Chapter 1

General Introduction
Introduction

The development of the brain is a highly complex and dynamic process. The initial development of the nervous system is orchestrated via molecular signals that instruct the neural plate to form the neural tube and to subdivide into several areas (Super et al. 1998; Rubenstein and Rakic 1999; Copp et al. 2003). Subsequently, a large number of regulatory signals are responsible for the generation and differentiation of neurons and glia cells, followed by the migration of these cells to their final position and, later in development, the formation of axonal pathways and connections (Rakic 1988; O’Leary et al. 1994; Rubenstein and Rakic 1999; Bystron et al. 2008; Tau and Peterson 2010). Postnatally, environmental experience becomes increasingly important for establishing neuronal connections (Katz and Shatz 1996; Hensch 2005; Tau and Peterson 2010). Although the mature brain is less sensitive to environmental influences it will remain susceptible to adaptations throughout life (Nithianantharajah and Hannan 2006; Holtmaat and Svoboda 2009).

During these stages of development, the brain is highly sensitive to adverse influences such as environmental stressors or exposure to drugs which interfere with the ongoing development of the brain resulting in psychopathological conditions (Gaspar et al. 2003; Caspi et al. 2006; Brunton and Russell 2008; Frederick and Stanwood 2009; Leonardo and Hen 2008; Thompson et al 2009). In the brain, serotonin is one of the first synthesized neurotransmitters and in a number of developmental events serotonin has been shown to play a regulatory role (Lauder 1993; Levitt et al. 1997; Gaspar et al. 2003). However, during neurodevelopment, external influences can cause alterations in serotonergic signaling leading to structural abnormalities in the brain together with behavioral changes reminiscent to psychopathology (Gaspar et al. 2003; Hornung 2003; Whitaker-Azmitia 2005; Daubert and Condron 2010).

Although disturbances in serotonergic signaling have been associated with a number of psychopathological disorders, the underlying mechanisms are still largely unknown and heavily depend on the developmental window in which serotonin levels are changed (Gaspar et al. 2003; Daubert and Condron 2010). In this dissertation I will focus on the regulatory role of serotonin during cortical development and investigate how external influences affect postnatal cortical development and what the consequences of these changes are.
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The serotonergic system

The monoamine serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that is present in almost every organism. In the mammalian brain, the cell bodies that synthesize 5-HT are located in the brain stem and are called the raphe nuclei (Jacobs and Azmitia 1992). The serotonergic raphe nuclei comprise a number of cell clusters (B1-B9) which can be divided into a caudal group (B1-B5) projecting to the brainstem and spinal cord and a rostral group (B6-B9) projecting to the telencephalon and diencephalon in particular the limbic areas, cortex, basal ganglia and hypothalamus (Jacobs and Azmitia 1992; Gaspar et al. 2003) (Figure 1).

![Figure 1. Serotonergic projections from the raphe nuclei to several regions of the rodent brain. Adapted from Kandel et al. 2000](image)

The synthesis of 5-HT requires two enzymatic steps. First, the amino acid tryptophan is converted into 5-hydroxytryptophan (5-HP) by the enzyme tryptophan hydroxylase. Then, 5-HP is converted into 5-HT by the enzyme 5-HP decarboxylase. After being released from presynaptic axon terminals into the synaptic cleft, 5-HT can bind to several 5-HT receptor subtypes. It can also be reuptaken by the serotonin transporter (5-HTT) and repacked into secretory vesicles by the vesicle monoamine transporter (VMAT) or metabolized into 5-hydroxyindoleacetic acid (5-HIAA) by the enzyme monamine oxidase (MAO) (Borue et al. 2007) (Figure 2).
Figure 2. Serotonin synthesis and breakdown. In the left panel the amino acid tryptophan is converted into 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase. Then, 5-HTP is converted into 5-HT by the enzyme 5-HTP decarboxylase. Subsequently, 5-HT can be converted into 5-hydroxyindoleacetic acid (5-HIAA) by the enzyme monamine oxidase (MAO). In the right panel 5-HT is released into the synaptic cleft from vesicles located in presynaptic axon terminals. In the synaptic cleft, 5-HT can bind to several 5-HT receptor subtypes. It can also be re-uptaken by the serotonin transporter (5-HTT) and re-packed into secretory vesicles by the vesicle monoamine transporter (VMAT) or metabolized into 5-hydroxyindoleacetic acid (5-HIAA) by the enzyme MAO. Adapted from Borue et al. 2007.

