The role of the serotonin 5-HT3 receptor in cortical development

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Chapter 6

General Discussion
Summary

During the development of the brain a large number of regulatory signals are responsible for the migration of neurons and the subsequent formation of neuronal connections. One of these signals that has a modulatory function during neurodevelopment is serotonin. Throughout life, but mainly during development, environmental factors influence brain structure and function. It has been shown that when environmental factors affect serotonin levels during development, several changes in the brain and in particular in the cortex can be observed. The ontogeny of the cortex starts early in development and around birth, the six-layered structure of the cortex can be distinguished. It has been reported that in the postnatal brain reelin controls dendritic maturation of cortical pyramidal neurons upon serotonergic activation of the serotonin 5-HT$_3$ receptor on Cajal-Retzius cells. However, how environmental factors affect this regulatory pathway involved in cortical development and what the consequences are is to date unknown. In this dissertation, I have investigated the consequences of alterations in serotonergic signaling during neurodevelopment. I specifically focused on the effect of alterations in a serotonin 5-HT$_3$ receptor-mediated regulatory pathway involved in postnatal dendritic maturation of cortical pyramidal neurons.

In the first two chapters I focused on the question: How do environmental influences interfere with the ongoing development of the cortex? In chapter 2, we used the maternal care model to investigate the effect of early-life experience on cortical development. In this study, we found that differential maternal care during the first week of life had a lifelong influence on dendritic maturation and spine density of cortical layer 2/3 pyramidal neurons. By recording sEPSC’s we showed that as a consequence of differential maternal care, an increase or decrease in dendritic complexity resulted in a corresponding increase or decrease in amplitude of the recorded events. The observation that differential maternal care also influenced cortical reelin levels, indicated that differential maternal care affected cortical development via a reelin-dependent regulatory pathway.

In chapter 3, we investigated the effect of an increase in serotonin levels due to prenatal exposure of the SSRI fluoxetine on cortical development. In this study, we found that alterations in serotonin levels during neurodevelopment had a lifelong effect on cortical development and that prenatal fluoxetine exposure resulted in a hypocomplex dendritic tree of cortical layer 2/3 pyramidal neurons.
More importantly, we showed that the effect of prenatal fluoxetine exposure on dendritic complexity and anxiety-related behavior was absent in 5-HT\textsubscript{3A} receptor knockout mice. In addition, we showed that in organotypic brain slices of mice prenatally exposed to fluoxetine, the decrease in dendritic complexity could be reversed by treating these slices with a 5-HT\textsubscript{3} receptor antagonist. Together, these results indicate that the 5-HT\textsubscript{3} receptor plays a critical role in modulating the effects of alterations in serotonin levels on cortical development and anxiety-related behavior.

After showing that environmental influences induce lifelong changes in cortical development via a reelin-dependent pathway and that the 5-HT\textsubscript{3} receptor plays an essential role in modulating these effects, I focused on the question: Are these changes in cortical development also accompanied by alterations in the columnar organization of the cortex? In chapter 4, we investigated the organization of dendritic bundles in the somatosensory cortex of 5-HT\textsubscript{3A} receptor knockout mice. By analyzing the spatial organization of MAP-2 immunostained apical dendrites in tangential sections from layer 3 of the somatosensory cortex, we compared dendritic bundle properties of 5-HT\textsubscript{3A} receptor knockout mice with wildtype mice. In addition, we analyzed in both groups the distribution of reelin-rich Cajal-Retzius cells in tangential sections from the somatosensory cortex, to determine whether the distribution of these Cajal-Retzius cells was related to the position of dendritic bundles. In this study, we showed that in the somatosensory cortex of both groups apical dendrites are organized in dendritic bundles, but that the average surface of the dendritic bundles in 5-H\textsubscript{3A} receptor knockout mice was larger than in wildtype mice. Furthermore, we showed that the distribution of reelin-rich Cajal-Retzius cells was random while the distribution of dendritic bundles was regular. Together with the previously observed differences in dendritic complexity of cortical layer 2/3 pyramidal neurons and cortical reelin levels, these findings suggest another important role for the 5-HT\textsubscript{3} receptor in cortical development.

