Serotonergic control of the developing cerebellum

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Citation for published version (APA):
Chapter 1

General introduction

Part of this chapter forms the basis of a review paper, published as:
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The role of serotonin in cerebellar development
Neuroscience, in press
1.1 Preface

Brains of all species undergo a precise and crucial developmental process, both before and after birth. In humans, development of the brain occurs until after the teenage years, during which the volume of the brain doubles and the cortical gray matter volume increases fourfold (Lodygensky et al., 2010). Even after that age, neurons and their neuronal networks keep changing. New neurons can be born in specific areas of the brain, and connections between neurons are adapted under the influence of experience.

During brain development, neurons are born and migrate to their final destination, they grow and start to form functional connections with the cells around them. In order for neurons to form these connections, both their axon and their dendrites must grow in the right direction and to the right location for synapses to form. It is therefore important that the dendritic formation, growth and maturation occurs in the right way. Under- or overshoot of the dendritic growth may lead to a malfunctioning of the neuronal network. Thus, having brain cells with bigger dendritic trees does not necessarily lead to a better brain functioning. Stop signals for the dendrites to grow are essential to establish the right neuronal connections and ensure a well-functioning brain.

One of the factors involved in brain development is the classical neurotransmitter serotonin (5-hydroxytryptamine, 5-HT; Lauder and Krebs, 1978; Lauder et al., 1983; Levitt et al., 1997; Gaspar et al., 2003). Serotonin provides low levels of tonic extrasynaptic neurotransmitter receptor activation and signaling in the developing nervous system (Lauder, 1993). Alterations of serotonin levels in the brain during development affect the connections between neurons which may produce long-lasting changes leading to neurodevelopmental disorders such as autism and schizophrenia (Migliarini et al., 2012). However, the role of serotonin in neurodevelopment is not yet fully known. It is hypothesized that the temporal pattern of serotonin levels during development may underlie its role in neurodevelopmental disorders. Children produce twice more serotonin in the brain during their first five postnatal years than adults, indicating its importance in development (Chugani et al., 2001). Members of
the serotonin receptor family are expressed during pre-and postnatal development, during which each serotonin receptor has its own specific temporal expression pattern (del Olmo et al., 1994; Gaspar et al., 2003). Furthermore, there is a developmentally critical window during which altered serotonin levels permanently influence neuronal circuitry (Daubert and Condron, 2010). This developmentally critical window is in rodents during the early postnatal period. An intriguing question is whether serotonin acts throughout the development of the serotonergic innervation and whether specific developmental phases exist. I suggest that through differential expression and function of serotonin receptors during different developmental phases, serotonin may have different effects throughout development. In order to be able to answer this question, it is important to investigate the expression and function of different serotonin receptors during each developmental phase.

One of the brain regions whose development occurs mostly postnatally is the cerebellum. The cerebellum is a highly organized brain area, whose tri-laminar structure and development were described in detail by Ramón y Cajal after his pioneer investigations (1890). Cerebellar development, i.e. migration of neurons, alignment of cells, and growth and maturation of the neurons all occurs in rodents in the first three weeks after birth. In humans, development of the cerebellum occurs until the first postnatal years (ten Donkelaar et al., 2003; ten Donkelaar and Lammens, 2009). So far, the cerebellum is known to be involved in motor coordination and motor learning, but is recently also thought to be involved in cognition and emotion (Middleton and Strick, 1998; Sacchetti et al., 2009; Strata et al., 2011; O’Halloran et al., 2012). This also includes involvement in autism and schizophrenia.

In adult animals, the cerebellum is richly innervated by serotonin: serotonergic fibres are the third main afferent fibres into the cerebellum. In order to gain a better understanding of the possible role of the cerebellum in neurodevelopmental disorders and the serotonergic system therein, it is of importance to understand the functioning of the serotonergic system in the cerebellum. However, the physiology of the serotonergic system and its functional significance are not fully known during development in the cerebellum. In this thesis I will focus on the serotonergic regulation of the cerebellum during postnatal development.
1.2 The cerebellum