To date, 15 different serotonin receptor subtypes have been discovered. The family of serotonin (5-HT) receptors consists of a large complement of G-protein coupled 7-transmembrane (7TM) receptor subtypes, except for the 5-HT₃ receptor which is a ligand-gated ion channel (Barnes and Sharp 1999) (See Box ).
Box  The 5-HT\textsubscript{3} receptor

The 5-HT\textsubscript{3} receptor is a ligand-gated ion channel belonging to the Cys-loop family of ligand-gated ion channels which include the nicotinic acetylcholine receptors, GABA\textsubscript{A} receptors and glycine receptors (Barnes and Sharp 1999; Chameau and van Hooft 2006). So far, two functional 5-HT\textsubscript{3} subunits have been cloned: the 5-HT\textsubscript{3A} subunit (Maricq et al. 1991) and the 5-HT\textsubscript{3B} subunit (Davies et al. 1999). In humans, also the 5-HT\textsubscript{3C}, 5-HT\textsubscript{3D} and 5-HT\textsubscript{3E} subunit have been cloned (Karnovsky et al. 2003; Niesler et al. 2003). However, in rodents, these subunits are absent. Functional receptors can be formed either as homo-oligomeric 5-HT\textsubscript{3A} or hetero-oligomeric 5-HT\textsubscript{3A} and 5-HT\textsubscript{3B} subunit complexes. Each subunit crosses the membrane four times, with one large extracellular N-terminal region. The 5-HT\textsubscript{3} receptor can be found in both the peripheral (PNS) and central nervous system (CNS). In the PNS, functional 5-HT\textsubscript{3} receptors have been observed in the myenteric plexus, submucous plexus, nodose ganglion, superior cervical ganglion, dorsal root ganglion and vagus nerve (Jackson and Yakel 1995). In the CNS, the 5-HT\textsubscript{3} receptor is located in several brain areas including the cortex, hippocampus, amygdala, ventral tegmental area, substantia nigra, nucleus accumbens, cerebellum and several nuclei of the brainstem such as the nucleus tractus solitarius, area postrema and dorsal motor nucleus of the vagus. In general, the overall distribution of the 5-HT\textsubscript{3} receptor is scattered (Barnes and Sharp 1999). Within the CNS, functional 5-HT\textsubscript{3} receptors are primarily present on GABAergic interneurons where they often co-localise with cholecystokinin (CCK) and the Ca\textsuperscript{2+} binding protein calbindin, but not somatostatin or parvalbumin. (Tecott et al.1993; Kawa 1994; McMahon and Kauer 1997; Morales and Bloom 1997; Roerig et al. 1997; Zhou and Hablitz 1999; Sudweeks et al. 2002; Ferezou et al. 2002; Inta et al. 2008). Functional 5-HT\textsubscript{3} receptors can also be found on presynaptic GABAergic nerve terminals in the amygdala, striatum, cerebellum and hippocampus (Nichols and Mollard 1996; Ronde and Nichols 1998; Nayak et al.1999; Koyama et al. 2000; Katsurabayashi et al. 2003; Turner et al. 2004). Apart from being expressed on interneurons, the 5-HT\textsubscript{3} receptor is also located on Cajal-Retzius cells (Chameau et al. 2009). It has been suggested that presynaptic 5-HT\textsubscript{3} receptors modulate neurotransmitter release whereas postsynaptic 5-HT\textsubscript{3} receptors can control the excitability of neuronal networks by mediating fast serotonergic transmission. The differences in physiological properties of pre- and postsynaptic 5-HT\textsubscript{3} receptors support this idea. Presynaptic 5-HT\textsubscript{3} receptors are able to induce an elevation of intracellular Ca\textsuperscript{2+} either via a Ca\textsuperscript{2+} influx or via activation of voltage-gated Ca\textsuperscript{2+} channels (Ronde and Nichols 1998). In contrast, postsynaptic 5-HT\textsubscript{3} receptor channels are blocked by calcium at negative membrane potentials, similar to the voltage-dependent block by Mg\textsuperscript{2+} of NMDA receptors (Kawa 1994; McMahon and Kauer 1997; van Hooft and Wadman, 2003; Noam et al. 2008). So far, the 5-HT\textsubscript{3} receptor has been implicated to play a role in a variety of functions including regulation of gut motility and peristalsis, urinary tract functioning, control of nausea induced emesis, regulation of nociceptive processing and although less clear in cognition and anxiety related behavior (Barnes and Sharp 1999). The clinical use of 5-HT\textsubscript{3} receptor antagonists has been particularly successful in controlling nausea induced emesis associated with cancer chemotherapy and irritable bowel syndrome treatment (Costall and Naylor 2004). Upon the discovery that drugs prescribed to control emesis acted as 5-HT\textsubscript{3} receptor antagonists a number highly selective ligands such as the antagonists MDL 7222, tropistetron (ICS 250930), ondansetron, granisetron, and the agonist 2-methyl-5HT have been developed (Barnes and Sharp 1999; Chameau and van Hooft 2006). To study 5-HT\textsubscript{3} receptor function several tools are available. In addition to the above described ligands, a 5-HT\textsubscript{3A} receptor knockout mouse was generated (Zeitz et al. 2002) and using RNA interference technology we developed another tool to study 5-HT\textsubscript{3} receptor function (see appendix).
The physiological function of serotonin

