Glial Cells

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12.1 Introduction

Rudolf Virchow made the first description of glial cells in the mid-nineteenth century when he described a neuroglia connective tissue ('nervenkitt') that embedded and maintained nerve cell structure [1]. Otto Dieters reported the first description and of an astrocyte [2], and after the invention of appropriate histological staining by Santiago Ramon y Cajal, Camilo Golgi, and Pio del Rio Hortega, it became possible to describe the morphology of astrocytes and begin to unravel their diversity and morphological states [3]. The term astrocyte, derived in English from Astrocyten, was from Michael von Lenhossek, who suggested that the star-shaped glial cells should be named either spider or star cells to describe the shape they made with multiple processes emanating from their centre [4]. Astrocytes are typically detected by antibodies to GFAP or s100β. Neither of these stain all astrocytes present and so we are likely not witnessing the total behaviour of these cells in schizophrenia and associated disorders. Furthermore, we are now seeing the further separation of these types in function subtypes, such as reactive or fibrillary astrocytes and non-reactive or gemistocytic astrocytes (see Figs. 12.1, 12.2 and 12.3), and seeing how these subtype changes may be responsible for the changes observed [5–7]. In oligodendrocytes, we have additional problems as a reliable antibody for oligodendrocyte identification in formaldehyde-fixed tissue has been elusive, although recently

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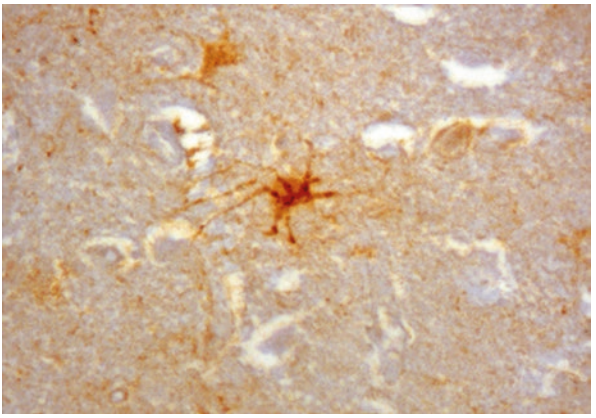

Fig. 12.1 A GFAP-immunohistochemical stained fibrillary or reactive astrocyte observed at x400 magnification. The multiple processes projecting from the cell body can clearly be observed

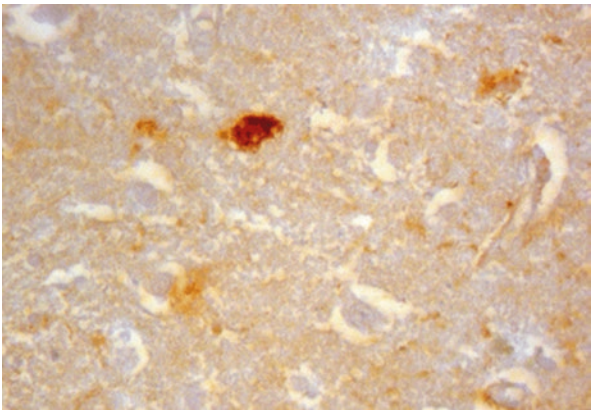

Fig. 12.2 A GFAP-immunohistochemical stained gemistocytic or reactive astrocyte observed at x400 magnification. The dark staining in the cell body is clear, with no processes observed. A gemistocytic astrocyte may sometimes have a single, large, darkly stained process

