Neurodevelopment, brain vasculature and schizophrenia

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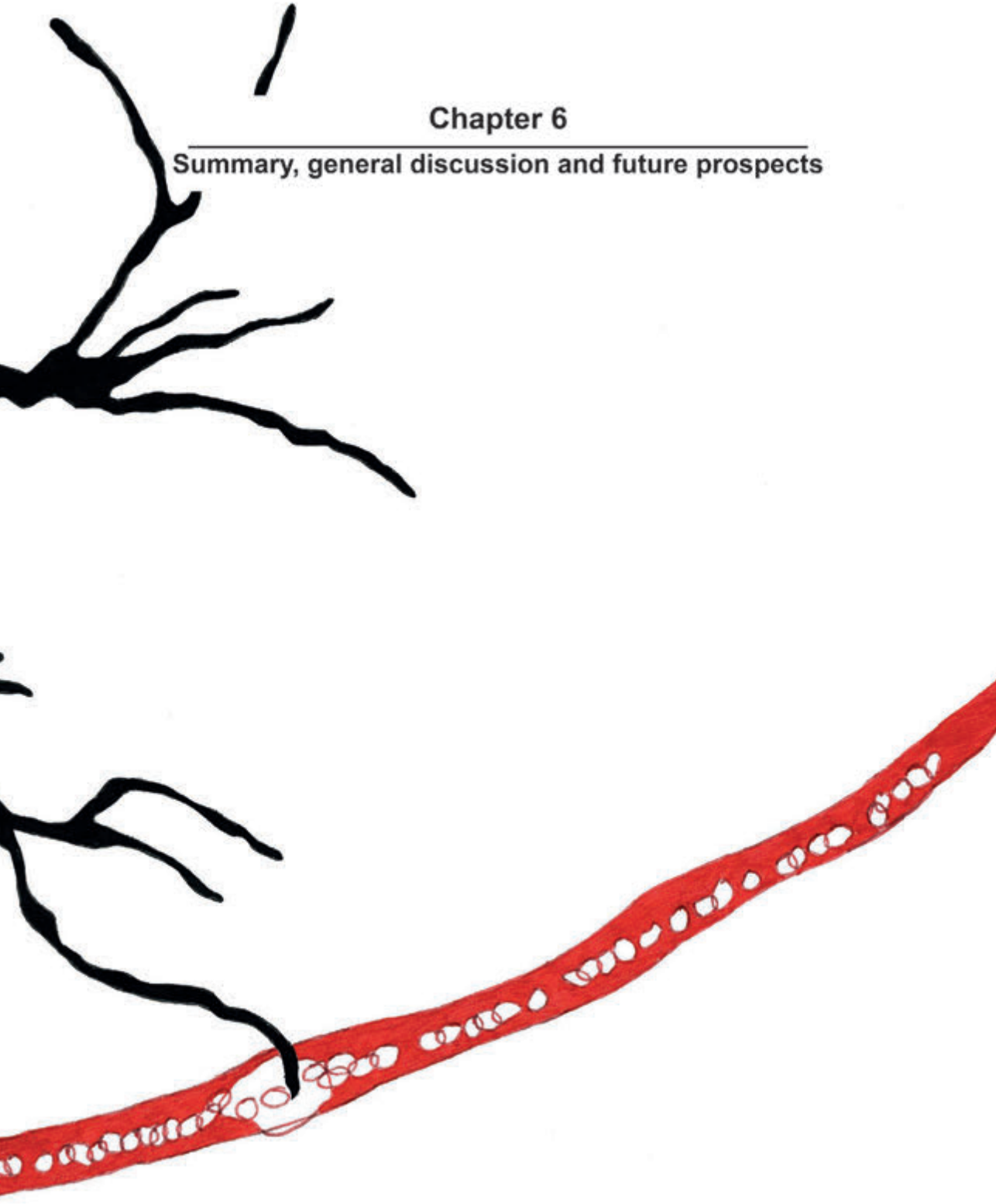
Chapter 6

Summary, general discussion and future prospects



Summary

Schizophrenia is a mental disorder with a neurodevelopmental origin and heterogeneous pathophysiology. Diverse lines of evidence have suggested the contribution of the nervous system and the vasculature to schizophrenia brain pathology; nonetheless, the cellular and molecular mechanisms that trigger and predict the progression of schizophrenia remain unknown.