The neurotransmitter 5-HT is involved in the regulation of motor output, food intake, pain, thermoregulation, sleep and in controlling several behavioral states including aggression, learning, attention and sexual behavior (Jacobs and Fornal 1997; Azmitia 1999). The serotonergic system has also been implicated to play a role in mood regulation. In humans, variations in genes encoding for the 5-HTT, MAOA and the 5-HT_{1A} receptor have been linked with an increased risk in developing anxiety and depression (Lesch et al. 1996; Caspi et al. 2003; Strobel et al. 2003; Leonardo and Hen 2008). It has been suggested that humans carrying one of these genetic variations are more vulnerable to early life gene-environment interactions which lead to structural changes in brain areas implicated with mood regulation (Gross and Hen 2004; Leonardo and Hen 2008).

The effects of disturbances in serotonergic signaling during neurodevelopment

In the developing brain, serotonin has been implicated to play a regulatory role in a number of events including cell division, neuronal migration, cell differentiation and synaptogenesis (Lauder 1993; Levitt et al. 1997; Gaspar et al. 2003; Hornung et al. 2003; Vitalis and Parnavelas 2003). To obtain a better understanding about the consequences of disturbances in serotonergic signaling during neurodevelopment, both pharmacological and genetic tools have been used. Several drugs that target the serotonergic system including selective serotonin reuptake inhibitors (SSRI’s), MAO inhibitors and tricyclic antidepressants have been developed and are commonly perscribed to treat patients who suffer from depression and anxiety-related disorders (Nemeroff and Owens 2002). A major advantage of SSRI’s in studying the effects of disturbances in serotonergic signaling in the developing brain is that SSRI’s such as paroxetine, fluoxetine and fluvoxamine pass the placenta and enter the bloodstream of the fetus were they inhibit the reuptake of serotonin by binding to the 5-HTT causing an increase in serotonin levels (Borue et al. 2007; Homberg et al. 2009). In humans, it was shown that prenatal exposure to SSRI’s leads to an increased risk of neurological abnormalities (Zeskind et al. 2004; Borue et al. 2007; Oberlander et al. 2008; Homberg et al. 2009; Oberlander et al. 2010). In mice, prenatal or early-life exposure to the SSRI fluoxetine has been reported to result in molecular and structural changes in the brain and a higher vulnerability to depressive and anxiety-related behavior later in life (Anzorge et al. 2004; Noorlander et al. 2008; Karpova et al. 2009).
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Using genetic mouse models including the MAOA, 5-HTT, VMAT2 and TPH2 knockout mouse it has been shown that alterations in serotonin homeostasis during development result in several abnormalities including anatomical alterations in the brain and/or behavioral changes in adult life reminiscent to psychopathological disorders (Cases et al. 1995; Fon et al. 1997; Alvarez et al. 2002; Lira et al. 2003; Alenina et al. 2009).

