Antidepressants and the adolescent brain: Changing the course of neurodevelopment?
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Citation for published version (APA):

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Chapter 12

SUMMARY, GENERAL DISCUSSION AND CONCLUSIONS
Summary, general discussion and conclusions

Summary

The main objective of this thesis was to gain more insight in the effects of SSRIs, and fluoxetine (FLX) in particular, on neurodevelopmental processes in the adolescent brain. In more detail, we were interested in the effects of chronic FLX treatment on neurotransmitter function, with a strong emphasis on serotonergic function, and on how these effects differed between adolescent- and adult exposure in the rat brain. Additionally, we focused on the usefulness of pharmacological MRI as an imaging tool for assessing 5-HT function.

Part I: Chemical imprinting after 5-HT manipulation

Chemical imprinting can occur when psychoactive substances are administered during developmentally vulnerable time windows. In part I of this thesis, two pilot studies looking at effects of 5-HT manipulation during adolescence are described. In chapter 2, MDMA exposure was used as a model for 5-HT neurotoxicity. In line with the literature, and likely due to ongoing plasticity in the developing brain, we observed that the neurotoxic effects of MDMA on SERT expression are less pronounced in juvenile-exposed rats than in adult-exposed rats. Additionally, we were interested to see if the effects of ecstasy in recreational users of this drug are also dependent upon age. Interestingly, we found that younger age-of-first-ecstasy-use predicted higher SERT density in adulthood, but only in individuals that started their abuse before the age of 18 years. Hence, age-at-first-exposure affected adult SERT availability only when this first exposure happened during youth, thus in a developing brain. Chapter 3 describes a combination of experiments in which we looked at the effects of chronic FLX on behavior, on SERT density and on phMRI readouts. After treatment cessation, an increase in anxiety-like behavior was seen in the adult-treated animals only, while there were no behavioral effects in the adolescent-treated animals. Age-by-treatment effects were seen in SERT density within all cortical areas and the hypothalamus, which confirmed earlier findings (Bock et al., 2005; Wegerer et al., 1999), but not in the phMRI results. Thus, there were clear indications of age-related effects following adolescent and adult SSRI exposure, which asked for further research with larger sample sizes.

Part II: Age-related effects of chronic fluoxetine in the rat brain

Several different research techniques were used to assess the age-dependency of the effects of chronic FLX exposure on 5-HT and related neurotransmitter systems. For each of these studies the same treatment protocol and study design was applied. Adolescent (postnatal
day 25 (PND25)) and adult (PND65) male Wistar rats were treated for 21 days with 5 mg/kg FLX via the oral route. After one week washout, 5-HT function was assessed. With 5-HT phMRI, we looked at effect of previous treatment on the brain response to an acute 5-HT challenge. As described in chapter 4, we observed a diminished brain response to this acute 5-HT challenge in adult treated animals when compared to control animals, whereas this response was increased in juvenile treated rats. Age-by-treatment effects were apparent in several 5-HT related brain areas, such as the frontal cortex, amygdala, hypothalamus and striatum. Subsequently, follow-up studies were performed to gain more insight in underlying processes of these phMRI results. In vivo concentrations of extracellular 5-HT, DA and NA and their metabolites were measured in the mPFC before and after an acute 5-HT challenge (chapter 5). Although previous treatment with FLX did increase the 5-HT and DA response to the acute challenge, no age-by-treatment effects were observed. Chapter 6 describes three exploratory experiments looking at the effects of chronic treatment on gene expression, SERT availability and TPH expression. Age-by-treatment effects were found in the mRNA expression of glutamatergic and GABAergic targets, but not in the expression of 5-HT, DA, and NA targets and also not in the expression of genes related to neuronal outgrowth and HPA-axis function. Surprisingly, we also found no age-by-treatment effects on SERT availability. The immunoreactivity of the rate limiting enzyme of 5-HT synthesis, TPH, was dependent on age-at-treatment, in that adolescent-treated animals showed heightened expression of TPH+ cells whereas adult-treated animals showed decreased expression compared to non-treated animals. Additionally, we looked at the impact of FLX on immunoreactivity of two markers of adult neurogenesis in the hippocampal dentate gyrus (DG), which is known to be positively influenced by 5-HT (Boldrini et al., 2009). Similar to the TPH findings, we found significant age-by-treatment effects on the expression of both DCX+ and Ki67+ cells (chapter 7). Overall, these findings give a clear indication that FLX can indeed have a differential effect on 5-HT neurotransmission dependent on the age of exposure.

