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## Development and modulation of mouse and human cortical circuitry

Kroon, T.

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# 1

## Introduction

We live and learn. We also see, hear, think, feel, make decisions, and contemplate whether we live inside the Matrix (Bostrom, 2003). All of these things would be impossible without our brains. Specifically, higher order cognitive processes have been ascribed to the neocortex, the outermost part of the telencephalon. The neocortex is a laminated structure, each layer containing cells that have specific properties and connectivity (Shipp, 2007). While the differences in cell type-specific characteristics and innervation of cortical layers have been extensively studied, the development of these properties across layers has received much less attention, and it is unclear to what extent the development of neurons in different layers is similar.

In recent years, it is increasingly being recognised that developmental processes may be very relevant for the pathophysiology and treatment of neurodevelopmental disorders (Kroon et al., 2013; Marín, 2016). Specifically, neuronal networks may undergo sensitive time windows during which they are highly susceptible to impairments in synaptic function (Meredith et al., 2012). Combining both of these concepts, I hypothesise that synaptic impairments may impact the development of cortical circuitry in a laminar-specific manner. In this thesis, I therefore aim to determine whether pyramidal neurons in layers 3 and 5 of medial prefrontal cortex develop similarly, and to establish whether knockout of Oligophrenin-1, a model for developmental disorders, differentially impacts the development of neurons and circuits in these layers.

### ***The neocortex***

Evolutionarily, the neocortex is the most recent part of the brain (Rakic, 2009) and is found exclusively in mammals, although the brains of birds and reptiles contain populations of neurons that are homologous in function and gene expression to certain mammalian neocortical neuronal populations (Dugas-Ford et al., 2012). The neocortex contains many different cortical areas, defined by their cytoarchitecture and thalamocortical and corticocortical connections, as well as their function in sensory modalities, cognitive processing and behaviour. The cellular and circuit makeup of different parts of the cortex follow the same general patterns, albeit with alterations reflecting their function of different areas and the type of information they process (Harris and Shepherd, 2015). The following description of neocortical circuitry is therefore necessarily simplified. A diagram summarising the following sections is shown in figure 1.

### ***Excitatory circuitry of the neocortex***

The neocortex generally consists of six cell layers, delineated by differences in neuronal morphology and cell density. The most prevalent neuronal cell type of the neocortex is the pyramidal cell. These cells have a rounded triangular soma and a prominent apical dendrite that extends toward the pia and ends in an apical tuft. The exact morphology, e.g.

the size and complexity of the apical tuft, depends on pyramidal cell subtype ([Oberlaender et al., 2012](#); [Spruston, 2008](#)). Short pyramidal cells in layer 6, on the other hand, have shorter apical dendrites that often do not reach the pia and rarely have a tuft ([Briggs, 2010](#)). The apical dendrite also gives rise to oblique dendrites, that branch off from the main apical trunk. The basal dendrites extend from the soma and are shorter and less complex than the apical dendrites. The axon of pyramidal cells protrudes from the base of the soma, and, depending on the subtype of the cell, forms extensive branches and projects to both intracortical and subcortical targets ([Kim et al., 2015](#)).

Pyramidal cells are present in layers 2, 3, 4, 5 and 6 of the cortex, although the exact composition of cell types varies between cortical areas ([Scala et al., 2019](#)). Layer 4 contains mainly spiny stellate cells, which receive most of the thalamic input to the cortex ([López-Bendito and Molnár, 2003](#)). In primary sensory regions, these thalamocortical projections pass on information directly from sensory organs. These types of inputs are known as “driver” inputs ([Sherman, 2016](#); [Sherman and Guillery, 1998](#)). In higher sensory cortices, thalamocortical projections to layer 4 also convey processed sensory information from layer 5B cells in primary sensory regions ([Theyel et al., 2010](#)). Layers 2 and 3 of the cortex receive primarily modulatory thalamic input ([Viaene et al., 2011a](#)), while layers 5 and 6 also receive mainly driver-like input ([Constantinople and Bruno, 2013](#); [Viaene et al., 2011b](#)), albeit less than layer 4. Neurons in layer 4 synapse onto cells in every layer of the cortex, although they receive little input from neurons in other layers ([Lefort et al., 2009](#)). Neurons in all layers form local circuits with other neurons in the same layer. Layers 2/3 and 5 project to each other, but rarely to layers 4 and 6. Layer 6 neurons primarily project to the thalamus and modulate thalamic function ([Harris and Shepherd, 2015](#); [Varela, 2014](#)), as do some neurons in layer 5B ([Hoogland et al., 1987](#)). In addition, neurons in layers 2/3, 5 and 6 receive diverse and widespread connections from other cortical areas ([Cauler et al., 1998](#); [Stehberg et al., 2014](#)). This reflects the fact that many regions of the cortex process information from several modalities ([Ghazanfar and Schroeder, 2006](#); [Serenó and Huang, 2014](#)), and even primary sensory areas encode more than just raw sensory information ([Macaluso, 2006](#); [Takahashi et al., 2016](#)). Moreover, neurons in several layers receive neuromodulatory inputs from various sources, which can significantly alter circuit function ([Hasselmo, 1995](#); [Marder, 2012](#)).