Based on previous findings which showed that depleting the serotonergic innervation to the cortex in the brains of neonatal mice resulted in changes in cortical organization, but also in changes in social behavior, in chapter 5, I addressed the question: Do 5-HT\textsubscript{3A} receptor knockout mice show impaired social behavior? In this study, we showed that although both male and female 5-HT\textsubscript{3A} receptor knockout mice showed deficits in social communication, other deficits in social behavior were sex-specific. Together, these results suggest that, similar to other models in which serotonergic signaling is changed during neurodevelopment, 5-HT\textsubscript{3A} receptor knockout mice show impaired social behavior.
In summary, by showing that alterations in serotonergic signaling during neurodevelopment result in lifelong changes in dendritic maturation and dendritic bundle organization in the cortex together with behavioral deficits via a 5-HT$_3$ receptor-dependent pathway, our results indicate that the serotonin 5-HT$_3$ receptor plays an essential role in modulating cortical development.
Chapter 6

General discussion

The effect of changes in serotonergic signaling on brain development

Several factors have been shown to influence serotonin levels during development including nutrition, infection, stress, genetic polymorphisms and exposure to drugs (Daubert and Condron 2010). Recently, the adverse effects of SSRI’s on the development of the brain have been subject to several studies (Borue et al. 2007; Homberg et al. 2009; Thompson et al. 2009). Initially, it was shown that early postnatal SSRI exposure resulted in increased anxiety reminiscent to the behavioral phenotype of the 5-HTT knockout mouse (Holmes et al. 2003; Lira et al. 2003; Ansorge et al. 2004). Further studies showed that both pre- and postnatal SSRI exposure have an effect on anxiety-related behavior (Lisboa et al. 2007; Noorlander et al. 2008). In chapter 3, we also showed that prenatal fluoxetine exposure resulted in increased anxiety, but in addition we showed that in mice lacking the 5-HT3A receptor these changes in anxiety-related behavior were absent, suggesting a novel role for the 5-HT3 receptor in modulating fluoxetine-induced anxiety. In this study, our main objective was to investigate the effect of increased serotonin levels on cortical development. The fact that an increase in serotonin levels resulted in alterations in dendritic maturation and reelin levels in the cortex opposite to what we previously observed in the 5-HT3A receptor knockout mouse, suggests that an increase or decrease in serotonin levels during development can have differential effects on postnatal dendritic maturation in the cortex. Based on these results, we propose that upon activation of the 5-HT3 receptor on Cajal-Retzius cells, an increase in serotonin levels causes an increase in reelin levels, which subsequently acts as a stop signal for ramifying apical dendrites, whereas a decrease in serotonin levels causes a reduction in reelin levels resulting in an increase in dendritic complexity (Figure 1).
Figure 1. The consequences of alterations in serotonin levels on dendritic maturation of cortical layer 2/3 pyramidal neurons. Upon activation of the 5-HT₃ receptor on Cajal-Retzius cells, an increase in serotonin levels causes an increase in reelin levels (left side) which in turn acts as a stop signal for ramifying apical dendrites, whereas a decrease in serotonin levels causes a reduction in reelin levels (right side) and thus an increase in dendritic complexity.

Using different pharmacological and genetic tools, previous studies have shown that both an increase and decrease in serotonin levels during embryonic or early postnatal development leads to cortical abnormalities (Table 1). However, in contrast to our model, the results summarized in Table 1 show similar effects as a result of an increase or decrease in serotonin levels during neurodevelopment. Yet, one needs to take into consideration that when using some of these pharmacological or genetic mouse models to study the developmental role of serotonin, robust changes in the brain cause these alterations in serotonin levels and in some cases also other neurotransmitter levels leading to other developmental changes (Fon et al. 1997; Alvarez et al. 2002; Alenina et al. 2009).
Table 1. Effects of alterations in serotonin levels during neurodevelopment on cortical morphology