Part III: Methodological issues of 5-HT phMRI

Being a relatively new imaging approach, the full range of applications for 5-HT phMRI is currently being explored. In chapter 8 and the accompanying video article (available at www.jove.com/video/3956), we presented a methodological overview on how to perform 5-HT phMRI in the alive and free-breathing rat. In addition, its strengths and shortcomings were discussed. While 5-HT phMRI makes it possible to assess cerebral neurotransmitter function in the live animal, it remains a challenging technique. The greatest possible effort should be put in assuring the maintenance of stable physiological parameters during the full length of image acquisition. For patient studies, especially studies concerning effects of medication
on the developing brain, 5-HT phMRI is still considered to be fairly invasive, particularly when the challenging drug is administered intravenously. Therefore, it would be beneficial if the same effects could be measured after oral administration of the drug. This specific method has been used in previous fMRI studies but test-retest reliability of this method was not yet determined. In chapter 9, we report that the repeatability of pulsed ASL phMRI with an oral citalopram challenge is low, limiting the technique’s sensitivity to time-dependent changes and consequently its use as a (clinical) research tool. Recently, ASL techniques with less signal variability, such as pseudo continuous ASL, have become available. It will of great interest to see if these techniques will give a better result. Nevertheless, we demonstrated that the effect of an oral SSRI challenge on cerebral blood flow is relatively small. Also with BOLD-based fMRI, we demonstrated that it is difficult to reliably detect the same effects of an oral SSRI challenge on task-related brain activity, as is described in chapter 10. Here, we found no reproducible effects of the challenge on brain activity related to emotional processing and sensorimotor activation. However, we did find reproducible effects on task-negative processes, in the so-called ‘default mode network’ (DMN). This network comprises of brain areas that are mainly activated during rest and become deactivated in response to goal-directed behavior (Buckner et al., 2008). Oral citalopram administration reliably diminished this deactivation, particularly in the medial frontal cortex. This finding could be indicative of 5-HT control on the default mode network. Overall, oral challenge 5-HT phMRI demonstrated to be a valuable imaging technique, but caution is needed in its appliance in terms of test-retest reliability. This is especially the case when a drug challenge with a relatively small effect size is used or when extra variability is introduced to your design, for example with anesthesia or if the challenge is given orally instead of intravenously. This requires sufficiently large sample sizes. Finally, in chapter 11, we combine the aims of Parts II and III of this thesis and describe the design and methodology of three ongoing clinical trials that should give us more insight in the effects of psychotropic medication use on the neurodevelopment of children and adolescents in the near future, using both prospective and retrospective approaches and several neuroimaging techniques including 5-HT phMRI.