The cerebellum, Latin for little brain, has been a subject of study because of its clear anatomy. The anatomy of the cerebellum was already described in the early 19th century (as reviewed in Glickstein et al., 2009), and knowledge of the neuronal circuitry within the cerebellar cortex made it an attractive brain area to study neuronal networks in the brain. The cerebellum consists of two hemispheres with the central vermis in the middle. The cerebellum can be divided in ten sagittal lobules, as described by Larsell (1952, 1970). The cerebellar cortex is a trilaminar structure, consisting of the outer molecular layer, the middle Purkinje cell layer and the inner granule cell layer (figure 1.1). In the molecular layer one finds the cerebellar interneurons, stellate, basket and Golgi cells, the dendrites of the Purkinje cells and the parallel fibres of the granule cells. The Purkinje cell layer is a monolayer of Purkinje cell somata aligned next to each other. In the inner granule cell layer one finds the granule cells, mossy fibres and Lugaro cells. The granule cells are small but numerous, in total accounting for approximately half of the neurons in the mammalian brain. Climbing fibres ascend from the white matter through the granule cell layer until they wrap around the soma and dendritic tree of one Purkinje cell in the Purkinje cell layer and the molecular layer.

Purkinje cells, first described by and named after the Czech anatomist and physiologist Jan Evangelista Purkyně (1837), are the sole inhibitory output of the cerebellum and project to the deep cerebellar nuclei. One Purkinje cell is innervated by two glutamatergic inputs: multiple parallel fibres and one climbing fibre. Parallel fibres are the axons from the granule cells, which receive excitatory inputs from mossy fibres originating from nuclei in the spinal cord and the brainstem. Parallel fibres extend for long distances in a transverse plane, innervating hundreds of Purkinje cells. One Purkinje cell gets inputs from thousands of granule cells via their parallel fibres. The other excitatory input to Purkinje cells, the climbing fibre, has a one to one contact with Purkinje cells and originates from the inferior olivary nucleus. Purkinje cells receive multiple inhibitory contacts from the molecular layer interneurons (the basket and stellate cells) and Lugaro cells. Other inhibitory cells are the
Golgi cells and unipolar brush cells but they do not project onto Purkinje cells. Input from parallel fibres can constitute a so-called simple spike in Purkinje cells, while input from one climbing fibre generates a complex spike in Purkinje cells. The cerebellum is connected to many regions of the cerebral cortex. Axons from the dorsal dentate nucleus innervate motor areas and axons from the ventral dentate nucleus innervate non-motor areas of the prefrontal cortex. In addition, the deep cerebellar nuclei also project to non-cortical regions such as the limbic system, the hypothalamus, thalamus, nucleus accumbens and the ventral tegmental area. The cerebellum plays a crucial role in this distributed circuit and coordinates or modulates aspects of cortical activity.

The cerebellum is associated with functioning of the motor system, and more specifically motor control and motor learning, such as the initiation of movement and the coordination of posture, head and eye movements, skilled motor activities, and language. One important theory of cerebellar functioning is the Marr-Albus-Ito hypothesis which predicted the existence of long-term depression (LTD) based on
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Hebbian principles (Marr, 1969; Albus and Branch, 1971; Ito, 1984). They hypothesized that activation of both parallel fibres and climbing fibres is necessary for LTD, during which climbing fibre activation acts as an error signal. LTD at the parallel fibre - Purkinje cell synapse was thought to be the key to motor learning (see also Linden, 2003). However, this view has recently been challenged (Schonewille et al., 2011).

1.3 Pre- and postnatal development of the cerebellum

Cerebellar development begins halfway the embryonic period. The first cells to be born are the neurons from the deep cerebellar nuclei and the Purkinje cells, whose birth start around embryonic day (E) 11 - 13 in rodents. The inhibitory interneurons are mostly born during the first ten postnatal days. The last cell type to be born in the cerebellum is the granule cell, whose development occurs completely postnatally, in rodents during the first three postnatal weeks. The timeframe of neurogenesis for different cerebellar cells is summarized in figure 1.2.

In rodents, the cerebellum is still developing during the first weeks after birth. This includes general growth of the cerebellum and development of the lobules, and organizational developments at a cellular level (figure 1.3). In general, the first postnatal week is characterized by stable immaturity of the cerebellar network, while there is a transitional stage during the second postnatal week. During the first two postnatal weeks, the inhibitory stellate cells, basket cells and Golgi cells proliferate and migrate to their final positions. Functional parallel fibre - Purkinje cell synapses are formed at the end of the first postnatal week (Altman, 1972b). During the third postnatal week, stellate and basket cells form feed-forward pathways from parallel fibres to Purkinje cells, and Golgi cells form a feedback pathway from parallel fibres to granule cells. By the third and fourth postnatal week the cerebellum has developed into a stable adult stage.