<table>
<thead>
<tr>
<th>Animal model</th>
<th>5-HT levels</th>
<th>Cortical phenotype</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAOA KO</td>
<td>↑</td>
<td>absent barrels</td>
<td>Cases et al. 1996, Vitalis et al. 1998</td>
</tr>
<tr>
<td>5-HTT KO</td>
<td>↑</td>
<td>barrel malformation decreased layer 4 thickness</td>
<td>Esaki et al. 2005, Persico et al. 2001</td>
</tr>
<tr>
<td>SSRI treatment</td>
<td>↑</td>
<td>impaired layer 4 innervation barrel malformation</td>
<td>Lee et al. 2009</td>
</tr>
<tr>
<td>P0-P6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VMAT2 KO</td>
<td>↓</td>
<td>reduced growth cortical layers barrel malformation</td>
<td>Alvarez et al. 2002</td>
</tr>
<tr>
<td>PCPA treatment</td>
<td>↓</td>
<td>decrease dendritic thickness and arborization pyramidal neurons</td>
<td>Vitalis et al. 2007</td>
</tr>
<tr>
<td>E12-E17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCPA treatment</td>
<td>↓</td>
<td>barrel malformation</td>
<td>Persico et al. 2000</td>
</tr>
<tr>
<td>P0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,7-DHT lesion P0</td>
<td>↓</td>
<td>increased cortical layer width</td>
<td>Hohmann et al. 2000, Boylan et al. 2007</td>
</tr>
</tbody>
</table>

Nevertheless, one aspect these studies have in common is that independent of the pharmacological or genetic tool used to modify the serotonergic system during brain development, all reported effects are lifelong and restricted to specific developmental time windows. For instance, the phenotype of MAOA knockout mice or of mice treated with a MAO inhibitor only appears when serotonin levels are changed during early postnatal life and can only be rescued during this developmental time window (Cases et al. 1996; Vitalis et al. 1998). Also, the increased levels of anxiety are only observed in mice in utero or postnatally exposed to SSRI’s and not in mice treated with SSRI’s from the second week of life onwards (Oh et al. 2009). Similarly, increased levels of anxiety in 5-HT1A knockout mice can only be reversed during the first three postnatal weeks (Gross et al. 2002). In line with these observations, we have found that the effects of alterations in serotonergic signaling on 5-HT3 receptor-mediated cortical development are lifelong and sensitive to alterations in serotonin levels only within a critical period (chapter 3 and unpublished observations). Taken together, the effects of alterations in serotonin levels on neurodevelopment heavily depend on the developmental time window in which changes occur, most likely because of a transient presence of local molecular factors that are critical for the development and maturation of specific structures in the brain.
General Discussion

Taken this into account, we suggest that to increase knowledge on the regulatory role of the serotonin during neurodevelopment, genetic approaches which specifically target a regulatory pathway during a specific developmental time window should be used.

Evidence for the link between disturbances in serotonin levels and cortical column abnormalities in psychopathological disorders

Several animal studies have shown that a decrease in serotonin levels during neurodevelopment results in cortical abnormalities and behavioral deficits reminiscent to autism, and a link between disturbances in serotonin levels and cortical malformations in autism has been suggested (Hohmann et al. 2000; Boylan et al. 2007; Hohmann et al. 2007; McNamara et al. 2008). Similar to these studies, we found in chapter 5 several deficits in social behavior in mice lacking the 5-HT$_{3A}$ receptor, in addition to changes in cortical development. Moreover in chapter 4, we report changes in dendritic bundle size in mice lacking the 5-HT$_{3A}$ receptor. In line with our observation in chapter 4, it was shown that in the presubiclar cortex of neonatal mice of which the serotonergic innervation to Cajal-Retzius cells was depleted, the columnar organization of the cortex was disrupted and reelin levels were changed (Janusonis et al. 2004). Changes in the vertical organization of neurons in the cortex have also been described in autism and schizophrenia, yet instead of changes in dendritic bundling, changes in the size and spacing between vertically aligned rows of cells, also referred to as minicolumns, have been reported (Casanova et al. 2002; Chance et al. 2008). Moreover, in autistic patients changes in reelin levels have been reported similar to what has been shown in the 5-HT$_{3A}$ receptor knockout mouse (Fatemi 2005). Together, these results suggest that in addition to changes in serotonin levels, changes in reelin levels can be linked with cortical abnormalities as observed in autism. In conclusion, by showing that alterations in serotonergic signaling during neurodevelopment result in lifelong changes in dendritic maturation and dendritic bundle organization in the cortex together with behavioral deficits via a reelin-dependent pathway, our results provide more knowledge about how alterations in serotonin and reelin levels lead to cortical abnormalities as observed in psychopathological disorders such as autism.
Chapter 6