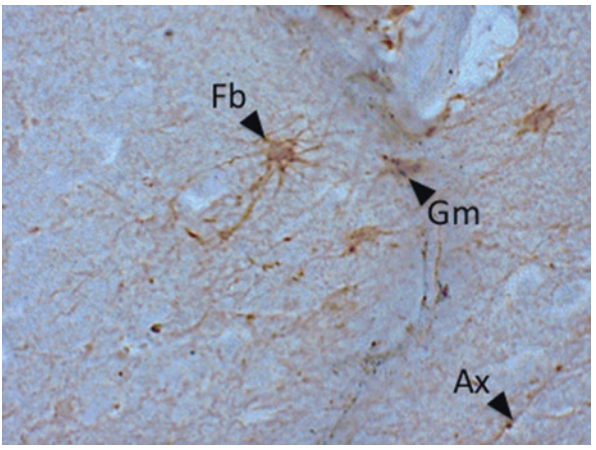

Fig. 12.3 GFAP-labelled section showing multiple stained astrocytes. Gemistocytic astrocytes are identified by the deep cell body stain, oval or rectangular shape and either no processes or a single, thick process. Fibrillary astrocytes are characterised by a clearly stained cell body with multiple, thinner processes. *Gm* gemistocytic, *Fb* fibrillary, *Ax* GFAP-reactive axon

ADAM-12 has been reported to have some success in this respect [8]. The conflicting findings described previously in dorso-lateral prefrontal cortex (dlPFC) white matter using different stains suggest this could be a key issue in the future of neuropathological investigation of schizophrenia. Microglia have not been shown to have a substantial role in schizophrenia using pathological methods, although some PET studies have suggested an elevation globally in cortical grey matter, although some studies have suggested an increase in subcortical microglia associated with acute psychosis [9–12]. More recent theoretical models of schizophrenia, including a link between microglia and oligodendrocytes in maintenance of white matter axons [10, 13–15], suggest the role of microglia in this disorder may be subtler than previously thought. This is demonstrated clearly in the white matter microglia density studies described previously, as it is suggested that neuroinflammation in schizophrenia may be associated with white matter pathology by linkage with axonal degradation, myelin phagocytosis and oligodendrocyte density.

Therefore, it is not functionally useful to pay attention to combined total glial but rather to separate into the glial types described. As the results of studies on neuropathological findings on glia cells populations have been described by anatomical location in previous chapters, here we examine a specific factor in each glial cell type biology that is related to the pathology of schizophrenia.

12.2 Astrocytes

Many symptoms of schizophrenia and associated psychosis have been suggested to have a biological cause originating in the cortex [16], although competing pathological and imaging studies are renowned for showing a complex mixture of results. In recent years, the link between cortical glutamate (GLU) and psychosis has been strongly supported by experimental evidence.

As glial cells have received more attention in research, it has become clear that the role of astrocytes in neuronal function is more diverse and critical than was originally assumed. Astrocytes were first described in 1893 by Michael von Lenhossek, the name reflecting the star-like structure of their fibrillary morphological form and are now understood to have many roles in normal neuronal function. They are thought to make up to 30% of CNS cells in the mammalian brain [17, 18], although they seem to be rarer in non-mammalian animals [19–23]. A critical function of astrocytes is the regulation of GLU, an amino acid and the most prevalent excitatory neurotransmitter in the brain [24]. GLU is released in the cortex by excitatory interneurons, found predominantly in cortical layers II and IV, and is a key neurotransmitter in most aspects of normal brain operation such as cognition, memory and learning and several biological processes including the control of synaptic plasticity, cell migration, differentiation and cell death. GLU is released from vesicles in presynaptic terminals by a Ca^{++}-dependent mechanism that involves N- and P/Q-type voltage-dependent Ca^{++} channels, and GLU-neurotransmission is mediated or affected by metabotropic and ionotropic GLU receptors. These receptors are each subdivided into three groups. Group I metabotropic glutamate receptors (mGluR1 and mGluR5) are mainly

post-synaptic, and groups II (mGluR2 and mGluR3) and III (mGluR4, mGluR6, mGluR7 and mGluR8) are primarily presynaptic and modulate neurotransmitter release. Ionotropic GLU receptors are amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) [25].