Post-mortem brain tissues from patients with schizophrenia may harbor information on the cellular and molecular mechanisms involved in the progression of schizophrenia brain pathology. On the other hand, studying neurodevelopment in schizophrenia may help to a better understanding of its etiology. However, because of the polygenic architecture of schizophrenia and the human-specific nature of the traits associated with the disease, there are no reliable animal models for schizophrenia. Therefore, the study of neurodevelopmental aspects related to schizophrenia has been limited. Recent advances in stem-cell culture techniques enabled recapitulation of human neurogenesis using human-induced pluripotent stem cells (hiPSCs), which has facilitated the investigation of human neurodevelopment under normal and pathological conditions.

In this thesis, we studied early neurodevelopmental alterations in schizophrenia using hiPSCs-derived neurons from schizophrenia and control donors. To investigate physiological adult neurogenesis and the possible contribution of the brain vasculature to the pathophysiology of schizophrenia, single-cell gene expression profiling of *post-mortem* brain tissues was performed.

Chapter 1 is an introduction to the subsequent chapters. Here, we mentioned and described the different cell types forming the brain. Also, a brief description of the development of the nervous system and brain vasculature is given. We then introduced schizophrenia and discussed a selection of relevant evidence indicating alterations in both the nervous system and cerebral vasculature of patients with schizophrenia. Finally, we proposed that research on neurodevelopmental aspects and heterogeneity of cerebral vasculature associated with schizophrenia may contribute to understanding the etiology and progression of this disease.

In **chapter 2**, we investigated physiological neurogenesis in adults by profiling the cellular composition of *post-mortem* human subependymal zone (SEZ) with single-nucleus RNA sequencing (snRNAseq). We identified different cellular clusters expressing neural stem cells (NSCs) marker genes, but none of the clusters exhibited particular expression of proliferation-related genes, suggesting that NSCs in the adult SEZ are quiescent.

The relative abundance of NSCs clusters was stable between early and middle adulthood, indicating that the pool of SEZ NSCs is not reduced during this time window. Nonetheless, the expression of genes related to nervous system development was higher in early adulthood, suggesting ongoing central nervous system development in early adulthood that may slow down in middle age. In addition to the transcriptional changes associated with age, the relative abundance of oligo progenitor cells (OPCs) declined along with age, indicating that the cellular composition of human SEZ changes between early and middle adulthood.

The transcriptional profiles of the identified cell types were used to evaluate whether some cell types of the neuroglia lineage could potentially be affected by genetic variation associated with different neurodevelopmental disorders. In this context, we identified several neuronal sub-types enriched for genes associated with schizophrenia, including somatostatin neurons, a cluster of neurons that co-expressed *DLX6-AS1* with *RELN* and two types of medium spiny interneurons. Conversely, the clusters expressing NSCs marker genes did not exhibit enriched expression of schizophrenia-related genes. These results suggested that genetic variation related to schizophrenia might negatively affect the performance of already differentiated neurons rather than glial or progenitor cells.

In **chapter 3**, we used hiPSCs-derived cultures to model neurogenesis and to study possible neuronal bases of brain functional connectivity impairments associated with schizophrenia. We quantified the expression of genes related to synapse maturation, registered spontaneous neuronal activity with calcium imaging, and adapted a functional connectivity analysis, which was previously designed to study whole-brain functional connectivity [1], to quantify resting-state functional connectivity at the neuronal level.

Along the neurodifferentiation process, neuronal networks derived from schizophrenia patients exhibited higher expression of glutamatergic synapse marker genes, suggesting a potential tendency to develop hyper-excitability in schizophrenia.

Schizophrenia and control networks adopted different and recurrent functional connectivity configurations during resting-state. This resembled what has been observed in the human brain using functional magnetic resonance imaging (fMRI), which shows much lower spatial and temporal resolution compared to our *in-vitro* methodology. The presence of dynamic and recurrent neuronal functional connectivity configurations could contribute to a basal state of neuronal activity that provides an adequate starting point to respond efficiently to a changing environment. However, schizophrenia neuronal networks exhibited a reduced repertoire of functional connectivity configurations and were slower in rearranging between different connectivity settings, reflecting reduced dynamism and flexibility of resting-state neuronal functional connectivity associated with schizophrenia. Of note, differences in functional connectivity dynamics between schizophrenia and control neuronal networks followed the same trend of previously described differences in whole-brain resting-state connectivity dynamics between controls and patients with schizophrenia [1]. The findings described in this chapter suggest that alterations in communicational dynamics at the neuronal level might contribute to brain functional connectivity alterations in patients with schizophrenia, by compromising the ability of their neuronal networks for rapid and efficient reorganization through different activity patterns.