The effect of changes in cortical morphology on cortical functioning

To understand the relation between cortical abnormalities and behavioral deficits as observed in disorders in which alterations in serotonergic signaling have lead to structural changes in the cortex, the functional consequences of changes in cortical structure need to be studied. Therefore, the first question that needs to be answered is: What are the functional consequences of alterations in dendritic complexity of apical dendrites of cortical layer 2/3 pyramidal neurons? In chapter 2, we have shown that differences in dendritic complexity of apical dendrites of cortical layer 2/3 pyramidal neurons affect the amplitude of sEPSC’s. However, what kind of inputs do these apical dendrites of cortical layer 2/3 pyramidal neurons receive? It has been shown that in the cortex, layer 2/3 pyramidal neurons receive excitatory input from layer 4, other layer 2/3 pyramidal neurons and to a lesser extent from layer 1 (Lübke and Feldmeyer 2007; Thomson and Lamy 2007). The majority of the synaptic connections between layer 4 to 2/3 and layer 2/3 have been observed on the basal dendrites although a small fraction has been observed on the proximal part of apical dendrites (Lübke and Feldmeyer 2007; Thomson and Lamy 2007). However, the distal parts of layer 2/3 apical dendrites do receive input from layer 1 originating from higher-order cortical regions (Cauller and Connors 1994). Apart from excitatory input, layer 2/3 pyramidal neurons receive inhibitory input from several types of interneurons which are present in all layers in the cortex and target several parts of the pyramidal neuron including axon initial segments, somata, proximal and distal dendritic shafts and spines, and dendritic tufts (Somogyi et al 1998; Markram et al. 2004). Interneurons are known to control excitation and by making connections with apical dendrites they influence integration of synaptic inputs. Furthermore, several other factors determine how incoming synaptic information becomes integrated resulting into a specific output pattern. Apart from dendritic morphology and the spatio-temporal patterning of excitatory and inhibitory synaptic inputs also active voltage-gated ion channels present in the dendritic membrane determine the output of a neuron (Magee 2001; Migliore and Shepherd 2002; Häusser and Mel 2003; Gulledge et al. 2005). Therefore, it is difficult to predict how alterations in dendritic morphology affect action potential output of a neuron.
Nevertheless, we still can speculate that an increase in complexity of apical dendrites of cortical layer 2/3 pyramidal neurons as observed in the 5-HT\textsubscript{3A} receptor knockout mouse, results in an increase in the number of synaptic contacts on various locations of the apical dendrite, resulting in an increased frequency of both excitatory and inhibitory synaptic input which together affect depolarization of the cell and thus action potential output.

In a local cortical circuit, layer 2/3 pyramidal neurons integrate and subsequently distribute excitatory signals arriving from layer 4 to layer 2/3 and 5. In addition, it has been suggested that horizontal layer 2/3 pyramidal neurons are functionally interconnected and involved in synchronizing activity of neuronal ensembles with similar functional properties extending to several cortical columns (Holmgren et al. 2003; Lübke and Feldmeyer 2007). Therefore, changes in dendritic complexity as observed in the 5-HT\textsubscript{3A} receptor knockout mouse would primarily affect these processes. However, another important morphological alteration that was observed in the 5-HT\textsubscript{3A} receptor knockout mouse was that the organization of dendritic bundles in the somatosensory cortex was different compared to wildtype mice (chapter 4). In the mouse somatosensory cortex, dendritic bundles consist of ascending apical dendrites of layer 5 pyramidal neurons and adjoining layer 2/3 pyramidal neurons (Peters and Walsh 1972; Fleischhauer et al 1972; Rockland and Ichinohe 2004). It has been proposed that pyramidal neurons within a dendritic bundle are functionally interconnected and form the basis of a cortical microcircuit (Peters and Sethares 1996). However, recently it was shown that synaptic connections between layer 5 pyramidal neurons are independent of apical dendrite bundling, yet intrinsic connections between layer 2/3 and layer 5 pyramidal neurons have never been studied (Krieger et al. 2007). As mentioned before, the cortex is organized in columns of vertically interconnected neurons showing long-range transcolumnar horizontal connections (Mountcastle 1957; Lübke and Feldmeyer 2007). Via repetitive patterns of local cortical circuits which extend through the several layers of the cortex, information is processed through and subsequently projected to other cortical areas or back to subcortical areas (Thomson and Bannister 2003; Douglas and Martin 2004). It has been speculated that in each cortical area and in each species, small cortical microcircuits show a stereotypic organization and show specific connections between neurons within and across cortical layers (Kozloski et al. 2001; Silberberg et al. 2002; Song et al. 2005). However, which connections together form a cortical microcircuit still needs to be elucidated (Thomson and Lamy 2007).
One could speculate that changes in layer 2/3 pyramidal neuron functioning and changes in dendritic bundle organization as observed in the 5-HT\textsubscript{3A} receptor knockout mouse (chapter 4) affect the way information is being processed in a cortical microcircuit leading to a different output. However, to understand the consequences of alterations in dendritic morphology of cortical layer 2/3 pyramidal neurons and dendritic bundle organization on the network level, one should perform additional studies and elucidate the several complex interactions that exist and determine the output of a cortical microcircuit. Ultimately, more knowledge on the functioning of cortical networks and the effects of morphological changes on information processing in the brain should lead to a better understanding about the contribution of cortical abnormalities in developing complex behavioral phenotypes.