General discussion

**Age-dependent effects of chronic FLX exposure on 5-HT neurotransmission**

Collectively, the animal studies presented in this thesis support the hypothesis that the effects of chronic FLX exposure on 5-HT transmission depend on the age-of-treatment. Despite a number of negative and unexpected findings, several significant age-by-treatment interaction effects on read-outs of the 5-HT system were identified. In the adolescent-
treated animals, we observed an increase of TPH⁺ cells in the raphe nucleus (chapter 6) and of the neurogenesis markers DCX and Ki67 in the DG (chapter 7), together with an increase in the BOLD-response to an acute 5-HT challenge (chapter 4). This is all indicative of enhanced 5-HT neurotransmission. On the other hand, mRNA expression of several glutamatergic and GABAergic targets, and to a lesser extent of the 5-HT (auto)receptors, was diminished in comparison to adult-treated animals (chapter 6). Considering the important role of those targets in 5-HT feedback control (Sharp, 2010), taken together, these results suggest that there is an increased responsiveness of the 5-HT system in adolescent-treated animals, possible due to diminished feedback control. This is somewhat surprising, since it is commonly believed that drug exposure to a developing environment will integrate the drug into this environment and, subsequently, development will be adjusted to depend on its presence (Andersen and Navalta, 2011). In case of the enhanced 5-HT neurotransmission caused by an SSRI, one would thus expect the 5-HT system to adapt to this artificial enhancement, which will lead to a deficit state upon drug cessation, and not, as is suggested by our results, to enhanced 5-HT neurotransmission. However, as pointed out before, the possibility to alter neurodevelopment is highly dependent on the so-called ‘sensitive period’ in which the developmental process is taking place. The occurrence of this timeframe might not only vary for each different part of 5-HT system (e.g. the expression of the variety of 5-HT receptors and SERT, synaptogenesis of 5-HT neurons, etc.) but even differs per brain region. So, timing is in this case everything. During the postnatal period that was chosen for our studies, representing the entire peri-adolescent period (PND21 to PND59; (McCormick and Mathews, 2007)), most of the 5-HT related developmental processes will be completed (Murrin et al., 2007). FLX exposure during this time window will most probably only affect the late-developmental processes such as synaptic pruning and changes in receptor expression, which are both relevant for the fine-tuning of 5-HT feedback control later in life. This would thus support our hypothesis of diminished feedback control after adolescent FLX treatment. Nevertheless, as long as it is unidentified when and where these specific developmental processes take place, making inferences about the effects of chronic SSRI exposure hereupon will remain speculative at best. Regarding the title of this dissertation, the question mark thus remains unresolved. While it was confirmed that chronic SSRI exposure can indeed affect the 5-HT system in a different manner within adolescent animals than in adult animals, and that this is most probably due to ongoing plasticity in the adolescent brain, it remains to be seen if these differences really reflect changes in neurodevelopment. More importantly, longer washout periods are needed to ensure that these effects are permanent, also considering the fact that ongoing effects of previous treatment were observed in the adult-treated rats as well.
Underlying pathways?

Although our findings of age-by-treatment interaction effects of chronic FLX treatment on several neurochemical properties of the 5-HT system are of interest in itself, the real challenge would be to explain differences between adolescent- and adult-treatment in terms of the underlying neurobiological pathways that have caused them. In order to do so, we should start with joining together the findings of our different experiments. To begin with, the heightened release of extracellular 5-HT (and DA) as a response to acute reuptake blockade after previous FLX treatment that we describe in chapter 5 is indeed indicative of increased 5-HT system responsiveness. However, this effect did not depend on age-of-treatment and was seen in both the adult and adolescent treated animals. And if indeed extracellular 5-HT release is directly responsible for changes in BOLD MRI signal, as is suggested by Preece et al. (2009), then why did we find age-at-treatment related differences in BOLD response with phMRI but not in the 5-HT release with in vivo microdialysis? To answer this question, we should consider in more detail the neural basis underlying the BOLD fMRI signal. Although it was recently confirmed that the regional activations measured with MR neuroimaging do indeed reflect local changes in neural activity, the precise underlying mechanisms of this neural basis of the fMRI signal are still not fully identified (Logothetis and Wandell, 2004).

The MRI signal changes that are observed in response to a 5-HT challenge are known to be influenced not only by extracellular 5-HT release directly (Preece et al., 2009; Sekar et al., 2011), but also indirectly by the (de)activation of a number of 5-HT receptors (Gozzi et al., 2010; Martin and Sibson, 2008; Sekar et al., 2011). This direct effect of extracellular 5-HT release on the BOLD response is however not straightforward. While Preece et al. (2009) showed signal decreases after 5-HT release in most brain regions, in our study (chapter 4) and that of Sekar et al. (2011) 5-HT release due to SERT blockade by an SSRI resulted in signal increase. BOLD signal change is dependent on neurovascular coupling, the relationship between local neural activity and subsequent changes in cerebral blood flow. This is not only influenced by oxygen and glucose consumption, but also by neurotransmitter release (Logothetis and Wandell, 2004). Additionally, 5-HT is a known modulator of vascular control, although the actions of 5-HT on blood flow are intricate and result from interactions with the multiplicity of 5-HT receptors (Martin, 1994). So, depending on the state of the 5-HT receptors, extracellular 5-HT can cause either vasoconstriction or vasodilatation. In this way, the phMRI response to increased 5-HT release can have both a specific and non-specific neurovascular origin (Preece et al., 2009; Scanley et al., 2001).