High activation of GLU receptors is dangerous as GLU is toxic in high concentrations inducing cell death. The mammalian nervous system has an evolutionarily conserved mechanism to regulate GLU uptake, with homeostasis of GLU maintained by synaptic release from glutamatergic cells and uptake to glial cells through GLU absorption. To regulate extracellular GLU levels, the CNS has a complete family of high-affinity excitatory amino acid transporters (EAAT1–5). EAAT1 and EAAT2 are expressed in glial cells and EAAT3 expressed peri-synaptically on neurons, possibly playing a more central role in regions where astrocytes are less dense such as in the hippocampus, or in situations where there is an excess of GLU such as ischemia. EAAT3 also transports cysteine, contributing to the synthesis of intracellular glutathione, an important antioxidant providing an additional neuroprotective effect especially in early CNS development [26]. EAAT4 and EAAT5 found mainly in the cerebellum and retina, respectively, are also expressed peri-synaptically on neurons and can act as uncoupled Cl ion channels providing a physiological clamp on membrane potential and may act more like inhibitory GLU receptors [27]. They have a much higher affinity for GLU than EAAT1, EAAT2 or EAAT3 but are situated much further away from synaptic junctions and are therefore thought to be involved in 'mopping up' excess GLU [28, 29]. The five trans-membrane GLU-transporters are encoded by solute carrier family SLC1 genes crucial in the regulation of GLU homeostasis in neurons in the brain and other organs (detailed in Table 12.1). The transporters are ATPase-coupled ion transporters; the influx of GLU coupled with 3Na+/1H and the efflux of 1K+ where it is converted to glutamine by glutamine-synthase and recycled to the pre-synaptic neuron. The transporters can also act as uncoupled chloride ion channels, typically in response to the build-up of extracellular K+, and so help to maintain the membrane potential at physiological levels [30–32].

GLU transporters form a transmembrane protein of eight helices with distinct regions involved in ion transport and an associated scaffold domain [33]. Functional transporters exist in the membrane as homo-trimers although there is some evidence that EAAT2 and EAAT3 can form hetero-trimers in the membrane. This alternative configuration has been shown to alter receptor localisation within the membrane, without appearing to affect functionality [34].

EAAT1 and EAAT2 are expressed at different levels in different brain regions and is selectively expressed in astrocytes in the brain [35]. EAAT1 is highly present in specialised glial cells such as the Bergmann glia of the cerebellum [36], the Müller cells of the retina and supporting glia in the vestibular end organ, inner ear [37]. EAAT1 is regulated in multiple levels with the results of important changes in the EAAT1 expression on the plasma membrane. All these changes are dependent on the phosphorylation status of EAAT1, which is regulated by several kinases. EAAT2 is the most abundant GLU transporter, so is also highly expressed in astrocytes in all parts of the brain and spinal cord.

Table. 12.1 The five primary glutamate transporters encoded by solute carrier family 1 (*SLC1*) genes crucial in the regulation of glutamate homeostasis in neurons in both the brain and other organs

Gene	Human gene locus	Gene size (bp)	Exons	Introns	Splice variants	Post-transcriptional modification	Protein name	Protein size	Isoforms	Substrate	Distribution in CNS
SLC1A1	9q24	97,043	14	13	3	Glycosylated (acetylation, phosphorylation, ubiquitination)	EAAC1 EAAT3	524 AA 57,100 Da	2	L-Glu, D/L-Asp L-Cys	Neurons
SLC1A2	11p13-p12	168,859	16	18	8	Glycosylation (acetylation, ubiquitination, sumoylation, palmitoylation, phosphorylation)	GLT-1 EAAT2	574 AA 62,104 Da	2–5	L-Glu, D/L-Asp	Astrocytes (some neuronal)
SLC1A3	5P13	81,980	12	17	12	Glycosylation Ubiquitination	GLAST EAAT1	542 AA 5972 Da	2–9	L-Glu, D/L-Asp	Astrocytes (some neuronal)
SLC1A6	19	72,957	15	19	9	Glycosylation (acetylation, dimethylation, phosphorylation)	EAAT4	564 AA 61,565 Da	2–5	L-Glu, D/L-Asp	Neurons (cerebellum, Purkinje cells)
SLC1A7	1p	55,435	13	11	4	Glycosylation	EAAT5	50 AA 60,658 Da	4	L-Glu, D/L-Asp	Neurons, glia (retina)

Once taken up by the astrocyte GLU is metabolised to glutamine- the GLU precursor. The GLU-glutamine pathway can occur in neurons but recent evidence shows it may primarily be a glial-determined process, mainly conducted by astrocytes around the synaptic cleft. This proximity allows fast GLU uptake and efficient recycling of astrocytic glutamine back to the neuron [38], and also regulate the diffusion of GLU into the extracellular space [39, 40].