Efferent projections from Purkinje cells to the cerebellar and vestibular nuclei occur during late embryogenesis and synaptic contacts are established around birth
Figure 1.2: Neurogenesis in the cerebellum.
Projection neurons, such as the Purkinje cells, are first born in the cerebellum. This is followed by the birth of local interneurons and granule cells. Adapted from Carletti and Rossi (2008).

(Eisenman et al., 1991). The exact timing of the development of projections from the cerebellar nuclei to the thalamus is not known, but it is likely that these connections are made before postnatal day (P) 21 as it is during this neonatal and juvenile period that systematic changes take place and the cerebellar network is formed (Altman and Bayer, 1996).

1.3.1 Purkinje cell development

Shortly after birth, developing Purkinje cells migrate to form a single layer of Purkinje cells just beneath the external granule cell layer. Around birth, Purkinje cells still form a multilayered zone (Uzman, 1960). They form a monolayer around P10 (Miale and Sidman, 1961). During the first few days that Purkinje cells are aligned in a monolayer, they do not yet form connections (Altman, 1972a). Once settled at their final position, Purkinje cell development occurs in different morphological and physiological stages (Fig. 1.4; Ramón y Cajal, 1911; Altman, 1972a; Kapfhammer,
Figure 1.3: Postnatal development of mouse cerebellum. During the first three postnatal weeks, granule cells migrate from the external to the internal granule cell layer along Bergmann glia cells, and Purkinje cells develop a vast dendritic tree. Abbreviations: BG, Bergmann glia cell; CF, climbing fibre; EGL, external granule cell layer; GC, granule cell; GCP, granule cell precursor; glomerulus; IGL, internal granule cell layer; MF, mossy fibre; ML, molecular layer; P, postnatal day; PC, Purkinje cell; PCL, Purkinje cell layer; PF, parallel fibre; rp, radial processes; sp, synapse. Adapted from Kagami and Furuichi (2001).
2004; McKay and Turner, 2005): an initial stable immature stage of minimal change from birth to P9, a transitional stage in which the Purkinje cells undergo major morphological and physiological maturation, and from P18 a stable adult stage with only minor refinements. In the first stage, Purkinje cells have small somata with multipolar peri-somatic dendrites. At P9, their soma size has increased threefold, and the input resistance decreases accordingly. During the second transitional stage (P9 - P12), Purkinje cell morphology is hugely variable, indicating a rapid change in Purkinje cell morphology which does not have a similar temporal pattern for each Purkinje cell. Input resistance is lower in more mature Purkinje cells. Purkinje cells in the vermis and the anterior and posterior hemispheres have different times of migration and settling (Goodlett et al., 1990; Altman and Bayer, 1996). Monoplanar arborization of Purkinje cells is coupled with functional development of the cerebellar circuitry. During the third postnatal week, expansion of the dendritic tree of Purkinje cells occurs in multiple sagittal planes. Dendrites then become confined to a single plane in the fourth postnatal week. In mice with abnormal Purkinje cell connectivity and motor discoordination, Purkinje cell arborization continues in multiple sagittal planes instead of the mature monoplanar organization (Kaneko et al., 2011). During the final developmental period, the Purkinje cell dendritic tree vastly increases in size and complexity (see figure 1.4).

### 1.3.2 Granule cell development

Granule cells migrate twice, in different directions: first tangential migration, and secondly radial migration, during and after which they form synaptic connections (figure 1.3). Between E15 and P15, granule cell progenitors start to migrate tangentially from the rhombic lip to form the external granule cell layer. In the external granule cell layer, the granule cells start to bilaterally extend long horizontal processes, the parallel fibres. During the first three postnatal weeks in rodents, the granule cells migrate radially along the Bergmann glia cells. They thereby migrate from the external granule cell layer through the Purkinje cell layer into the internal granule cell layer. Finally, migration terminates in the internal granule cell layer, where the
Figure 1.4: Morphological maturation of Purkinje cells.

Purkinje cells mature slowly from P0 to P9, underwent rapid dendritic growth between P12 and P18, and gradually complete development by P90. Two examples are shown at P9 and P12 to indicate significant heterogeneity in Purkinje cell morphology at these ages. Adapted from McKay and Turner (2005).

Granule cells form synapses with Golgi cells and mossy fibres and become physiologically mature: their voltage-gated Na\(^+\) currents become larger and they have an increased ability to fire action potentials (see also chapter 2). The extended parallel fibres, whose terminals are still in the top 2/3rd of the external granule cell layer, start to form functional connections with the Purkinje cell dendrites. Three weeks after birth, granule cell migration and maturation is complete.