Interestingly, several researchers have linked alterations in serotonergic signaling in the developing brain with autism, a severe psychological disorder characterized by deficits in communication and language, repetitive behavior and impaired social behavior as described in DSM IV (Palmen et al. 2004). In addition to these core features, autistic patients often also have co-morbid disorders including intellectual impairment, seizures and anxiety (Amaral et al. 2008). The heterogeneous phenotype of autism makes it difficult to identify the neurological origin of the disease, although twin studies have shown that it is a highly inherited disorder (Walsh et al. 2008). Over the years, neuroanatomical differences in brains of autistic patients have been reported including an increase in cortical volume and an altered width of minicolumns (Bailey et al. 1998; Casanova et al. 2002; Carper et al. 2005). Another interesting observation is that autistic patients show decreased serotonin synthesis in the brain, whereas in healthy subjects serotonin synthesis is 200% compared to adult levels during the first five years (Chugani et al. 1997; Chugani et al. 1999). In addition, it has been reported that 30% of the autistic children show high blood and platelet levels of serotonin (Anderson et al. 1987; Chugani, 2002; Lam et al. 2006). According to the hyperserotonemia hypothesis, these high levels of serotonin enter the developing brain via the blood-brain barrier, which at an early stage of development has not yet fully been formed, and cause a loss of serotonin terminals in the brain through a negative feedback function of serotonin (Whitaker-Azmitia, 2005). Researchers studying the effects of 5-HT depletion in the brains of neonatal mice, reported changes in the width of cortical layers together with several deficits in social behavior reminiscent to autism (Boylan et al. 2007; Hohmann et al. 2007). Similar changes in cortical organization together with changes in reelin levels, which has also been implicated to play a role in autism, were observed in mice of which the serotonergic innervation to Cajal-Retzius cells was disrupted at birth (Janusonis et al. 2004; Fatemi 2005). Taken together, the results from these pharmacological and genetic studies suggest that alterations in serotonergic signaling during neurodevelopment result in several structural abnormalities in the brain and in particular in the cortex together with behavioral deficits later in life.
However, to elucidate how disturbances in serotonergic signaling in the developing brain lead to these behavioral changes later in life, it is important to obtain a better understanding about the functional organization of the affected brain areas such as the cortex.

The organization of the cortex

The cerebral cortex is a complex and highly organized part of the brain. It is involved in cognitive function, sensory processing and movement control. In humans, the cerebral cortex can be subdivided into four major lobes: the frontal, parietal, temporal and occipital lobe. The largest part of the cerebral cortex is part of the neocortex, which is from an evolutionary perspective the most novel part (Rakic 2009). Both the neocortex of humans and rodents has a six-layered structure consisting of neurons and glia cells with different inputs and outputs (Mountcastle 1995; Rakic 2009) (Figure 3).