Thus, while its computational organisation is not fully understood, certain patterns of connections between excitatory neurons can be found throughout the neocortex. How these patterns of connectivity translate into cortical functions is unclear. However, recent studies have begun to shed light on the function of certain connections. For example, motor learning in rodents involves strengthening of recurrent connectivity between specific spinal-projecting layer 5B pyramidal neurons ([Biane et al., 2019](#)).

### ***Inhibitory circuitry of the neocortex***

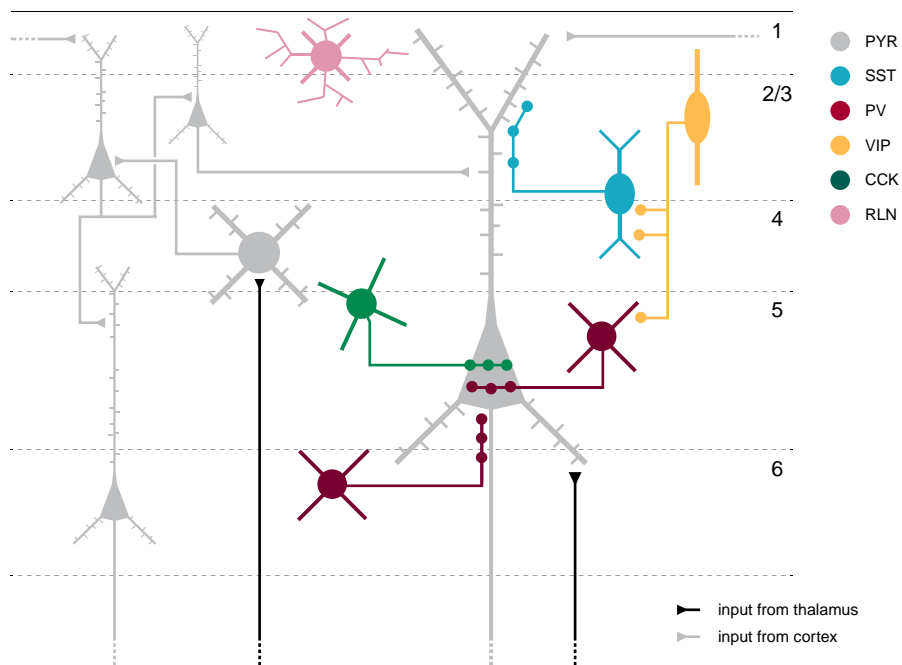
While excitatory neurons make up roughly 80% of all neurons in the cortex, their impact on computation is rivalled by the other 20%, the GABAergic interneurons. GABAergic interneurons inhibit other neurons by releasing  $\gamma$ -amino-butyric acid (GABA), and most have local axonal arborisations (Tamamaki and Tomioka, 2010). Interneurons in the neocortex can be characterised by a wide range of physiological, morphological and molecular properties (Ascoli et al., 2008; DeFelipe et al., 2013; Markram et al., 2015). In addition to differences in intrinsic properties and intracortical connectivity between interneuron types, inhibitory neurons receive different levels of thalamic input (Cruikshank et al., 2012; Tan et al., 2008; Wall et al., 2016). A comprehensive scheme for classifying these neurons is not yet available. However, promising new techniques involving the sequencing of full single-cell transcriptomes are being developed and may allow full-scale classification of neuronal subtypes in the near future (Poulin et al., 2016; Tasic et al., 2018; Zeisel et al., 2015). On the basis of molecular markers, cortical interneurons can be divided into three main groups: those expressing parvalbumin (PV), those expressing somatostatin (SST), and those expressing the serotonin receptor 5HT3aR (Rudy et al., 2011; Tremblay et al., 2016).

Most, but not all, parvalbumin-expressing interneurons are fast-spiking (Blatow et al., 2003), and the fast-spiking group typically consist of basket cells that target the soma and proximal dendrites, and chandelier cells that target the axon initial segment. These cells are thought to have particularly strong inhibitory effects due to their inhibition of cells close to the location of action potential generation (Fishell and Rudy, 2011). The fast spiking behaviour of PV interneurons, coupled with short synaptic latency, allows them to control the timing of action potential firing in post-synaptic cells (Hu et al., 2014). Although it is widely recognised that PV interneurons are involved in the generation of cortical oscillations (Hu et al., 2014; Sohal et al., 2009), and their activity is related to specific behavioural states (Brown et al., 2015; Kvitsiani et al., 2013), clear understanding of their role in regulating behaviour is still lacking (Tremblay et al., 2016).

The main group of somatostatin-expressing interneurons are the Martinotti cells, which target layer 1 and, in contrast to other interneuron types, receive excitatory inputs that are facilitating rather than depressing (Beierlein et al., 2003). These cells target dendrites of pyramidal cells (Silberberg and Markram, 2007) and also target virtually all other interneuron types (Jiang et al., 2015). Martinotti cells are involved in lateral inhibition (Lee and Huguenard, 2011; Silberberg and Markram, 2007), allowing them to perform functions such as surround suppression of sensory input (Adesnik et al., 2012). However, recently somatostatin-expressing interneurons in the somatosensory cortex have been shown to be either activated or inactivated by whisking in a layer-specific manner, showing that these neurons may have opposing functional roles depending on their location and axonal arborisations (Muñoz et al., 2017).