1.3.3 Climbing fibre elimination

During the maturation process of the Purkinje cells, synapses formed with both excitatory and inhibitory inputs can change based on the amount of activity from these inputs: activity-dependent synaptic plasticity takes place. After the formation of first climbing fibre - Purkinje cell synapses, and then parallel fibre - Purkinje cell synapses, connections are dynamically modified, resulting in the elimination of climbing fibres until each Purkinje cell is innervated by only one persisting climbing fibre, in parallel with ongoing synaptogenesis onto Purkinje cells from parallel fibres (Eccles, 1967; Crepel et al., 1976; Mariani and Changeux, 1981; Chedotal and Sotelo, 1993; Sotelo,
Figure 1.5: Climbing fibre elimination.
During the first postnatal week in rodents, Purkinje cells are polyinnervated by multiple climbing fibres, which are all but one eliminated during the next two postnatal weeks. In adult rodents, Purkinje cells are innervated by a single climbing fibre. From De Grujil et al. (2013).

At P0, a Purkinje cell is innervated by multiple climbing fibres which will all but one be removed during the first few postnatal weeks (Crepel et al., 1976; see figure 1.5). The maximum degree of polyinnervation is reached between P1 and P7 where nearly 50% of the Purkinje cells bear at least four climbing fibres (Kano et al., 1995). In the first few postnatal days, climbing fibres are building synapses onto the Purkinje cells as shown by the fact that multiple climbing fibre innervation index increases (Crepel et al., 1981; Mariani and Changeaux, 1981). During this time, climbing fibres only contact Purkinje cell somata, and from P8 they start to innervate the Purkinje cell dendrites (Mason et al., 1990). Climbing fibre competition is already ongoing from P3, before dendritic translocation of the 'winning' climbing fibre takes place (Hashimoto et al., 2009; Carrillo et al., 2013).
Interestingly, parallel fibre input has a highly dominant role in climbing fibre regression during development. Morphological and electrophysiological data demonstrate that parallel fibres synapses appear at P7 and their development is significantly correlated with the time course of the climbing fibre regression (Scelfo and Strata, 2005). Partial loss of granule cells results in innervation of a Purkinje cell by multiple climbing fibres even in the adult stage (Mariani et al., 1990; Bravin et al., 1995; Sugihara et al., 2000). However, details on how parallel fibre - Purkinje cell synapse activation may lead to climbing fibre regression are unknown. I will thus investigate the effect of different serotonin receptors on parallel fibre - Purkinje cell activity and the subsequent effect on climbing fibre elimination during postnatal development.

### 1.4 Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) was first described in 1948 (Rapport et al.) as the 'serum tonic factor', and was therefore named serotonin. In 1953 serotonin was detected in the brain (Twarog and Page). There is a great diversity in serotonin receptors, of which serotonin 1 - 2 and 4 - 7 receptors are all G protein coupled receptors. The serotonin 3 (5-HT$_3$) receptor is the only ligand-gated ion channel for serotonin.

#### 1.4.1 The 5-HT$_3$ receptor

The 5-HT$_3$ receptor is the only serotonin receptor which does not belong to the G protein receptor superfamily. The 5-HT$_3$ receptor is a ligand-gated cation channel, enabling fast synaptic transmission. The 5-HT$_3$ receptor has two known subunits: the 5-HT$_{3A}$ subunit and the 5-HT$_{3B}$ subunit (Derkach et al. 1989; Maricq et al. 1991; Davies et al., 1999). The 5-HT$_3$ receptor is a pentamer with two different natural forms known: the homomer receptor, consisting of only 5-HT$_{3A}$ subunits and mainly located in the brain, and the heteromer receptor: a combination of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits which is only found in the peripheral nervous system. 5-HT$_3$ receptors are permeable to cations such as potassium, calcium and sodium (Yakel et al. 1990). The 5-HT$_3$ receptor is expressed throughout the CNS, in particular by GABAergic
interneurons, with high levels of expression in the cortex, hippocampus, amygdala, several brainstem nuclei and the spinal cord (reviewed by Chameau and van Hooft, 2006). In addition, 5-HT$_3$ receptors are expressed postsynaptically by Cajal-Retzius cells, located in layer I of the cortex during the first two postnatal weeks (Chameau et al., 2009). The 5-HT$_3$ receptor is also expressed in a subset of GABAergic interneurons, such as those expressing calretinin, cholecystokinin (CCK; Morales and Bloom, 1997), and calbindin (Bloom and Morales, 1998). Neurotransmitter release is mediated by the 5-HT$_3$ receptors on the presynaptic sites by increasing the intracellular Ca$^{2+}$ concentration via Ca$^{2+}$ entry either through the 5-HT$_3$ receptor-operated ion channel or indirectly via activation of voltage-gated Ca$^{2+}$ channels. Generally, presynaptic 5-HT$_3$ receptors modulate neurotransmitter release and postsynaptic 5-HT$_3$ receptors control neuronal excitability. 5-HT$_3$ receptors are also interesting for clinical reasons: 5-HT$_3$ receptors are involved in nausea, emesis, nociception, and possibly also in cognition and anxiety (Barnes and Sharp, 1999). So far, 5-HT$_3$ receptors have not been found in the cerebellum (see also section 2.1 for a more detailed literature discussion on this topic).