In **chapter 4,** we reviewed recent findings reflecting the structure and function of the brain vasculature in schizophrenia. We discussed evidence derived from diverse methodologies, such as *post-mortem*, *in-vitro,* blood and cerebrospinal fluid analyses, imaging techniques, genomic, and transcriptomic studies. According to the reviewed literature, on average, 17% of the patients with schizophrenia exhibit increased blood-brain barrier (BBB) permeability, reflected by higher levels of albumin in the cerebral spinal fluid. The patients with schizophrenia who also exhibited an increased pro-inflammatory signature, exhibited alterations in the brain vasculature and BBB permeability more consistently than the general schizophrenia population. However, it remains unclear whether intrinsic alterations in the brain vasculature of patients with schizophrenia may lead to neuroinflammation, by facilitating the ingression of toxins

and immune cells into the brain parenchyma, or if preceding neuroinflammation affects the brain vasculature and the functioning of the BBB in schizophrenia.

In **chapter 5,** we investigated potential alterations of the cells comprising the BBB in schizophrenia *post-mortem* midbrain tissue with snRNAseq analysis. The relative abundance of the different BBB cell types and subpopulations was similar between schizophrenia and control tissue, suggesting overall preservation of cellular proportions and phenotypes of BBB cells in schizophrenia. In addition, transcriptional differences between schizophrenia and control BBB were limited and specific to ependymal cells and pericytes, suggesting that the cell types of the BBB are not broadly affected in schizophrenia.

General discussion and future prospects

Schizophrenia brain pathology may originate during early nervous system development; nonetheless, overlying schizophrenia-related genes with the transcriptomic profiles of the different cell types in the human SEZ (chapter 2) suggested that neuronal progenitors may not be affected by genetic predisposition to schizophrenia, whereas some sub-types of already differentiated neurons are likely to be affected. In accordance with this, the study of differentiated neurons during early neurodevelopment (chapter 3), modeled with hiPSCs-derived neurons, indicated alterations in neuronal communicational dynamics associated with schizophrenia, which may contribute to brain functional connectivity anomalies in schizophrenia patients. Our findings suggested that impairments in neuronal communication may already be present during early developmental stages in schizophrenia, compromising the ability of the nervous system for fast and efficient reorganization. This could converge in the development of neural circuits that are more sensitive to harmful or stressful external factors.

Given the strong relationship between the establishment and functioning of the nervous and brain vascular system, altered neuronal communication dynamics during early development might have a negative influence on the correct functioning of the brain vasculature in patients with schizophrenia. In contrast to this hypothesis, our study of *post-mortem* brain tissue (chapter 5) identified few transcriptional changes between schizophrenia and control vasculature, which were limited to pericytes and

ependymal cells, suggesting no major involvement of the brain vasculature in schizophrenia.

BBB functioning could vary along with schizophrenia onset and progression

Previous studies using hiPSCs-derived BBB models pointed to impaired barrier function in schizophrenia, reflected by reduced transendothelial electrical resistance and increased permissiveness to monocyte transmigration [2-5]. hiPSCs-derived BBB models may resemble early developmental stages of the brain endothelium; therefore, the impaired functioning of hiPSCs-derived BBB from patients with schizophrenia could suggest that genetic variation associated with schizophrenia negatively affects the brain vasculature and the functioning of the BBB during early stages of development. Brain endothelial gene expression and performance vary along with development. Thereby, the expression of schizophrenia-related genes might also vary along with the establishment of the BBB. For instance, the strongest independent genetic association with schizophrenia has been reported in *FZD1* [6], a hypoxia-response gene that is expressed 600-fold higher in early development endothelium compared to endothelium in the adult brain [7]. Thus, some possible alterations in the brain vasculature and in the functioning of the BBB of people with schizophrenia could have occurred in earlier stages of development and may not be detectable in the adult brain, which could partially explain the lack of extensive differences in gene expression profiles between schizophrenia and control *post-mortem* tissue (chapter 5).

Modulation of the integrity and permeability of the BBB is dynamic and varies along with physiological changes, such as normal development and during pregnancy, and in response to extrinsic environmental factors, such as oxidative stress (reviewed in [8]). In addition, it has been demonstrated that microglia-vasculature interactions can modulate BBB permeability. For instance, in response to peripheral pro-inflammatory signals, microglia accumulate around cerebral vessels and protect the integrity of the BBB through CLDN5-mediated tight junctions between microglia and endothelial cells. After sustained inflammation, microglia become activated and disrupt BBB integrity; thereby, increasing BBB permeability [9].