One can imagine that the MRI signal is influenced by the available amount of SERT as well, since an acute SSRI challenge will block the available SERT, leading to an increase of extracellular 5-HT and resulting in BOLD signal changes. Adjustments in the amount
of available SERT might thus influence the effect size of the challenge, depending on the level of occupancy. Although we did find age-at-treatment related differences in the SERT binding potential in our initial pilot study (chapter 3), with a relatively small sample size and after intraperitoneal drug administration, we were not able to replicate these findings with a larger sample size and oral administration (chapter 6). It thus remains elusive if chronic FLX divergently changes SERT availability with adolescent or adult treatment. Yet, if the SERT expression in younger animals would have been increased after FLX treatment, as suggested by our results in chapter 3 and by Wegerer et al. (1999), SERT blockade might have been less effective and this would have led to a diminished response to an acute SSRI challenge in comparison to control animals, though we observed an increased 5-HT release after chronic exposure in both adolescent as adult in our microdialysis study (chapter 5). On the other hand, the occupancy of SERT by SSRIs is known to be relatively high with approximately 80% occupancy at the minimum clinical dose in humans (Meyer, 2007) and possibly even higher levels of occupancy in the rat (Geldof et al., 2008). This high level of occupancy could have concealed the effects of SERT density in both the microdialysis and the phMRI experiment. Nevertheless, basal 5-HT levels were also not different between treated and untreated animals (chapter 5).

The age-by-treatment interaction effect on the expression of TPH in the dorsal raphe, as described in chapter 6, suggests enhanced 5-HT responsiveness after adolescent treatment as well, as the increased TPH expression is assumed to be indicative of augmented 5-HT synthesis. On the other hand, this is still not fully supported by our measures of extracellular 5-HT (chapter 5), which indeed showed enhanced 5-HT release after FLX treatment, though no age-by-treatment effects were seen in this enhancement. Once more, there might be an important role for the negative feedback mechanisms that regulate the 5-HT homeostasis. At any rate, it does show that TPH expression in the DRN is apparently not predictive of the level of 5-HT release in the mPFC. Additionally, previous literature suggests a decrease of TPH after prolonged SSRI exposure, both after adult and early developmental exposure (MacGillivray et al., 2010; Maciag et al., 2006). In normal developing rats, TPH activity reaches its peak between 24-30 days after birth and TPH mRNA expression is known to increase vastly until around PND 22 after which it again decreases with about 40% before it reaches adult values around PND 61 (Park et al., 1986; Rind et al., 2000). We hypothesize that this normal decrease in TPH during adolescence is countered by the decrease due to SSRI exposure and that therefore TPH expression is heightened compared to the same-age control animals after drug withdrawal, in line with the ‘use it or lose it’ philosophy of brain development, as postulated in Andersen and Navalta (2011).
Similar age-by-treatment effects were observed in the expression of adult neurogenesis markers in the DG (chapter 7). As 5-HT is known to stimulate cell proliferation, this is in line with our suggestion of enhanced 5-HT function in the adolescent-treated rats. Although it is still not clear how 5-HT transmission regulates adult neurogenesis, this process is again mediated by 5-HT (auto)receptors (Banasr et al., 2004; Benninghoff et al., 2012; Jha et al., 2008; Klempin et al., 2010; Soumier et al., 2010). Even though mRNA expression of the 5-HT2A was significantly lower in the HC (chapter 6), it was a main effect of treatment and did not depend on the age-at-treatment. With phMRI, there was a treatment effect on BOLD reactivity in the HC as well, but only in adult animals. At this moment, our results can thus not explain the age-by-treatment effect seen in the expression of adult neurogenesis markers.