### 1.4.2 Serotonin in the cerebellum

Serotonergic fibres are the third largest population of afferent fibres extending in the cerebellum, after mossy fibres and climbing fibres. Serotonergic fibres which innervate the cerebellum originate mainly in the medullary- and pontine reticular formation, although some serotonergic inputs originate from the raphe nuclei and the gigantocellular reticular formation adjacent to the raphe nuclei (Chan-Palay, 1975; Takeuchi et al., 1982; Weiss and Pellet, 1982; Bishop and Ho, 1985). These serotonergic fibres form a dense network in the granular layer and are found around the somata of Purkinje cells, and in the overlying molecular layer, which contains the dendrites of Purkinje cells (Andén et al., 1967; Hökfelt and Fuxe, 1969; Takeuchi et al., 1982; Beas-Zarate et al., 1984; Bishop and Ho, 1985; Triarhou and Ghetti, 1991). Serotonin axon terminals innervate Purkinje cell dendrites (Sotelo and Beaudet, 1979), granule cell dendrites (Chan-Palay, 1975; Chan-Palay et al., 1977; Sotelo and Beaudet, 1979;
Beaudet and Sotelo, 1981), parallel fibres (Sotelo and Beaudet, 1979), and basket, stellate and Golgi cells (Chan-Palay, 1975; Chan-Palay et al., 1977). Different serotonin receptors have distinct functions and can mediate both excitatory and inhibitory neurotransmission. In the cerebellum, serotonin depresses Purkinje cell discharges and excites the granule cells (Bloom et al., 1972; Strahlendorf et al., 1979; Weiss and Pellet, 1982; Strahlendorf et al., 1984). Serotonin can tonically inhibit endogenous glutamate release in the cerebellum, via 5-HT\textsubscript{1} and 5-HT\textsubscript{2} receptors (Maura et al., 1988). Application of serotonin results in long-lasting enhancement of GABA-mediated inhibitory postsynaptic currents observed in Purkinje cells (Mitoma et al., 1994).

Different receptors for serotonin are present in the cerebellum (figure 1.6). Purkinje cells express different 5-HT receptors, including the 5-HT\textsubscript{1A}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B}, 5-HT\textsubscript{2C}, 5-HT\textsubscript{5A} and 5-HT\textsubscript{7} subtypes (Pazos and Palacios, 1985; Maroteaux et al., 1992; Matthiessen et al., 1993; Ruat et al., 1993; Boschert et al., 1994; Choi and Maroteaux, 1996; Maeshima et al., 1998; Wu et al., 1998; Cornea-Hebert et al., 1999; Eastwood et al., 2001a; Geurts et al., 2002; Li et al., 2004). Cerebellar granule cells express 5-HT\textsubscript{6} receptors (Geurts et al., 2002; Ward et al., 1995; Gérard et al., 1997). In addition, very low densities of 5-HT\textsubscript{1} receptors in the molecular and granule cell layer in the adult rat cerebellum have been found (Pazos and Palacios, 1985). Golgi cells express 5-HT\textsubscript{2A} and 5-HT\textsubscript{5A} receptors, and molecular layer interneurons express 5-HT\textsubscript{5A} receptors (Geurts et al., 2002). Lugaro cells are sensitive to serotonin and express 5-HT\textsubscript{2} receptors, but not 5-HT\textsubscript{1}, 5-HT\textsubscript{3} or 5-HT\textsubscript{4} receptors (Dieudonné, 2001). Excitation of Lugaro cells by serotonin accounts for the serotonin-driven inhibitory activity recorded from Golgi cells (Dieudonné and Dumoulin, 2000).

