clinical implications

In **Part I** of this thesis, we concluded that MPH treatment in patients with ADHD who were first exposed at an early age, i.e. during a period of ongoing brain development, may have induced possible lasting effects on their DAergic and GABAergic systems. Using both a clinical trial (**Chapter 3** and **4**) and a cross-sectional study (**Chapter 2**) reported in this thesis, it is unlikely that pre-existing differences between the subject groups, such as age, medication history, or comorbid disorder, could explain the results. In addition, different methodologies were used in this thesis (MRS in **Chapter 2**, fMRI in **Chapter 3** and actigraphy in

Chapter 4), while also preclinical studies support the conclusions from **Part I** of this thesis.

These conclusions are of considerable clinical relevance. First, they underpin the need for a correct diagnosis of ADHD at the initiation of pharmacotherapy[36]. Ideally, diagnosis should be made by an experienced psychiatrist, or by a specialized ADHD center. Given the effects of MPH on the developing brain, misdiagnosis of ADHD in children is an important concern for both the community and professionals[37]. Our findings will increase the available evidence on treatment of ADHD with MPH, not only in children but adults as well. Caregivers and parents need to be able to make an informed decision on whether or not to treat a child with MPH, and whether it will do more harm or good. Such knowledge is essential for a better understanding of ADHD and its proper management[36]. Although we do not know, and will most likely never know for sure whether MPH also affects the DAergic and GABAergic system in children without an ADHD diagnosis, preclinical studies on MPH effects were, in absence of a reliable animal model for ADHD, all performed in healthy animals.

Based on this, and on the increased prescription rates of MPH among children and young adolescents[38], future studies are necessary to investigate whether the current effects on the DAergic and GABAergic neurotransmitter system persist throughout the rest of the subjects' life. If this is indeed the case, the diagnostic process of ADHD might need to be reevaluated in order to prevent misdiagnosis of ADHD and thereby avoid a situation where children are exposed to medication that do not fulfill the ADHD criteria.

The results in **Chapter 2** further highlight the role of the GABAergic neurotransmitter system in ADHD, and the effects of MPH has on this system outlasting treatment duration. Although the role of GABA in ADHD was already investigated by other studies[15,16,39,40], and GABAergic and glutamatergic gene sets are found to be involved in ADHD[41], it is currently not considered as a potential drug target in the treatment of ADHD. Benzodiazepines, e.g., a class of psychoactive drugs that enhance the effects of GABA, were not effective in treatment of ADHD[42], also because of their addictive properties. As the GABAergic system continues to develop throughout childhood and adolescence, age is a factor that needs to be taken into account also here[3,43], which makes it difficult to include also the GABAergic system as drug target for both children, adolescents and adults with ADHD. Although no other studies have looked into the possibility of GABA as a potential drug target in ADHD, it would however be interesting to consider

GABA as an additional or secondary drug target for the treatment of ADHD in the future, especially in view of the upcoming personalized medicine era[44].

The possibly lasting effects of MPH on the GABAergic and DAergic neurotransmitter system are expected to be positive in theory, as ADHD is linked to decreased levels of these two neurotransmitters[15,35,39,40,45]. However, longitudinal follow-up studies are necessary to examine whether these changes in the DAergic and GABAergic neurotransmitter systems persist longer after treatment cessation, and whether these alterations are associated with positive changes on behavioral level and symptomatology as well. Furthermore, our results from **Chapter 3** point towards a possible long term negative effect of MPH, as a heightened amygdala activation, as we observed after early MPH treatment, has been linked to emotion dysregulation[4] and a possible increase in the risk to develop depression, six years after treatment onset[6]. The ePOD 2.0 follow-up study will report on the three-year follow up of this heightened amygdala activation. Only after those results, or perhaps after an additional follow-up study (ePOD 3.0, six years after the initial trial) have been obtained and completed, will we learn whether the direct benefits of early MPH treatment outweigh the possible lasting effects of MPH treatment on depressive symptoms and possible other, unknown, lasting effects. Obviously, such follow-up studies should include results on the behavioral impact and disease symptomatology.

Conclusions

The preclinical studies supporting the concept of *neurochemical imprinting* have highlighted the need for clinical studies to investigate whether there long-term alterations occur also in human brain after early treatment with psychotropic medication. Although the safety and efficacy of these psychotropic medications are extensively documented, surprisingly little is known about the possible long-term effects of these medications on the developing brain.

In this thesis, we have shown, using several imaging approaches and methodologies, that treatment with MPH induces age-dependent effects on the DAergic and GABAergic neurotransmitter systems in human subjects, that may possibly be lasting. Early treatment with SSRIs, however, did not induce such lasting, age-dependent effects on the 5HTergic neurotransmitter system. Considering the amount of recent prescriptions issued of these medications, together with the preclinical studies highlighting the possible neurochemical imprinting effects, our results are of considerable clinical relevance. This thesis will

contribute to a better understanding of the impact of psychotropic medications on the developing brain, and may hopefully initiate more longitudinal follow-up studies into the effects of psychotropic medications on the human brain, as the *neurochemical imprinting* theory likely applies also to other medications prescribed to children and adolescents.

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