Taken together, we believe that differences in 5-HT receptor function and thus in 5-HT feedback mechanisms are responsible for age-by-treatment effects seen in the phMRI study, in the expression of TPH in the DRN and in the neurogenesis markers in the DG. This is supported by the results of the other studies, with no apparent age-related effects on 5-HT release in the mPFC and on SERT expression, and the age-related effect on mRNA expression of targets involved in this feedback control. Of course, these assumptions are largely hypothetical. The underlying working mechanisms of 5-HT transmission and the effects of SSRIs thereupon are complex and only a small part has been unraveled. This makes it extremely hard, if not impossible, to exactly pinpoint the differences of SSRI exposure on 5-HT transmission in the developing or adult brain. One should keep in mind that there is a broad range of variables that could potentially affect 5-HT function, which is not only limited to the exact age at evaluation but is also dependent on species, strain, brain region, rearing conditions and other environmental influences, etcetera.

Enduring effects of SSRI exposure in adult animals

In literature, it is often suggested that the time needed to remove a drug from the animal’s system is enough to end its effects. However, while we hypothesized to find little to no effects on 5-HT transmission in the adult-treated animals one week after drug withdrawal, we did find several effects of previous treatment in the adult rats as well (e.g. on BOLD response (chapter 4) and the 5-HT response to acute reuptake blockade (chapter 5)). In some cases, these effects were opposite to the known effects during or directly after SSRI treatment, such as the decrease in adult neurogenesis (chapter 7). In case of SERT expression, an effect of previous treatment was even only seen five weeks after treatment cessation (chapter 6). Our data thus indicate that the effect of adult FLX exposure is not only apparent during or directly after treatment, but that longer-lasting effects are present as well. It is likely that
those enduring effects reflect the recovery of ‘normal’ 5-HT neurotransmission, possibly in the form of compensatory actions, as suggested by the decrease in neurogenesis after the synthetic enhancement by chronic SSRI exposure (chapter 7). This recovery might take longer than generally thought. Surprisingly, little is reported on these restorative processes following discontinuation of SSRIs in the adult brain, although recent studies do suggest that for example the replacement of down-regulated SERT will take more than 10 days in an adult rat brain (Descarries and Riad, 2012). This uncertainty raises questions about the washout period chosen in our studies. Even in the fast metabolizing rat, one week may well be too short to fully restore 5-HT homeostasis, and will thus also be too short to adequately assess lasting changes after adolescent exposure. It is therefore recommended in future studies to assess the effects of FLX at several different time intervals after drug cessation, in order to learn more about the time period needed for full recovery of the affected system.

5-HT phMRI: a useful tool for studying developmental drug exposure?

The use of phMRI for the assessment of 5-HT neurotransmission is a relatively new imaging approach. The fact that this technique can be readily used in both animals and human subjects makes it especially suited for studies in which the translation from basic to preclinical and clinical research is a key factor (Martin and Sibson, 2008). Additionally, repeated assessment within the same individual is unrestricted, unlike for example with PET and SPECT imaging. The review papers by Anderson et al. (2008) and Martin and Sibson (2008) already give a good overview of the strengths and limitations of 5-HT phMRI in both humans and animals, so we kindly refer to those for considerations on the validity of phMRI for the assessment of 5-HT function. In the light of this thesis, we aimed to determine whether 5-HT phMRI is a useful tool to study the effects of developmental drug exposure on the 5-HT system. Indeed, we were able to pick up the effects of an acute 5-HT challenge and to observe differences herein between adolescent- and adult-treated animals, thus to demonstrate the age-dependency of the chronic effects of FLX treatment on brain function in rats (chapter 4). A great advantage of phMRI over other techniques used in this thesis is that it enables in vivo measurement of an acute pharmacological intervention on functional parameters in the entire brain at once. Application of this technique to study age-related effects in human subjects might nevertheless hold greater challenges than those of animal 5-HT phMRI (chapter 8). Although phMRI is less invasive than PET and SPECT imaging, there is still the issue of (repeated) drug administration. Administering drugs to healthy minors is not ethically accepted and, in case of juvenile patients, the drug challenge is ideally not given intravenously, while this is methodologically preferred. We have shown that with an oral 5-HT challenge the effect size of the drug on blood perfusion (chapter 9) and on task-
related BOLD responses (chapter 10) is relatively small, not only hampering the detecting of potential 5-HT related differences but also the technique’s test-retest reliability. It is therefore recommended to use an intravenous instead of oral challenge, as is being issued in our current patient trial, in order to assure adequate statistical power, and to use a mixed between-group and within-subject design (‘mixed factorial design’) to reduce between-subject variation (chapter 11).