The remaining group of interneurons express the serotonin receptor 5HT3aR (Lee et al.,

2010). These neurons are more heterogeneous and are therefore less well characterised than other types. A major subtype of these neurons are the ones expressing vasointestinal peptide (VIP), which are primarily found in layer 2/3 and are bipolar (Prönneke et al., 2015). They preferentially inhibit somatostatin-expressing interneurons, which leads to disinhibition of pyramidal cell dendrites (Pfeffer et al., 2013; Pi et al., 2013). This may in turn augment synaptic plasticity and facilitate learning (Letzkus et al., 2015). Neurogliaform cells are primarily found in layer 1, and have short dendrites and large, elaborate axons, and often show late-spiking firing patterns (Kawaguchi and Kubota, 1997). These cells typically produce slow inhibition on many neighbouring cells (Oláh et al., 2007) and can cause volume transmission of GABA (Oláh et al., 2009). The behavioural relevance of neurogliaform cells remains unclear (Overstreet-Wadiche and McBain, 2015). Cholecystikinin-expressing basket cells resemble large PV-positive basket cells in their morphology and synaptic targeting, but are not fast-spiking (Kawaguchi and Kubota, 1998; Kubota, 2014). Although their function in behaviour is largely unknown, they have recently been shown to play a role in the coding of spatial information (Del Pino et al., 2017).



**Figure 1.** Schematic overview of cell types and connections in the neocortex. PYR, pyramidal neuron; SST, somatostatin-expressing neuron; PV, parvalbumin-expressing neuron; VIP, vasoactive intestinal peptide-expressing neuron; CCK, cholecystikinin-expressing neuron; RLN, reelin-expressing neuron. Grey triangles indicate intracortical excitatory synapses. Black triangles indicate thalamocortical excitatory synapses. Circles indicate inhibitory synapses.

In short, there are many subtypes of interneurons, providing selective inhibition to different cellular compartments and cell types. Each of these is likely to have a specific function in behaviour that is dependent on location in the cortex, connectivity and even state of the animal. Although they make up a minority of the neurons in the cortex, GABAergic interneurons are crucial for proper brain function. Neuronal networks require a precise balance of excitation and inhibition (Haider et al., 2006; Landau et al., 2016) and excitatory/inhibitory imbalance has been linked to several neurological disorders (Eichler and Meier, 2008; Gao and Penzes, 2015; Žiburkus et al., 2013), as will be discussed later.

### ***The relationship between neuronal morphology and function***

The balance between excitatory and inhibitory input onto a neuron (E/I balance) is heavily influenced by the relative locations of excitatory and inhibitory synapses. Inhibitory synapses are placed at optimal locations along the dendrite, allowing them to control dendritic excitability more effectively (Gidon and Segev, 2012). For example, inhibitory synapses on dendritic spines often only inhibit a single excitatory synapse, while those positioned on dendritic shafts can decrease or abolish the propagation of dendritic spikes and back-propagating action potentials (Boivin and Nedivi, 2018). Similarly, where an excitatory synapse is located on the dendrite impacts its ability to evoke a somatic action potential (Spruston, 2008). More distal synapses have less impact on somatic membrane voltage than do proximal inputs, but lower the threshold of spike initiation by proximal synapses (Behabadi et al., 2012). Thus, the morphology of a neuron is an important factor in computations occurring within its dendrites.

There is a large diversity of neuronal morphology, between different neuron types (Ascoli et al., 2008) as well as between species (Mohan et al., 2015). The shape of the dendrites can have profound effects on the type of input a neuron receives, as well as the processing of that input. The reach of the dendritic tree determines with which axons it can form synapses. Inputs from specific sources tend to make connections with particular dendritic compartments (Petreanu et al., 2009). Moreover, these dendritic compartments are capable of performing computations (Magee, 2000; Poirazi and Mel, 2001) and synapses that convey similar input tend to cluster together within compartments (DeBello et al., 2014; Gökçe et al., 2016). Therefore, the morphology of the dendritic tree has a direct impact on the way synaptic inputs are processed (Srinivas et al., 2017). For example, modelling studies have shown that two electrophysiological functions – linear summation of synaptic inputs and spike-order detection – are performed optimally in very different neuronal morphologies (Stiefel and Sejnowski, 2007; Torben-Nielsen and Stiefel, 2009). In addition, the shape of the dendritic tree can impact the firing capabilities of a neuron (Elburg and Van Ooyen, 2010; Mainen and Sejnowski, 1996; Van Ooyen et al., 2002). Although the exact organisation of dendritic morphology and the mechanisms behind placement of synapses remain poorly understood, a neuron's dendritic morphology thus influences its function in several ways, and it is therefore

crucial that neurons develop proper dendritic morphology (Lefebvre et al., 2015).