EAAT dysfunction has been implicated in a variety of neurodegenerative and neurological diseases, with a very special downregulation of EAAT2 in amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, ischemia and epilepsy. The perturbation of glutamatergic neurotransmission by a decrease/loss of GLU transporter activity/expression can be regarded as a major cause for a number of neurological disorders and to be crucial for distinct neurodegenerative diseases. However, GLU abnormalities may also disturb brain function and underpin psychotic symptoms and cognitive impairments.

Genetic depletion in EAAT1 & EAAT2 double knockout mice has shown they are both required for brain development through regulation of extracellular GLU concentration [41]. EAAT2 deletion in mice has shown a very important reduction of GLU uptake activity, increased levels of extracellular GLU and induce hyperactive, epileptic phenotype accompanied by moderate mild sensorimotor impairment, hyperlocomotion lower anxiety, better learning of cue-based fear conditioning [36]. EAAT1 deletion in mice has shown insufficient GLU uptake in all region where EAAT1 is the predominant transporter, and display poor nesting behaviour, abnormal sociability and impaired learning, proposing that gene deletion of EAAT1 could be the source of select abnormalities related to the negative and cognitive symptoms of schizophrenia [42]. In humans the reduced expression of EAAT1 and EAAT2 have been correlated with impaired cognitive functions in schizophrenia patients [43]. GLU transporter expression and function are regulated both pre- and post-transcriptionally via a variety of mechanisms which have been widely characterised for EAAT2 and EAAT 1 in both rodents and humans both in vivo and ex vivo; less is known about the regulatory mechanisms of EAAT3, 4 and 5. The most basic regulatory mechanism is via a physiological feedback loop as glutamate transporter function optimally at normal membrane potentials [31].

Transcriptional upregulation of EAAT1 and EAAT2 resulting in increased protein expression and/or function has been documented in response to a variety of growth factors including oestrogen, TGF-alpha [44], IGF-1 [45], EGF [46] and PACAP [47]. The SLC genes contain promoter regions in the 5′ region that contain consensus sites to numerous nuclear transcription factors such as NFκB, CREB, c-Jun and mTOR. These can be activated by growth factors via downstream receptor signalling pathways such as PI3K/AKT, ERK/MAPK or Protein Kinase A, resulting in increased gene transcription. Growth factors such as TGF-alpha have also been shown to bind directly to regulatory transcription factor binding sites within the gene additionally promoting transcription.

Downregulation of the SLC genes is less well characterised; however, manganese is known to decrease the function, localisation and expression of transporters and is thought to act via the Protein Kinase C-induced activation of the novel

Transcription Factor YY1 [44, 48]. TNF-alpha and IL-1 beta have also been shown to down regulate transcription although the pathways remain unclear [44].

Post-transcriptional regulation has been shown to occur via alternative splicing of the gene and epigenetic modification. Multiple splice variants of EAAT1 and EAAT2 have been characterised (Genecard: GC05P036606 and GC11M035272) [33, 49]. Functionally these splice variants are not different, but they have been shown to have differential localisation within neurons and astrocytes which may affect function.

Histone acetylation has been shown to increase/stabilise GLU transporter mRNA whilst DNA methylation has differential effects on protein expression [50]. Hypo- and hyper-methylation have been associated with both increased and decreased EAAT2 expression in vivo, a discrepancy that may be due to the specific site being modified by methyl-transferase within the gene. MicroRNA is usually associated with the destabilisation of mRNA and decreased translation, although miR-124a has been shown to consistently increase EAAT2 protein expression [51]. miR-124a can transfer the astrocytes from the neuron via exosomes which precipitate direct interaction with mRNA. Whilst the exact mechanism remains unclear, this indirect interaction could explain this unusual effect. All GLU transporters are post-translationally glycosylated within the endoplasmic reticulum to allow insertion into the plasma membrane and formation of homotrimers. EAAT2 has also been shown to be additionally palmitoylated and sumoylated [50]. EAAT1, EAAT2 and EAAT3 also undergo ubiquitination. These modifications effect membrane cycling and therefore regulate cell surface expression and function. Ubiquitination and sumoylation both result in the retention of glutamate transporters intracellularly and subsequent degradation [52] [50]. Ubiquitination has been shown to be promoted by Protein Kinase C which also has a role in transcriptional downregulation.