Methodological considerations

A major strength of the animal studies in Part II of this thesis, is that in each experiment, the same study design and treatment protocol was used, making a direct comparison possible (with exception of drug administration in drinking water instead of by oral gavage in the microdialysis study in chapter 5). There are however also a number of general restrictions to our studies that should be considered, apart from the earlier mentioned issues with the length of the chosen washout period, and the strengths and limitations of each individual study which are mentioned per chapter.

To start with, one might argue that our animal work is not directly representative for the human situation, since antidepressants are not normally prescribed to healthy subjects and no animal model of depression was used. SSRIs are meant to normalize a dysfunctional state and may well work differently in a healthy brain. Although we acknowledge the validity of this issue, much is still unclear about the etiology of depression as well as on the exact working mechanisms of SSRIs and it is therefore almost impossible to study age-related differences herein. We argue that, it is better to first focus on what is happening in a ‘normal’ state before complicating things with a diseased state. We can also turn this issue around; studying these effects in healthy subjects is the only way to get insight of the effects of the drug independent of the underlying disease state (Andersen and Navalta, 2011). Since our interest is primarily in the age-dependency of the effects of SSRIs, it is likely that if such age-related differences occur in a healthy brain, they will also occur in the depressed brain. Of course, it remains to be seen if these differences are equivalent and also if they are beneficial to the diseased state or not? Furthermore, there is yet an ongoing debate about the validity of animal models for depression. Although new models are still being developed (Neumann et al., 2011), there is at the moment no model that can capture the heterogeneity of symptoms expressed in MDD (Berton et al., 2012; Dzirasa and Covington III, 2012). Even so, it remains to be seen if such a model also adequately captures the specific aspects of pediatric depression, and more importantly, one cannot rule out that the model itself will affect either the effects of SSRI exposure or neurodevelopment itself.
Another issue of great relevance is the drug dosing and pharmacokinetics in children and young animals. All aspects regarding the effectiveness of a drug, such as bioavailability, distribution, and drug metabolism, but also the pharmacodynamics, can all differ with developmental state (Kearns et al., 2003). In our experiments, we chose to use the same weight-dependent dosage in the adolescent animals as in the adult animals (5mg/kg), as this is considered to be a clinical relevant dosage (chapter 3). However, as the plasma concentrations presented in chapter 5 show, drug clearance is about two-fold faster in the younger animals. This will result in less of a steady state in drug plasma levels, especially with in case of the once daily administration via oral gavage. Although the active and slower eliminating metabolite of FLX, norfluoxetine, presumably remains present in the rat brain over 24h (Caccia et al., 1990), also in the adolescent animals, we cannot rule out that this difference in elimination rate has affected our results. Additionally, these developmental differences in drug properties and effectiveness seriously impede the translation of our and related studies between species. As said before, we consider the used dosage to be clinically relevant, but this is based upon adult data. This is not only an issue in (our) animal studies, but in the clinical setting as well, as often the dosage of SSRIs issued in children and adolescents is not evidence-based. Findling and colleagues (2006) stated that dosing strategies that are being employed in placebo-controlled efficacy studies in juvenile MDD are not supported by the data available from juvenile PK studies. Not only might data derived from adults be inapplicable, it was even suggested that children and adolescents require different dosages of FLX based on PK data (Findling et al., 2006).