I assess the development of dendritic morphology of pyramidal cells in layer 3 and 5 of the rodent medial prefrontal cortex in chapter 2.

### ***Structure and function of the prefrontal cortex***

The prefrontal cortex (PFC) serves a wide array of functions, as is shown by patients with frontal lobe damage, who often suffer profound and diverse behavioural and personality changes (Szczepanski and Knight, 2014). The PFC is involved in long-term and working memory (Barbey et al., 2013; Euston et al., 2012), emotional processing (Vertes, 2006), executive control and decision-making (Domenech and Koechlin, 2015; Miller, 2000) and attention (Dalley et al., 2004). It is especially vital in the latter, allowing the organism to focus on and respond to less inherently salient stimuli that are more relevant to the task at hand, and to ignore stronger but irrelevant stimuli (Miller and Cohen, 2001).

The function of the prefrontal cortex depends heavily on its connectivity, both local and inter-areal. After all, information about both internal states and the external cues must be integrated in order to produce adaptive behaviour. In accordance with this, the prefrontal cortex receives input from virtually all higher order sensory and motor cortices and paralimbic cortex (Hoover and Vertes, 2007; Van Eden et al., 1992), as well as hippocampal CA1 (Jay and Witter, 1991) and amygdala (Hoover and Vertes, 2007). It is also extensively innervated by numerous thalamic nuclei (Hoover and Vertes, 2007; Hunnicutt et al., 2014). Specific functions of the PFC rely on afferent connections from other brain regions. For instance, the role of the PFC in attention relies on cholinergic input from the basal forebrain (Bloem et al., 2014; Parikh et al., 2007), whereas its role in working memory is dependent on its interactions with the basal ganglia (O'Reilly and Frank, 2006).

Equally important for proper PFC function is its efferent connectivity. The prefrontal cortex projects to various brain structures, including the amygdala (McDonald et al., 1996), nucleus accumbens (Öngür and Price, 2000), thalamus (Mitchell, 2015), and sensory and motor cortex (Bedwell et al., 2014). Especially its projections to the motor cortex have been proposed to underlie the control of the PFC over behaviour (Miller and Cohen, 2001). Besides projections to and from other brain areas, regions within the prefrontal cortex make extensive connections with each other (Barbas and Pandya, 1989; Hoover and Vertes, 2007). However, the functional relevance of the connections between different areas of the prefrontal cortex is only now starting to be elucidated.

### ***Areas of the prefrontal cortex across species***

The structure of the prefrontal cortex differs considerably across species (Wallis, 2012). The rodent medial prefrontal cortex (mPFC) is generally considered to be homologous to dorsolateral prefrontal cortex (dlPFC) in primates (Uylings et al., 2003). However, the extent





to which rodent prefrontal cortex is homologous to that in primates has long been – and still is – subject of debate (Kolb, 2007; Preuss, 1995; Seamans et al., 2008; Uylings et al., 2003; Wise, 2008). Particularly, the prefrontal cortex in humans and monkeys consists for the most part of granular cortex, which contains a layer 4, while that of rats and mice is agranular cortex, which lacks layer 4. Yet, the rodent mPFC seems to incorporate anatomical and physiological aspects of both the anterior cingulate cortex (ACC) and dlPFC in primates, although it does so in a more rudimentary fashion, and without the ability to encode abstract information (Seamans et al., 2008).

In rodents, the mPFC consists of the ACC, the prelimbic cortex (PrL) and the infralimbic cortex (IL). These areas are defined by cytoarchitectonic criteria. However, there seems to be a functional division between the dorsal mPFC, consisting of the ACC and the dorsal part of the PrL, and the ventral mPFC, made up of the IL and the ventral part of the PrL. There is strong connectivity between cytoarchitectonic areas within both the dorsal and ventral mPFC, while connectivity between these functional areas is less strong (Heidbreder and Groenewegen, 2003). Moreover, these functional areas have different projections, with the dorsal mPFC projecting more to sensorimotor area, and the core of the nucleus accumbens, while the ventral part projects more strongly to the thalamus and nucleus accumbens shell (Berendse et al., 1992; Heidbreder and Groenewegen, 2003; Room et al., 1985; Wright and Groenewegen, 1995). Likewise, the ventral and dorsal parts of the mPFC are also differentially involved in a number of behavioural tasks (Gisquet-Verrier et al., 2000; Levin et al., 2017; Luchicchi et al., 2016).

### ***Development of the prefrontal cortex across species***

The principal neurons and glial cells of the neocortex develop in the ventricular zone, a temporary developmental structure, and then migrate through the intermediate zone to their final position in the cortical plate (Rakic, 2009). The GABAergic interneurons, which make up the inhibitory system in the cortex, do not arise from the subplate, but instead come from the subcortical ganglionic eminences and migrate to their final positions within the cortex and other structures of the brain (Anderson et al., 1997, 2002; Lavdas et al., 1999). Growth of axons and dendrites subsequently allows for the formation of synapses. Historically, the prefrontal cortex is seen as the area of the brain with the slowest development (Alexander and Goldman, 1978; Dumontheil et al., 2008; Kolb et al., 2012). This idea dates back to the turn of the twentieth century, when the prefrontal cortex was shown to be the last area of the cerebral cortex to become myelinated (Flechsig, 1901). Further examination of this phenomenon has been frustrated by opposing findings that may be dependent on species (Bourgeois et al., 1994; Elston et al., 2009; Huttenlocher and Dabholkar, 1997; Oga et al., 2017; Petanjek et al., 2011; Van Eden et al., 1991), although protracted development is clear in human prefrontal cortex.