The effect of gene-environment interactions on brain development

In various ways, environmental influences including exposure to drugs and environmental stressors, can interfere with the ongoing development of the brain and lead to an increased vulnerability to psychopathological conditions (Dioro and Meaney 2007; Thompson et al. 2009; Tau and Peterson 2010). To date, a number of genetic variations have been identified that have been associated with an increased risk in developing psychopathology upon adverse early life events including childhood neglect or maltreatment (Caspi and Moffitt 2006). Which mechanisms underlie these gene-environment interactions has been subject to many studies. A typical example of how early life experiences can influence brain development via gene-environment interactions is the maternal care model (Fagiolini et al. 2009; Dioro and Meaney 2007; Szyf et al. 2007). In this model, it has been shown that differential maternal care influences DNA methylation of genes sensitive to epigenetic modulation (Weaver et al. 2004). The observation that reelin, which has been shown to regulate postnatal dendritic maturation, is also sensitive to epigenetic modulation, made us to select this model to study the effect of environmental influences on cortical development (Niu et al. 2004; Weaver et al. 2006; Levenson et al. 2008; Chameau et al. 2009). Although in chapter 2, we did not investigate DNA methylation of the reelin promoter, the observation that differential maternal care affected not only dendritic maturation in the cortex, but also cortical reelin levels, supported the idea that as a result of gene-environment interactions between maternal care and reelin, cortical development was changed.
In a number of neurological disorders including schizophrenia, autism, lisenchephaly and Alzheimer’s, changes in DNA methylation in the promoter region of reelin have been reported, suggesting that gene-environment interactions which affect reelin expression contribute to the development of neuropathology (Fatemi 2005). However, it has to be noted that a large number of complex interactions proceed, until the first signs of these neurological disorders appear, yet many of these interactions are still not understood.

The consequences of disturbances in serotonergic signaling in humans

As mentioned before, evidence is increasing that several external influences such as exposure to drugs and environmental stressors play a contributory role in the etiology of a number of psychopathological disorders (Dioro and Meaney 2007; Thompson et al. 2009; Tau and Peterson 2010). In addition to gene-environment interactions, it has been suggested that adverse influences cause disturbances in serotonergic signaling during neurodevelopment leading to psychopathology. For instance, it has been shown that humans carrying a variation in a gene encoding for the 5-HTT, MAOA and the 5-HT\textsubscript{1A} receptor show an increased risk in developing psychopathology upon adverse early life events including childhood neglect or maltreatment (Lesch et al. 1996; Caspi et al. 2003; Strobel et al, 2003; Caspi and Moffitt 2006; Leonardo and Hen 2008). In addition, it has been observed that prenatal or early life exposure to drugs that affect serotonergic signaling lead to neurological abnormalities and given the results in animal studies could lead to an increase in anxiety-related behavior later in life (Homberg et al. 2009; Thompson et al. 2009). However, so far no reports exist about how these alterations in serotonergic signaling lead to psychopathology. In autism, alterations in serotonin levels during neurodevelopment are accompanied by several anatomical abnormalities including increased brain volumes, changes in cortical thickness and cortical minicolumns, alterations in anterior cingulate cortex lamination and increased cerebral lobe volumes which together have been linked to the behavioral phenotype of autism (Kemper and Bauman 1993; Piven et al. 1995; Bailey et al. 1998; Courchesne et al. 2001; Carper et al 2002; Casanova et al. 2002; Carper et al. 2005; Courchesne et al. 2005; Hazlett et al. 2005). However, why serotonin levels are altered in autism and what the link is between the observed structural abnormalities and the typical behavioral deficits is to date unknown.
Together with the results from other studies investigating the effects of alterations in serotonergic signaling, the findings in this dissertation suggest that environmentally-induced alterations in serotonin levels cause changes in cortical development leading to an increased risk in developing psychopathology. However, what remains to be answered is: During which developmental time window in humans do these events take place? In humans, cortical development occurs in a similar way as in rodents, except that the time window in which the cortex develops in humans is different.