Schalbetter et al., 2022, demonstrated in mice the presence of critical developmental time windows of higher vulnerability, such as adolescence, when transient depletion of microglia causes synaptic and cognitive sequelae in later life stages, as opposed to

adulthood when transient microglia depletion failed to induce cognitive effects [10]. The percentage of microglia directly contacting the brain capillaries changes during lifespan, with higher percentage of juxtavascular microglia during CNS development compared to adulthood [11]. Therefore, there could also be time windows of increased sensitivity to perturbations in BBB permeability, as exist for microglia perturbations, possibly during CNS development when the barrier is not yet fully established. Indeed, maternal inflammation, a risk factor for schizophrenia, was recently shown to disrupt BBB formation in the fetuses through the activation of juxtavascular microglia, leading to chronic inflammation that persists throughout the offspring's lifespan [12]. Thus, disturbances in the functioning of the BBB during particular stages of development, or particular stages of disease progression, might be sufficient to contribute to schizophrenia brain pathology and neuroinflammation, even though these alterations are not chronically observed. Future longitudinal assessment of the BBB permeability in people with high-risk for developing schizophrenia, with positron emission tomography (PET) scanning or measuring cerebral spinal fluid albumin, would contribute to understand some potential changes in the brain vasculature that could dynamically vary along with disease onset and progression.

Identifying the potential contribution of different cell types to schizophrenia brain pathology throughout development

To identify the particular cell types that might contribute to schizophrenia neurovascular dysfunction during development, it may be useful to overlap schizophrenia-related genes, obtained from genome-wide association studies, with the transcriptomic profiles of the different cellular components of the neurovascular unit, obtained at distinct neurodevelopmental stages with single-cell transcriptomics. This approach would shed light on the cell types or cell states that, during particular developmental stages, may express the genes conferring higher risk for schizophrenia. Nonetheless, *post-mortem* tissue is rare and difficult to obtain, especially if it is desired to come from humans at certain developmental stages. Given that hiPSCs differentiation may serve to model human physiological development [13, 14], and that all major brain cell types, such as neurons, glial, pericytes, and brain endothelial cells, can now be differentiated from hiPSCs, they can serve as a valuable source to obtain the transcriptomic profiles of the neurovascular cells throughout development [145].

Understanding the effect of gene-environment interactions associated with schizophrenia

Besides a genetic predisposition conferring higher risk for schizophrenia, the etiology and progression of schizophrenia are driven by diverse environmental factors. The two-hit hypothesis of schizophrenia posits that a genetic predisposition to schizophrenia interacts with a perturbed intrauterine environment, affecting the normal neurodevelopmental trajectory and leading the organism prone to a pourer outcome after second stressful events during post-natal life, which would trigger schizophrenia during adulthood [15-18]. Although several risk factors have been identified, for instance intrauterine hypoxia, maternal inflammation, obstetric complications, among others [18], it is still not clear how these lead to schizophrenia. *In-vitro* models of schizophrenia, such as patient-derived hiPSCs, resemble a genetic predisposition to the disease and enable the manipulation of culture growth conditions, which could help to decipher the effect of risk factors that interact with the genetic variation associated with schizophrenia. For instance, our neuronal functional connectivity analysis may be conducted in hiPSCs-derived neuronal networks challenged with different risk factors. This would help elucidating potential effects of environmental interactions with a schizophrenia genetic predisposition particularly on brain connectivity, which seems widely affected in schizophrenia. This culturing methodology could also be applied to hiPSC-BBB models derived from schizophrenia patients. Challenging hiPSC-BBB models derived from schizophrenia and controls with pro-inflammatory signals may help understand the possible interaction between inflammation and the genetic predisposition to schizophrenia on BBB functioning (discussed in chapters 4 and 5).

Furthermore, recent advances in hiPSCs culturing allow three-dimensional modelling of human neurodevelopment, and even more, the possibility of three-dimensionally reconstruction of the neurovascular unit, by combining different cell types differentiated from hiPSCs into vascularized brain organoids [19]. Exposing control and schizophrenia-derived vascularized organoids to different experimental conditions, simulating different risk factors, could provide an overview of the potential effects of gene-environment interactions associated with schizophrenia on the neurovasculature. This experimental model could be studied with single-cell transcriptomics, which would reveal how the interaction between a genetic

predisposition to schizophrenia and risk factors affects, perhaps differentially, the distinct cell types comprising the neurovascular unit.

Conclusion

In conclusion, this thesis showed that neuronal communication may be impaired during neurodevelopment in schizophrenia, while the adult brain vasculature seems not greatly affected by the disease. Nonetheless, it remains unclear whether the brain vasculature might be affected during particular timings of development or disease progression. Future longitudinal measurements of BBB permeability in high-risk individuals and experiments with *in-vitro* models of schizophrenia would contribute to a better understanding of schizophrenia pathophysiology.

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