During development, the cortex undergoes a period of synaptic overproduction, followed

by a period of synaptic pruning that is essential for normal brain function (Katz and Shatz, 1996; Tau and Peterson, 2010). Although the initial overproduction appears to occur simultaneously in all cortical areas, at least in primates (Rakic et al., 1986), it is this process of synaptic pruning that is prolonged in human prefrontal cortex, lasting beyond puberty into adulthood (Gogtay et al., 2004; Petanjek et al., 2011). Consequently, there has been considerable interest in comparing different brain regions and species. In addition, studies have been done to compare cells throughout cortical layers in the same brain region (Rojo et al., 2016; Tsumoto and Suda, 1982) and detail the development of a single cell type (Romand et al., 2011; Zhang, 2004). Direct comparisons of the development of different cortical layers, on the other hand, have been scarce, and in animal models have mostly focussed on the growth of axons and differential innervation of cortical layers (Hand et al., 2015; Larsen and Callaway, 2006), although some have also assessed layer-specific development of dendritic spines (Tjia et al., 2017) and dendrites in humans (Petanjek et al., 2008). However, there has yet to emerge a clear and precise picture of the layer-specificity of neuronal development, with regards to both morphology and physiology. Furthermore, it is unclear whether neuronal development in the rodent prefrontal cortex differs from other areas, and whether it takes place in a layer-dependent manner.

In this thesis, I provide a detailed description of the development of pyramidal cells in both layers 3 and 5 of the mouse mPFC. Chapter 2 describes the development of dendritic morphology, while chapter 3 addresses the development of intrinsic physiology and synaptic inputs of these cells.

### ***Neurodevelopmental disorders***

As mentioned earlier, damage to the prefrontal cortex leads to severe changes in personality and behaviour in humans. Unsurprisingly, the prefrontal cortex has been implicated in many cognitive disorders, including chronic stress-related disorders (Negrón-Oyarzo et al., 2016) and schizophrenia (Honea et al., 2005; Schubert et al., 2015). More and more, schizophrenia is being recognised as a neurodevelopmental disorder (NDD; Brown, 2012; Heyer and Meredith, 2017; Schmitt et al., 2014; Selemon and Zecevic, 2015; Weinberger, 1987). NDDs comprise a wide range of disorders whose aetiology lies in aberrant development of the nervous system. Besides schizophrenia, they include autism spectrum disorders (Volkmar and Pauls, 2003) and many types of syndromic intellectual disability (ID), such as Down syndrome (Vicari et al., 2013), Fragile X syndrome (FXS; Fung et al., 2012), Rett syndrome (Neul and Zoghbi, 2004) and non-syndromic forms of ID (Ba et al., 2013).

A myriad of genes have been linked to neurodevelopmental disorders, and the majority of molecular pathways affected by NDDs are involved in synaptic function (Kroon et al., 2013; Van Bokhoven, 2011). Many NDD gene products interact with Rho GTPases, which are involved in the development and maintenance of dendritic spines (Ethell and Pasquale, 2005;

Tashiro et al., 2000), as well as synaptic plasticity (Murakoshi et al., 2011; O’Kane et al., 2004). Rho GTPases are regulated by two types of proteins, the guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), of which dozens have been identified (Ba and Nadif Kasri, 2017). GTPases need to be bound to GTP in order to exert their function. GEFs activate GTPases by exchanging hydrolysed GDP for GTP, while GAPs deactivate GTPases by stimulating their hydrolysis of GTP, transferring them to their inactive, GDP-bound state (Sasaki and Takai, 1998). Several GEFs and GAPs have been associated with ID (Ba et al., 2013).