The number of transporters in the plasma is a key regulatory mechanism, their subsequent localisation also affecting function. EAAT2 and to a lesser extent EAAT1, EAAT3 and EAAT4 are associated with cholesterol-rich lipid rafts found on both neurons and glia [33, 53]. This could aid in the effective formation of homotrimers or concentrate receptors in the synaptic cleft during nerve transmission. Membrane cholesterol depletion has been shown to result in decrease GLU uptake suggesting a regulatory function.

Electron microscopy shows EAAT1 and EAAT2 is found in astrocytic soma and processes, specifically in small astrocytic processes adjacent to axon terminals forming asymmetric GLU synapses. Whilst EAAT2 labelling was most prevalent in these astrocytic processes, EAAT1 labelling was also present in neuronal processes including the soma, axons and dendritic spines, showing the dual, but weighted, regulatory pathways involved in cortical GLU regulation [54]. Direct immunohistochemical examination of mouse cortex shows co-localisation of EAAT4 with cellular GFAP, a classic astrocytic marker [55].

C6 cell lines, astrocyte-like cells obtained from rat gliomas, are often used to study cellular functions such as GLU uptake and glutamine synthetase activity. Treatment of C6 cells with Riluzole, an approved drug for the treatment of amyotrophic lateral sclerosis, has shown to stimulate GLU uptake and augmented the

expression of the GLU EAAT1, directly supporting the role of astrocyte-type cells in regulating GLU [56]. RT-PCR analysis has also demonstrated mRNA expression of EAAT4 in astrocyte cultures [55], with additional cell culture studies demonstrating that EAAT1 and EAAT2 are expressed by morphologically distinct glial-fibrillary-acid-protein (GFAP)-positive astrocytes following their transformation from gemistocytic to fibrillary type [56, 57], and their expression correlates with the status of neuron differentiation, maturation and activity [57]. Up-regulation of lipocalin-2 (LCN2) marker has also been reported in reactive astrogliosis, although not to the same extent as GFAP. The function of this is not clear, as LCN2 is more typically involved in immune regulation, although this has not been shown to be its endogenous function in astrocytes [58, 59].

Activation of reactive astrogliosis is a complex process not well understood. Extracellular nucleotide binding to astrocytic P2 purinergic receptors (P2Y) has been shown to be a key trophic signalling pathway, particularly for the induction of stellation—the astrocyte formation of the long processes distinctive in the fibrillary state. Nucleotide binding to P2Y acts mainly through mitogen-activated protein kinase (MAPK) cascades, with trophic signal activated via the extracellular signal-regulated protein kinases (ERK) pathways, and mediating signals regulated via stress-activated pathways c-Jun N-Terminal kinases (JNKs) and p38 [60]. The trophic signals, after activation via ERKs, are transmitted through c-Fos, Elk1, STAT3 and GSK-3β pathways within the astrocyte to regulate cell behaviours such as stellation and movement. Nucleotides can also act in concert with polypeptide trophic factors such as glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) to magnify trophic signalling on astrocytes [61].

Functional differences linked to astrocyte morphology have been described in astroglial scars, as the astrocytes within derive almost entirely from newly proliferated cells with elongated shapes, fitting the description of gemistocytic astrocytes [62]. Fibrillary astrocytes are identified by the presence of many visibly stained processes emanating from a single cell body, although more recent findings suggest there may be two distinct populations of fibrillary astrocytes that could exist either in a continuous heterogeneous state or in multiple distinct activation states [63].