![Figure 3. The organization of the cortex. The cortex has a six-layered structure and contains pyramidal neurons and interneurons which are both horizontally and vertically interconnected. In the cortex of several species, bundles of ascending dendrites of pyramidal neurons from layer 5 and adjoining layer 2/3 pyramidal neurons can be discerned, which are believed to form the core of a group of interconnected neurons that extend through all vertical layers of the cortex and send their axons to the same target. Whereas layer 5 and layer 2 pyramidal neurons have ascending dendrites reaching until layer 1 of the cortex, layer 6 pyramidal neurons do not reach further than layer 4. Adapted from Ramon y Cajal, 1904 and Polleux 2000.](image-url)
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The neocortex receives input from the thalamus or from other cortical regions from both hemispheres and projects to the thalamus, striatum, superior colliculus, pons and other cortical areas (Thomson and Bannister 2003). Information processing in the neocortex occurs through local circuits that pass different layers of the cortex. Although the patterns of connections are rather complex and vary amongst different cortical areas, a simplified example of a cortical circuit through the different layers could start with a thalamocortical input arriving in layer 4, then excitatory neurons in this layer project to layer 3 and 2, followed by excitatory neurons in layer 3 which project to layer 5, which project back to layer 3 from where information is projected to other cortical regions (Figure 4). However, input from the thalamus or other cortical areas can also arrive at other layers such as layer 1 and 6 and also output can come from other layers (Thomson and Bannister 2003; Douglas and Martin 2004; Tau and Peterson 2010).

Figure 4. A simplified example of a cortical circuit. In the left panel input from the thalamus arrives in layer 4. Projection neurons from layer 4 project to layer 3, which then project to layer 5. Projection neurons from layer 5 either project to layer 6 or back to layer 3 from where information is projected to other projection neurons in layer 3. Projection neurons from layer 6 either project back to layer 4 or to the thalamus. In the right panel it is shown how inhibitory interneurons (black circles) located in layer 3 to 5, control excitation via local connections. Adapted from Thomson and Bannister 2003 and Douglas and Martin 2004.
In the neocortex, both interneurons and projection neurons also known as pyramidal neurons can be found. The pyramidal neurons are present in layer 2/3, 5 and 6 of the cortex, use in most cases the excitatory neurotransmitter glutamate and project to other pyramidal neurons within a local cortical circuit or to other cortical and subcortical areas (DeFelipe and Farinas 1992; Spruston 2008). The interneurons, which are mostly inhibitory, are present in all six layers, use the neurotransmitter GABA ($\gamma$-amino-butyric acid) and make connections within local cortical circuits via which they control excitation (Markram et al. 2004). Via local feedforward and feedback inhibition they can suppress excitation and thereby have an influence on the output of a cortical circuit (Somogyi et al 1998; Markram et al. 2004).

In a local cortical circuit information is processed through both interneurons and pyramidal neurons which are functionally interconnected. As shown in the simplified example of Figure 4, these connections pass several layers of the cortex and output can be projected to a similar local cortical circuit. It has been suggested that in the neocortex repetitive patterns of local cortical circuits also known as microcircuits exist, suggesting a stereotypic organization of the cortex (Silberberg et al. 2002). However, although across cortical areas and species repetitive patterns of structurally interconnected cells can be discerned, the functional relation between these neurons is still subject to investigation.