### ***Oligophrenin-1***

Among these is Oligophrenin-1, a Rho-GAP that was first identified in human intellectual disability in 1998 (Billuart et al., 1998). Mutation of *OPHN1* is the cause of a syndromic form of ID that is associated with clinical and neuroanatomical features such as cognitive impairments, seizures, strabismus and cerebellar hypoplasia (Billuart et al., 1998; Portes et al., 2004). In mice, knockout of *Ophn1* causes enlarged ventricles and hyperactivity and learning impairments (Khelifaoui et al., 2007). Like many ID-linked GAPs, OPHN1 regulates spine development by negatively regulating RHOA, RAC1 and CDC42 (Billuart et al., 1998; Govek et al., 2004; Khelifaoui et al., 2007). It is interesting in that it has several domains that regulate distinct aspects of neuronal function. OPHN1 interacts with Homer1b/c to stabilise AMPA receptors and regulate basal excitatory transmission (Nadif Kasri et al., 2009; Nadif Kasri et al., 2011). It also binds to endophilin A1 through one of its proline-rich domains, and through this interaction regulates endocytosis of synaptic vesicles (Nakano-Kobayashi et al., 2009). It is likely through this mechanism that synaptic function upon repeated stimulation is affected when *Ophn1* is knocked out (Powell et al., 2012, 2014). This interaction is also necessary for long-term depression (LTD) when elicited both by low-frequency stimulation (Khelifaoui et al., 2009) and activation of metabotropic glutamate receptors (Nadif Kasri et al., 2011). Interestingly, OPHN1 also mediates long-term potentiation (LTP) through its GAP-domain (Nadif Kasri et al., 2009). These different and sometimes opposing synaptic phenotypes may reflect the precise spatial and temporal regulation of GTPase activity (Duman et al., 2015; Soderling and Van Aelst, 2014). Accordingly, OPHN1 may mediate LTP at some synapses and LTD at others, depending on its subcellular distribution and local interactions with particular GTPases and the many other GAPs, GEFs and effector proteins.

### ***Sensitive periods and neurodevelopmental disorders***

Early brain development is likely genetically hard-wired (Chilton, 2006; Marín et al., 2010). At later stages, circuit development is influenced by neuronal activity (Lendvai et al., 2000; Spitzer, 2006), which can either be intrinsic (Golshani et al., 2009; Rochefort et al., 2009) or extrinsic, i.e. due to sensory stimulation (Siegel et al., 2012). While plasticity remains possible in the adult brain of both humans and rodents (Verhoog et al., 2013), there are time-windows

during development during which brain circuitry is particularly malleable and sensitive to external input. These time-windows are known as critical or sensitive periods (Hensch, 2005; Knudsen, 2004). While there are certain clearly defined critical periods, such as imprinting in chicks (Tzschentke and Plagemann, 2006), many brain-related periods that were thought to be critical – having a clearly defined end point – have later been shown to extend into adulthood. I therefore use the term *sensitive period* here.

The most well-studied sensitive period is that of ocular dominance plasticity. When one eye is deprived of input during early postnatal development, neurons in the binocular part of the visual cortex will respond more to the open eye. This effect lasts into adulthood, even if regular function of the eye is restored. The same effect is not seen if the eye is closed during adulthood (Hensch, 2005; Wiesel and Hubel, 1963). Although it was later shown that adult ocular dominance plasticity occurs, it does so to a lesser degree and through different mechanisms (Hofer et al., 2006; Sato and Stryker, 2008). Hence, sensitive periods reflect stages of development during which the potential for plasticity is increased.

We have previously proposed that sensitive periods of development may underlie neurodevelopmental disorders (Kroon et al., 2013; Meredith et al., 2012). The concept of sensitive periods can be applied to neurodevelopmental disorders in two ways. First, NDD pathology could hinder normal plasticity during a neurotypical sensitive period, as molecular pathways involved in NDDs often affect synaptic function. For example, many NDDs change the densities or morphology of dendritic spines (Kaufmann and Moser, 2000; Ramakers, 2002), although the extent to which this occurs has recently been challenged when assessed with superresolution imaging methods (Wijetunge et al., 2014). NDD genes can also affect synaptic plasticity (Huber et al., 2002; Meredith and Mansvelder, 2010; Nadif Kasri et al., 2011). Furthermore, proper GABAergic signalling is necessary for sensitive period plasticity (Fagiolini et al., 2004; Hensch et al., 1998). As many NDDs show aberrant GABAergic transmission (Chattopadhyaya and Di Cristo, 2012), this may lead to altered sensitive period plasticity.

The second way the concept of sensitive periods can be applied is to the expression patterns of NDD genes themselves. Here, the sensitive period is defined as a period during which the gene in question is upregulated during normal development. The impact of dysfunction or absence of the gene is more deleterious during such a period than during times when the gene would normally not be expressed, or be expressed at a much lower level.

Disruption of synaptic function during these sensitive periods could lead to cellular phenotypes that are only apparent at specific ages, or to phenotypes that are dependent on the presence or absence of the NDD gene at specific time points during development. Indeed, such phenotypes have been found, for example, in a *Drosophila* model for FXS. FXS is caused by dysfunction of the Fragile X Mental Retardation Protein (FMRP), the gene product of *FMR1*. Reintroduction of dFMRP, the *Drosophila* homologue of FMRP,

in a *Drosophila* model for FXS is only effective during a specific two-day period, but not in the adult or earlier during development (Gatto and Broadie, 2009), showing dFMRP to be essential during a particular sensitive period. The development of seizure-like behaviour in *Drosophila* can be induced by manipulation of neuronal activity during a specific embryonic sensitive period (Giachello and Baines, 2015). There have also been several studies showing temporary phenotypes in constitutive knockout mice (Bureau et al., 2008; Cruz-Martín et al., 2010; Testa-Silva et al., 2011).

Thus, sensitive periods of increased plasticity could be more vulnerable to disruption of synaptic processes. This may have important implications for treatment of neurodevelopmental disorders (Marín, 2016). In chapter 4, I assess whether knockout of Oligophrenin-1 leads to transient phenotypes in prefrontal cortex.