In contrast, gemistocytic astrocytes have substantially larger more-swollen cell bodies, often with a rectangular or cylindrical shape, and either no visible processes or a single, thicker process. With clear staining and microscopy, these can be distinguished clearly by eye. Astrocytes that become activated and undergo a series of morphological and functional changes termed astrogliosis. Morphologically, the continuous process of transformation, causing a relatively high intra-group heterogeneity, means that there can be individual cells which can be difficult to classify, although fractal analysis has suggested there are three types of astrocyte morphology [64], the difference is only detectable using detailed mathematical analysis and not visible to the eye, and therefore is of no use to researchers or clinicians using pathological methods. Astrocytes have also been found to have distinct ‘domains’ in pathological samples, with only the most distal ends of the processes overlapping between adjacent cells. The overlap of the process tips shows connecting gap

junctions when examined using ultrastructural methods, allowing cell–cell communications. This organisation has been shown in grey and white matter and means that the entire brain is covered in a continuous network of astrocytic processes [65, 66].

There are also methodological issues, such as astrocyte staining in frozen a formalin-fixed tissue requiring different antibodies and protocols. Astrocytes are typically identified using S100β and glial fibrillary acid protein (GFAP) antibodies in pathological samples, although neither antibody binds to even a majority of target cells S100β 10–30% of astrocytes, GFAP 25% [18, 67]. This distinction is potentially functionally important as S100β, a member of an EF-hand-type family of Ca2+−binding proteins found diffused throughout the cytoplasm, cytoskeleton and in cell membranes, has been implicated in the regulation of the morphological state of astrocytes [68]. Similarly, GFAP is likely involved in control of astrocyte morphology and movement [69].

Reports go back some decades of an increase in fibrillary gliosis using Holzer's stain in the periventricular structures of the diencephalon, the periaqueductal region of the mesencephalon, the basal forebrain, hypothalamus, midbrain tegmentum, and substantia innominata in schizophrenia cases [70], although subsequent analysis has suggested that this may well be a result of schizophrenia cases having a concurrent disorders with their own neuropathologies [71–73]. More recently, morphological astrocyte change in the human brain has been directly reported in the subgenual cingulate cortex in schizophrenia with psychosis where fibrillary, but not gemistocytic, astrocytes were decreased [5, 7], and in the dorsolateral prefrontal cortex where astrocyte morphology is changed in schizophrenia along with density [74]. As both these structures are heavily implicated in schizophrenia symptomatology, these pathological findings do support the hypothesis presented. This suggests that not only astrocytes are critical in the pathophysiology of psychosis via the GLU pathway but that the different morphological states observed correspond to very different functional roles, an under-examined area of pathology for some time [75]. Whilst this research is at an early stage, we would suggest that future studies should attempt to take morphological state into account along with cell numbers, clustering and density as is currently a normal practice.

12.3 Oligodendrocytes

Oligodendrocytes are highly specialised glia whose primary role is the wrapping of the axon in a myelin sheath to aid action potential conduction [76], a process conducted by mature oligodendrocytes spiral-wrapping plasma membrane extensions around axon internodes [77, 78]. They were discovered by Pío del Río Hortega (1882–1945) along with microglia [79].

Research into oligodendrocytes has been less consistent than other glial cell types. The main role of oligodendrocytes has always been thought to be in myelinating CNS axons [76, 79, 80], and as schizophrenia is not a typically demyelinating disorder, these cells may have less relation to the causes of the illness than

astrocytes or immune cells. The underlying mechanisms behind oligodendrocyte are still poorly understood, but to maintain axonal integrity, mammalian myelin-forming cells require the expression of some glia-specific proteins, such as CNP, PLP and MAG, as well as intact peroxisomes. None of these are necessary for myelin assembly. Loss of glial support causes progressive axon degeneration and possibly local inflammation, both of which are likely to contribute to a variety of neuronal diseases in the central and peripheral nervous systems [80].

Secondly, there are practical staining problems. Oligodendrocytes are more routinely identified using immunohistochemistry, with probes such as Olig1, NOGO, MOG and CPNase used by research groups and tissue banks worldwide. However, they are only effective in frozen tissue, and obtaining good-quality well-matched frozen tissue for schizophrenia research has been an insurmountable block for many research groups. Overwhelmingly, histopathological research is performed on formalin-fixed paraffin-embedded tissue which has so far proved resistant to antibodies for labelling oligodendrocytes.