**The columnar structure of the cortex**

Throughout the neocortex, units of vertically interconnected neurons can be observed which are often referred to as the columnar structures of the cortex (Mountcastle 1957) (Figure 3). Large populations of vertically interconnected neurons that together form a structural column have been found in the visual, auditory, motor and somatosensory cortex (Mountcastle 1997). For example, in the primary somatosensory cortex large “barrel” shaped columns are visible which all functionally represent a whisker (Woolsey and Van der Loos 1970). Some neuroscientists state that these large columns consist of smaller anatomical columnar structures. This theory is known as the minicolumnar hypothesis (Mountcastle 1997; Buxhoeveden and Casanova 2002). According to this theory, minicolumns are the smallest anatomical units in the cortex consisting of a group of 80-100 heavily interconnected neurons that vertically transverse all layers of the cortex.
It has even been suggested that these minicolumns act as functional units and have common inputs and outputs (Mountcastle 1997; Buxhoeveden and Casanova 2002). In several areas of the human and primate cortex, minicolumns can be discerned as vertically aligned rows of cells (Buxhoeveden and Casanova 2002; Rockland and Ichinohe 2004). However, in rodents and other mammals these vertically aligned rows of cells are often difficult to detect in contrast to ascending apical dendrites of pyramidal neurons that together form dendritic bundles. In these dendritic bundles, layer 5 pyramidal neurons form the core of a small group of interconnected neurons that extend through several vertical layers of the cortex (Peters and Walsh 1972; Fleischhauer et al 1972; Rockland and Ichinohe 2004). Although some researchers also refer to these dendritic bundles as minicolumns, the size and number of neurons that belong to these units is different from the cellular columns observed in humans (Figure 5).

![Figure 5](image)

**Figure 5.** Examples of vertically aligned columns of cells (left) and ascending dendritic bundles (right) in the human cortex. Both anatomical structures extend vertically through several layers of the cortex and are referred to as minicolumns. Adapted from Casanova et al. 2002 and Rockland and Ichinohe 2004.

The organization of dendritic bundles has been extensively studied in the visual, motor and somatosensory cortex of several species and it has been shown that the size of these bundles and the number of interconnected neurons is highly heterogeneous (Rockland and Ichinohe 2004). It has been proposed that ascending apical dendrites of cortical pyramidal neurons form the center of a unit of vertically interconnected neurons that share functional properties and project to the same target (Peters and Sethares 1996; Lev and White 1997).
However, the question whether dendritic bundles share functional properties has lead to much debate. The current state is that dendrites belonging to one bundle only have a common output and do not receive a common input, yet research is still ongoing (Mountcastle 1997; Krieger et al. 2007; Innocenti and Vercelli 2010).

Another question that remains to be answered is how vertical units of interconnected neurons originate. It has been postulated that during embryonic development, radial glia cells from the ventricular zone migrate towards the cortical surface in the form of ontogenetic columns. Later in development, these radial glia cells guide most of the migrating future cortical cells to their destination giving rise to the columnar structure of the cortex (Rakic 1972; Rakic 1988).

The development of the cortex

The ontogeny of the cortex starts early in development when around embryonic day 10 (E10) in mice out of the neuroepithelium, also known as the ventricular zone of the telencephalic vesicles, undifferentiated germinal cells are formed. These germinal cells produce pluripotent progenitors generating neuronal and glial precursors (Super et al. 1998). From the ventricular zone the first postmitotic cells radially migrate to the surface of the cerebral vesicles to form the primordial plexiform layer or preplate, also named early marginal zone which later in development becomes the marginal zone (Nadarajah and Parnavelas 2002).

Meanwhile, the subplate becomes separated from the ventricular zone by cells that will form the intermediate zone followed by another layer of proliferating cells which appear between the ventricular zone and intermediate zone to form the subventricular zone (Super et al. 1998). Via radial glial cells cortical neurons that are formed in the ventricular zone migrate through the intermediate zone to form the cortical plate between the marginal zone and intermediate zone after E13 (Noctor et al. 2001; Rakic 2003; Marin and Rubenstein 2003; Garcia-Moreno et al. 2007). Between E14-18 more waves of cortical neurons migrate sequentially into the cortical plate until they are stopped before entering the marginal zone to form layer 2-6 of the cortex (Rakic 1988; Super et al. 1998; Nadarajah and Parnavelas 2002; Bystron et al. 2008). Since later migrating neurons pass the earlier generated layers of cortical neurons to form the superficial layers, the formation of the cortex is called inside-out (Figure 6).
In humans, a similar sequence of events occurs during the formation of the cortex starting around E33 (Bystron et al. 2008). The migration of cortical neurons which will lead to the formation of the 6 layers of the cortex takes places between the third and seventh month of gestation (Sidman and Rakic 1973; Bystron et al. 2008; Tau and Peterson 2010).