1.5 Serotonin as a regulator of neuronal development

There is growing evidence that throughout the brain serotonin regulates the connectivity of the brain by modulating cellular migration and cytoarchitecture during development (Daubert and Condron, 2010). Serotonin levels during development can be altered by a number of external factors, including stress (Papaioannou et al., 2002), nutrition (Serfaty et al., 2008), infection (Winter et al., 2009) and pharma-
logical compounds such as selective serotonin reuptake inhibitors (SSRIs) (Xu et al., 2004; Smit-Rigter et al., 2012). Whereas a role for serotonin in brain development has been suggested for some time, the molecular mechanisms responsible for serotonin’s effects on physical restructuring of the brain are only beginning to be elucidated (Daubert and Condron, 2010). One of the brain areas to study this in is the cerebellar cortex, in which the neuronal network is well known and whose development is of interest for both the learning of motor skills and possible cognitive functions.

During postnatal development the cerebellum is already innervated by serotonergic fibres. Lidov and Molliver (1982) have studied the development of serotonergic innervation of the cerebellum in rodents. From E21, serotonergic fibres have reached the white matter, and terminal fields are formed in the internal granule cell layer around P1 - P3. At P6, there are multiple extensions of serotonergic fibres into the internal granule cell layer, occasionally penetrating the Purkinje cell layer. The first functional serotonergic contribution to the circuitry of the cerebellar cortex occurs around P10, when serotonergic fibres are fully extended into the Purkinje cell layer and occasionally penetrating the molecular layer. At two and three weeks after birth,
there is abundant arborization of serotonin axon terminals in the cerebellar cortex.

In cerebellar neural progenitors derived from primary cultures of cerebellar granule cells from 7-day old rats, presence of mRNAs for tryptophan hydroxylase (the rate-limiting enzyme in 5-HT synthesis), the serotonin transporter, and various subtypes of 5-HT receptors, including 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ was found (Zusso et al., 2008). This indicates that the neural progenitors are capable of synthesizing serotonin. There is some evidence suggesting presence of 5-HT$_{2}$ receptors on dissociated cerebellar granule cells at P8 from rat cerebellum (Xu and Chuang, 1987; Akiyoshi et al., 2005), while these receptors are not detected in the mature cerebellum (Pazos and Palacios, 1985; Geurts et al., 2002). In this thesis I will investigate the role of serotonin via differential expression of its receptors in the postnatal development of the cerebellum.

1.6 Scope and aim of this thesis

The aim of the research described in this thesis is to study the mechanism with which serotonin modulates the maturation of the cerebellum during postnatal development. In order to achieve this, I studied the localization and temporal expression pattern of three serotonin receptors (5-HT$_{1}$, 5-HT$_{2}$ and 5-HT$_{3}$) in the developing cerebellum. Given their specific and unique expression pattern, I investigated the role of serotonin, mediated via these receptors, in the physiology of the developing cerebellar cortex.

In chapter 2, I investigate the expression of 5-HT$_{3}$ receptors in the developing cerebellum using 5-HT$_{3A}$/Enhanced Green Fluorescent Protein (EGFP) transgenic mice. Using pharmacological tools I study the role of the 5-HT$_{3}$ receptors in synaptic plasticity in the cerebellum during the first three weeks after birth.

In chapter 3 I elaborate on the findings presented in chapter 2. I first test the hypothesis that 5-HT$_{3}$ receptors are involved in the maturation of Purkinje cell morphology via reelin, using both 5-HT$_{3A}$ receptor knockout mice and organotypic slice cultures. Continuing with electrophysiological recordings, I confirm the findings in
chapter 2 which were based on pharmacological blockade of 5-HT$_3$ receptors by using 5-HT$_{3A}$ receptor knockout mice. My aim is further to investigate whether the 5-HT$_3$ receptors only affect the physiology at the synapse where it is expressed, or whether it also affects the formation and functioning of the surrounding neuronal network.

In chapter 4 a characterization of 5-HT$_1$ and 5-HT$_2$ receptor expression in the developing cerebellar cortex is undertaken. Since 5-HT$_2$ receptor expression is sustained in the mature cerebellum (see Maeshima et al., 1998; Cornea-Hebert et al., 1999; Geurts et al., 2002; and the findings in chapter 4 of this thesis), I investigate its role in short- and long-term synaptic plasticity both in the developing and in the mature cerebellum.

In chapter 5 I provide an extensive discussion on the role of serotonin in the developing cerebellum and the mechanism behind this, based on the findings presented earlier in this thesis.