### ***E/I imbalance in neurodevelopmental disorders***

In addition to excitatory synapses, OPHN1 affects inhibitory synapse function (Powell et al., 2012, 2014). As mentioned earlier, proper balance of excitation and inhibition is crucial for the functioning of neurons and neuronal circuits. The concept is somewhat problematic, because what constitutes proper E/I balance changes between circuits, neurons, and even different parts of the same dendrite. However, many neurodevelopmental disorders show a change in the overall E/I balance (Nelson and Valakh, 2015; Rubenstein and Merzenich, 2003, Selten et al., 2018). It is often difficult to pinpoint the exact cause of E/I imbalance, as excitation and inhibition are intricately linked in circuits, and changes in one often lead to changes in the other. E/I balance is maintained in part by homeostatic plasticity (Turrigiano et al., 1998), which, consequently, has been implicated in neurodevelopmental disorders (Mullins et al., 2016). Evidently, GABAergic signalling plays an important role. Several NDDs have been shown to affect GABAergic synapse function and thereby disrupt the balance between excitation and inhibition [Autism spectrum disorders (Baat and Kooy, 2015; Nelson and Valakh, 2015); Fragile X (Paluszkiwicz et al., 2011a; Sabanov et al., 2017; Wahlstrom-Helgren and Klyachko, 2015); Tuberous sclerosis (Bateup et al., 2013); Down syndrome (Best et al., 2012; Kleschevnikov et al., 2004); Rett syndrome (Durand et al., 2012; Ure et al., 2016)]. Recently, it has been shown that E/I balance is affected in several genetic mouse models for autism, but that this change in E/I balance is a homeostatic mechanism that normalises synaptic membrane depolarisation and spiking in response to stimuli *in vivo* (Antoine et al., 2019). Regardless, inhibitory dysfunction is a major factor in many NDDs, including that caused by *OPHN1* mutation. In chapter 4, I assess whether *Ophn1* knockout affects inhibitory synaptic transmission and E/I balance in the medial prefrontal cortex.

### ***Metabotropic glutamate receptors***

Aberrant excitatory transmission can also lead to E/I imbalance. A form of excitatory

signalling that is particularly relevant to neurodevelopmental disorders is through metabotropic glutamate receptors (mGluRs) (Bear et al., 2004; Conn et al., 2009). mGluRs are G-protein-coupled receptors that modulate neuronal activity through intracellular cascades. The family of mGluRs consists of three groups, comprising a total of eight receptors (Conn and Pin, 1997; Niswender and Conn, 2010). Group 1 consists of mGluR1 and mGluR5. These are bound to G proteins Gq and G11, and via hydrolysis of phosphatidylinositol (PI), and synthesis of inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) cause mobilisation of intracellular calcium, as well as activation of protein kinase C (PKC) (Abe et al., 1992; Houamed et al., 1991; Niswender and Conn, 2010) among a myriad other processes, including those influenced by MAP/ERK and mTOR signalling (Hermans and Challiss, 2001; Hou and Klann, 2004; Page et al., 2006). Both members of group 1 are expressed throughout most of the brain (Baude et al., 1994; Petralia et al., 1997; Romano et al., 1995, 1996; Shigemoto et al., 1992, 1993). However, while the expression of mGluR5 peaks early during development and then decreases to adult levels, mGluR1 levels are highest in adult (Boer et al., 2010; Catania et al., 1994; López-Bendito et al., 2002; Minakami et al., 1992; Scheiwe et al., 2002; Shigemoto et al., 1992). There are also differences in the neuronal populations in which mGluR1 and mGluR5 are expressed. mGluR1 is primarily found in interneurons (López-Bendito et al., 2002; Petralia et al., 1997; Stinehelfer et al., 2000), while mGluR5 is expressed widely in pyramidal cells, but also in some types of interneurons (López-Bendito et al., 2002; Van Hooft et al., 2000). Subcellularly, both mGluR1 and mGluR5 are located perisynaptically around glutamatergic synapses (Ferraguti et al., 1998; López-Bendito et al., 2002; Luján et al., 1996).

Group 1 mGluRs regulate several neurophysiological processes. mGluR5 is primarily involved in the induction of mGluR-mediated LTD (Huber et al., 2001), which has been suggested to be the main component of FXS pathology (The “mGluR theory of Fragile X”; Bear et al., 2004; Huber et al., 2002). mGluR1 activation, on the other hand, depolarises both pyramidal cells (Chuang et al., 2000; Stratton et al., 1989) and interneurons (Chu and Hablitz, 1998; McBain et al., 1994; Van Hooft et al., 2000; Zhou and Hablitz, 1997). Activation of group 1 mGluRs agonists leads to rhythmic activity in several types of interneurons (Beierlein et al., 2000). This effect is mediated by low-threshold-spiking interneurons, which is in agreement with the localisation of mGluR1 in somatostatin-positive interneurons (Stinehelfer et al., 2000), which often have low spiking thresholds (Silberberg and Markram, 2007). However, the distinction between functions of mGluR1 and mGluR5 is not clear-cut. mGluR1 plays a minor but distinct role in mGluR-LTD (Mannaioni et al., 2001; Volk et al., 2006) and mGluR5 can depolarise certain interneurons (Van Hooft et al., 2000). Furthermore, mGluR5 activation regulates gene expression (Wang and Zhuo, 2012; Yang et al., 2006), and mGluR-mediated LTD depends on rapid local protein synthesis (Huber et al., 2000). At the same time, mGluR activation acts on FMRP to negatively regulate local translation of mRNA (Antar et al., 2004; Bassell and Warren, 2008). Thus, FMRP would constitute a brake on mGluR-induced protein synthesis, and without this brake, mGluR-mediated LTD is exaggerated (Bear et al., 2004; Huber et al., 2002).