Clinical implications and future directions

Although this thesis has not provided direct proof of neurodevelopmental effects of FLX in depressed children and adolescents and does not answer whether these effects will outweigh the clinical benefits of SSRIs in pediatric depression, our findings in the adolescent rat brain have again stressed the importance of uncovering the effects of SSRI exposure on human brain development. In addition, more insight is gained on the complexity of the 5-HT system, which primarily points out that much more research is needed to unravel the working mechanisms of SSRIs before any conclusions can be drawn on their influence on neurodevelopment. At the same time it is of great importance to learn more about the what, when, where and why of 5-HT related developmental processes, especially in the late developing brain. In the future, hopefully with our research as a starting point, this information could be ultimately used to generate more effective antidepressant treatments specifically designed for children and adolescents, as is previously suggested by Andersen and Navalta (2011). They stated that: “a greater understanding of neurodevelopment can
lead to improved treatment that intervenes early in the progression of a given disorder and prevents symptoms from manifesting [...] given the remarkable plasticity of the immature brain”.

In addition, our results suggest an important role for the feedback mechanisms that control 5-HT homeostasis. Although the significance of the 5-HT receptors is generally acknowledged, their exact function is not yet fully understood and, especially in regard to the age-related effects of SSRIs, not extensively studied. Moreover, the focus of future research should not be solely on 5-HT, considering the extensive interplay with other neurotransmitter systems such as the glutamate and GABA systems, as is pointed out in chapter 6. Also our findings of longer lasting effects of SSRI treatment in adult subjects, such as shown in chapter 5, and of possible effects of 5-HT on the default mode network, as was presented in chapter 10, will hopefully encourage new lines of research.

In the coming years, methodological improvements will also offer new opportunities. For one thing, the constant development of MRI sequences with a better signal-to-noise ratio will increase their capability to detect smaller effects, which will for instance improve the detection of the effects of an oral 5-HT challenge. With regard to neural basis of the BOLD response to 5-HT alterations, it would be of interest to combine MR imaging and neurochemical measurements of 5-HT release. In this light, much can be expected from advances in magnetic resonance spectroscopy or of the development of devices such as the Wireless Instantaneous Neurotransmitter Concentration Sensing System (WINCS), which enables real-time analysis of extracellular neurotransmitter concentrations simultaneous with MR imaging (Kimble et al., 2009).

Conclusions

Even though the precise working mechanisms of psychotropic drugs such as antidepressants are still not identified, we continue to increasingly prescribe them to children and youngsters whose brains are not yet fully developed. Yet, there is reason to believe that changing neurotransmitter function in an immature brain can potentially influence ongoing neurodevelopmental processes, leading to altered neurotransmission in later life. In this thesis, the occurrence of this so-called ‘chemical imprinting’ was first demonstrated with the age-at-exposure dependent effects of the potent 5-HT releaser and neurotoxic agent MDMA on 5-HT transporter density in both rats and humans. The main focus of this dissertation was however to gain more insight in the effects of SSRIs, and of FLX (Prozac®) in particular, on serotonergic signaling. Together, the animal studies described in this dissertation support the notion that the effects of chronic FLX exposure on 5-HT transmission depend
on the age of exposure. We observed an increased responsiveness of the 5-HT system in adolescent-treated animals while no or opposite effects were seen in adult-treated animals. This enhancement of 5-HT function may be due to imprinting effects on 5-HT feedback control. Future studies should confirm the non-transient nature of these findings in order to ascertain that these age-dependent effects actually represent neurodevelopmental changes in 5-HT transmission and related neurotransmitter systems. The role of the 5-HT (auto) receptors herein should be further elucidated as well. In general, a greater understanding of 5-HT neurodevelopment and the exact mechanisms of action of SSRIs is therefore clearly needed. Furthermore, our findings in the healthy rat brain ultimately require translation to the depressed human brain. In this respect, pharmacological MRI demonstrated to be a potentially useful research tool, as it enables the assessment of in vivo 5-HT function in both animals and humans in a relatively non-invasive way. With this technique, we were able to demonstrate the age-dependency of the chronic effects of FLX treatment on brain function in rats. Upcoming technical developments will most likely further increase the sensitivity of this technique to pick up 5-HT related differences in neurotransmission, so that the existence of ‘human FLX imprinting’ can be investigated even better in future studies.