In humans, the typical six-layered structure of the cortex can be discerned around gestation week 32 (Kostovic et al. 1995). Around this period, serotonergic fibers have entered the cortex which contains a large number of pyramidal neurons and GABA-ergic and interneurons (Tau and Peterson 2010). From the third trimester onwards, synaptogenesis takes place and dendritic arborization accelerates causing a thickening of the cortex (Huttenlocher and Dabholkar 1997). Although around birth, different cortical areas including the motor, somatosensory, visual and auditory cortex can be identified, the development of cortical neurons is still ongoing (Shankle et al. 1999). In fact, in the first year of life, the cortex shows an increase in volume of 88% and of 15% the second year of life, mainly due to dendritic arborization of pyramidal neurons and GABA-ergic interneurons (Knickmeyer 2008; Tau and Peterson 2010). Taken together, based on the increasing evidence from previous studies in humans and rodents showing the influence of external factors on cortical development due to alterations in serotonergic signaling, we suggest that caution should be taken in exposing the developing fetus or child to environmental factors known to affect serotonin levels until the second year of life instead of the first postnatal weeks in rodents.
Concluding remarks and future directions

In this dissertation I have investigated how environmental factors influence the ongoing development of the brain. In particular, I have shown that changes in serotonergic signaling during neurodevelopment do not only lead to changes in the morphology of cortical layer 2/3 pyramidal neurons and organization of dendritic bundles, but also result in behavioral deficits later in life and that the serotonin 5-HT$_3$ receptor plays an essential role in mediating these effects.

Many similarities between the changes we report in mice lacking the 5-HT$_{3A}$ receptor or in mice in which serotonin levels were changed during development and psychopathological disorders such as autism or anxiety disorders can be observed. Yet, to understand how these changes in the brain such as the ones observed in the cortex lead to behavioral changes later in life, one should obtain a better understanding about the functional consequences of alterations in serotonin levels. It is very likely that changes in dendritic complexity and dendritic bundle organization affect information processing in cortical microcircuits. Studying these functional circuits should lead to more knowledge on which structural changes underlie which behavioral deficits.

Nevertheless, apart from investigating the effects of structural changes in the cortex on cortical functioning, it is important to also investigate how these structural changes can be prevented or rescued. In this dissertation we showed that in vitro the effects of increased serotonin levels on dendritic maturation could be rescued by applying a 5-HT$_{3A}$ receptor antagonist. In addition, we developed a siRNA against the 5-HT$_{3A}$ receptor, although it still needs to be tested in vivo. We think that these kind of strategies can be promising for treating the consequences of alterations in serotonergic signaling during development. Nevertheless, these therapeutic agents would only be effective when subscribed during a specific moment in development, because later in development these changes are irreversible. In addition, it is important to bear in mind that in humans developmental processes in the cortex occur during a different time window. Therefore, a perhaps even better way to challenge these changes in cortical development due to alterations in serotonergic signaling is to protect the developing fetus or child from environmental factors known to have an adverse effect on serotonergic signaling.
In conclusion, the results from this dissertation have shed more light on how environmentally-induced alterations in serotonergic signaling affect 5-HT\textsubscript{3} receptor-mediated cortical development and provide more knowledge on the link between changes in cortical development due to alterations in serotonin levels and psychopathology.