**Figure 6.** The several stages of cortical development. The preplate consists of one of the first generated neurons: the Cajal-Retzius cells. As soon as the subplate becomes separated from the ventricular zone, the intermediate zone can be discerned. Later in development the preplate becomes the marginal zone and cortical neurons that are formed in the ventricular zone migrate via the radial glia cells towards the marginal zone through the intermediate zone to form the cortical plate. During the next stage of development, waves of cortical neurons sequentially migrate via the radial glia cells into the cortical plate and pass the earlier generated layers of cortical neurons until they are stopped before entering the marginal zone, thereby forming the six-layered structure of the cortex. Abbreviations: preplate (PP), Cajal-Retzius cells (CR), subplate (SP), ventricular zone (VZ), intermediate zone (IZ), radial glia cells (RG), marginal zone (MZ), cortical plate (CP). Adapted from Fatemi 2005.

In the developing cortex, serotonergic axon terminals enter the marginal zone and intermediate zone around E17 (Lidov and Molliver 1982). The first serotonergic neurons are generated as early as E10 to E12 (Levitt and Rakic 1982; Rubenstein 1998). Only one day after the generation of these serotonergic neurons, axons start projecting to either the spinal cord or prosencephalon.

Within the cortex, Cajal-Retzius cells are among the first neurons generated. Around E10, Cajal-Retzius cells have their origin at multiple sites including the cortical hem and ventricular zone from where they migrate into the part of the preplate which later becomes the marginal zone or layer 1 of the cortex (Meyer et al. 2002; Garcia-Moreno et al. 2007).
Cajal-Retzius cells play an important role during the formation of the cortex by secreting the glycoprotein reelin, which functions as a stop signal for newly formed neurons that migrate from the ventricular zone towards the marginal zone or layer 1 (Marin-Padilla, 1998). When reelin is absent for example in the reeler mouse, an autosomal recessive mutant in which the gene for reelin is defective, the position of neurons in the cortex is altered and their branches are abnormal directed (Caviness et al. 1972; D'Arcangelo 2005).

The glycoprotein reelin is a large extracellular protein of approximately 450 kDa secreted by a select population of cells in the brain. Once released into the extracellular space reelin is cleaved at two sites located C terminal to domain 2 and 6 respectively, resulting in several fragments of which only the central and N-terminal fragment have been shown to bind to a receptor (Jossin et al. 2007). The central fragment of reelin binds to the very low density receptor (VLDR) and the apolipoprotein E receptor 2 (ApoER2) (D'Arcangelo et al. 1999). The cytoplasmic domains of these receptors induce tyrosine phosphorylation of the disabled-1 (Dab1) protein, which leads to the activation of an intracellular signaling cascade (Hiesberger et al. 1999; Trommsdorff et al. 1999). The N-terminal fragment of reelin, on the other hand, interacts with the α3β1 integrin receptor (Dulabon et al. 2000).

**The role of serotonin in postnatal cortical development**

In the postnatal brain, when all migrating neurons have reached their position, reelin controls dendritic maturation in the hippocampus and cortex and regulates the formation of cortical columns in the presubicular cortex (Nakajima et al. 1997; Nishikawa et al. 2002; Janusonis et al. 2004; Niu et al. 2004; Chameau 2009). From E17, when the first serotonergic axons enter the cortex, serotonin is the main excitatory drive for Cajal-Retzius cells which express the 5-HT3 receptor and secrete reelin until they disappear around P14, most likely as a consequence of differentiation, degeneration or cell death (Derer and Derer 1990; Mienville et al. 1999; Chameau et al. 2009).