Indeed, protein synthesis is higher in *Fmr1*-knockout mice, in which FMRP is absent (Qin et al., 2005). This phenotype could be reversed by reduction in mGluR5 expression levels (Dölen et al., 2007). Furthermore, blocking mGluR5 could reverse behavioural and dendritic spine phenotypes (De Vrij et al., 2008; Yan et al., 2005). These promising preclinical results led to clinical trials being conducted in FXS patients. Unfortunately, these trials using mGluR5 antagonists proved ineffective (Berry-Kravis et al., 2016; Jeste and Geschwind, 2016).

There are several explanations for the ineffectiveness of mGluR5 antagonists in these trials. First, similar to what was discussed above, sensitive periods may occur during which proper function of mGluR5 is necessary for the development of neuronal circuits. These clinical trials were conducted on patients that were either adolescents or adults, when most of the neuronal circuitry may already have developed. The preclinical trials mentioned above also used adolescent or adult animals, but the discrepancy may be due to differences in brain development between humans and rodents, or differences in the development of circuits involved in the behaviours tested in mice versus those tested in the clinical trials.

Second, while the GABAergic system is affected in models for FXS (Curia et al., 2009; D'Hulst et al., 2006; El Idrissi et al., 2005), inhibitory function is omitted from the mGluR5 theory of Fragile X. Strikingly, *Fmr1*-knockout mice show reduced spiking of somatostatin-positive interneurons after mGluR activation (Paluszkievicz et al., 2011b).

Third, very little is known about mGluR function in human cortex. One study has shown that mGluR-mediated LTD exists, notably, in human fast-spiking interneurons (Szegedi et al., 2016). There have been several recent papers that show a difference in physiology between human and rodent neurons (Eyal et al., 2016; Testa-Silva et al., 2014; Verhoog et al., 2013). Therefore, in order to determine to what extent the mGluR theory of Fragile X is applicable to human patients, it is crucial to know whether mGluRs function in humans as they do in rodents.

In chapter 5, I confirm the existence of mGluR-mediated LTD in human cortical pyramidal cells. I further assess the effect of mGluR stimulation on spiking behaviour in both pyramidal cells and interneurons, and the consequences thereof on basal excitatory and inhibitory synaptic transmission.

## SYNOPSIS

The cerebral cortex is organised into cell layers that form distinct parts of the cortical circuitry. Although much progress has been made in understanding the structure, organisation and function of neurons in different cortical layers, very little is known about whether neurons within different layers show similar patterns of development, or whether disruption of neuronal function during development affects neurons in different layers in a similar manner.

In this thesis, I aim to determine whether pyramidal neurons in layers 3 and 5 of medial prefrontal cortex develop similarly, and to establish whether *Oligophrenin-1* differentially impacts the development of neurons and circuits in these layers.

In chapter 2 and 3, I assess the development of mPFC pyramidal neurons in wild-type mice. In **chapter 2**, I reconstruct the morphology of pyramidal neurons in layers 3 and 5 of the mPFC to study the development of their dendrites. I show that the development of dendritic morphology of pyramidal neurons shows no major differences between both layers. In **chapter 3**, I use *in vitro* electrophysiology to study the development of intrinsic membrane properties and synaptic connections of layer 3 and 5 pyramidal neurons. I show that while, like dendritic morphology, intrinsic membrane properties develop in similar fashion in both layers. In contrast, synaptic excitation and inhibition show distinct laminar developmental patterns.

In **chapter 4**, I determine whether deletion of *Ophn1* differentially affects pyramidal neurons in mPFC layers 3 and 5. I show that deletion of *Ophn1* does not affect general development of morphology or intrinsic properties of pyramidal neurons in either layer. However, both excitatory and inhibitory synaptic transmission are transiently affected, first in layer 5, and later in layer 3. However, analysis of miniature events and responses to high-frequency stimulation indicate that these phenotypes are a consequence of dysfunction of the presynaptic, rather than postsynaptic, cell.

Finally, in **chapter 5**, I shift focus to the physiology of human cortical neurons. Using electrophysiology, pharmacology and immunohistochemistry, I show that human pyramidal neurons undergo group 1 mGluR-mediated synaptic depression, that Martinotti cells are activated by mGluRs and subsequently increase synaptic inhibition onto pyramidal neurons. These results confirm earlier findings in rodents.