In neonatal mice of which the serotonergic innervation to these Cajal-Retzius cells was disrupted, reelin levels were decreased and cortical column organization was changed (Janusonis et al. 2004).
In another study, it was specifically shown that blocking the N-terminal fragment of reelin or the 5-HT$_3$ receptor in P0 organotypic brain slices, resulted in an aberrant growth of apical dendrites of cortical layer 2/3 pyramidal neurons (Chameau et al. 2009). Similar changes in dendritic complexity of cortical pyramidal neurons in addition to a decrease in reelin levels were found in mice lacking the 5-HT$_{3A}$ receptor (Chameau et al. 2009). Together, these findings suggest that in the postnatal cortex, reelin controls cortical column organization and acts as a stop signal for ramifying apical dendrites of pyramidal neurons upon serotonergic activation of the 5-HT$_3$ receptor on Cajal-Retzius cells. However, no reports exist of how alterations in serotonergic signaling affect the above described regulatory pathway and what the consequences are of alterations in postnatal cortical development.
Aim and outline of the thesis

Our main objective was to investigate how alterations in serotonin levels affect neurodevelopment with emphasis on the role of the 5-HT$_3$ receptor in cortical development.

Previous studies showed that reelin controls dendritic maturation in the cortex. In chapter 2 we used the maternal care model to study the effect of environmental influence on reelin levels and postnatal dendritic maturation in the cortex. This study was based on the previous observation that differential maternal care has an influence on reelin expression levels in the hippocampus. Using the maternal care model we investigated the effects of environmental influence on cortical reelin levels and the effects on dendritic development of cortical layer 2/3 pyramidal neurons. In addition, we focused on the functional consequences of alterations in dendritic complexity of cortical layer 2/3 pyramidal neurons.

In chapter 3 we specifically investigated the effects of alterations in serotonergic signaling on cortical development. By treating pregnant mice with the drug fluoxetine for several days until delivery, we studied the effect of an increase in serotonin levels during neurodevelopment. In offspring, we specifically analyzed the effect of prenatal fluoxetine exposure on dendritic development of cortical layer 2/3 pyramidal neurons. In addition, we investigated the role of the 5-HT$_3$ receptor in mediating the effects of prenatal fluoxetine exposure on cortical development. We hypothesized that fluoxetine influences cortical development via a 5-HT$_3$ receptor-mediated pathway and used several 5-HT$_3$ receptor antagonists and the 5-HT$_{3A}$ receptor knockout mouse to test this hypothesis. Moreover, we investigated the role of the 5-HT$_3$ receptor in mediating prenatal fluoxetine induced anxiety-related behavior later in life.

Following the results of the first two chapters, in the next chapter our aim was to investigate whether the observed changes in dendritic development of cortical layer 2/3 pyramidal neurons were accompanied by alterations in the columnar organization of the cortex similar to other studies in which depletion of the serotonergic innervation to the cortex resulted in changes in cortical cytoarchitecture. In chapter 4 we specifically examined the effect of lacking the 5-HT$_3$ receptor with respect to apical dendrite bundling. In this study, we compared the organization of dendritic bundles of ascending apical dendrites of pyramidal neurons in tangential sections of the somatosensory cortex of 5-HT$_{3A}$ receptor knockout mice with wildtype mice.
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In addition, we investigated the density and spatial distribution of reelin-positive Cajal-Retzius cells in wildtype and 5-HT_{3A} receptor knockout mice.

Based on the results of the previous chapters and the knowledge that changes in serotonergic signaling during neurodevelopment have been implicated with changes in social behavior together with alterations in cortical cytoarchitecture, in chapter 5, we examined the behavioral phenotype of mice lacking the 5-HT_{3} receptor. In this study, we subjected both male and female 5-HT_{3} receptor knockout mice and wildtype mice to several social behavior tests including the social transmission of food preference, social interaction and social approach test. In addition, we tested these mice for anxiety using the novelty suppressed feeding test. In chapter 6 we summarize the main findings and discuss the results from the above described studies.