Even with these experimental limitations, oligodendrocytes have shown to be disrupted in schizophrenia in the prefrontal cortex, cingulate and deep white matter (see Chaps. 4, 7 and 11).

There has been considerable interest in one oligodendrocyte-specific protein, 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) has been implicated in the maintenance of axonal integrity and is essential in generating and maintaining cytoplasm within the myelin compartment. CNP directly organises the actin cytoskeleton, providing an intracellular scaffold that acts to resist membrane compaction by myelin basic protein (MBP). A system of cytoplasmic channels within the growing myelin sheath enables membrane trafficking to the leading edge. The majority of these channels close as development progresses but can be reopened in adults by experimentally raising phosphatidylinositol-(3,4,5)-triphosphate levels, a phospholipid found in the plasma membrane, which has the effect of reinitiating myelin growth, suggesting the assembly of myelin as a multi-layered structure [77, 78, 81–83].

There is evidence that the exonic single-nucleotide polymorphism rs2070106 is associated with CNP expression, with under-expression of CNP mRNA in schizophrenia and with the lower-expressing A allele significantly associated with schizophrenia [84]. Prior studies have found decreased mRNA expression of oligodendrocyte-associated genes in the dlPFC of patients with schizophrenia. However, it is unclear which specific genes are affected and whether the changes occur in the cortical white or grey matter. Examination of mRNA expression levels of four oligodendrocyte-related genes, myelin-associated basic protein (MOBP), myelin-associated glycoprotein (MAG), CNP and oligodendrocyte-lineage transcription factor 2 (OLIG2) in dlPFC white and grey matter using quantitative-PCR and high-risk polymorphisms in CNP and OLIG2 on mRNA levels of these genes revealed changes between schizophrenia and control cases. Genetic polymorphisms in CNP (rs2070106) and OLIG2 (rs1059004 and rs9653711), previously linked with schizophrenia, predicted low expression. Expression of MAG, CNP and OLIG2 between schizophrenia and controls in the grey or white matter, and MOBP

and CNP protein in the white matter was unchanged between the experimental groups [85]. This is of particular interest as OLIG2 targets chromatin remodellers to enhancers to initiate oligodendrocyte lineage progression and maturation [86].

Even if this field is still in an early stage, there is compelling evidence that common single-nucleotide polymorphisms with alleles associated with schizophrenia have a distinct role in the development and function of oligodendrocytes and that this may have a similar effect in their morphology. The difficulty of using oligodendrocyte morphology in neuropathology studies in this field has been discussed in several papers in various contexts [7, 74, 87–96], although recent ultrastructural examination of oligodendrocytes in the dlPFC by morphometry and electron microscopy showed oligodendrocyte swelling, vacuolation, paucity of ribosomes and mitochondria and accumulation of lipofuscin granules in the schizophrenia as compared to controls. Morphometry detected a significant reduction of mitochondria and the increase in lipofuscin-granules and vacuoles in oligodendrocytes in the schizophrenic group as compared to controls [97]. Given these findings, it may be worth future neuropathological studies refocusing their attention on these glial cells to identify consistent morphology traits that could be linked with schizophrenia.

12.4 Microglia and Macrophages

Macrophages were first discovered late in 1884 by Ilya Metchnikoff. They are an evolutionarily conserved phagocytic cell type thought to have been present for over half a billion years [98, 99]. Whilst the idea that all macrophages in body tissues are derived from the blood has now been superseded by the understanding that many tissue types have their own resident macrophage populations [100–105], microglia have been termed the tissue-specific ‘macrophages of the brain’, and what neuroscientists call macrophages are those larger immune cells that move from the blood population [106, 107]. Microglia are extremely active cells within the CNS. Even in their resting state, microglia are mobile with the most active processes of any cell in the central nervous system, which may call into question the term ‘resting’ used to describe them [108], and have been found to have an active role in supporting synaptic activity via the microglia projections in a manner similar to astrocytes [109].

Microglia were first identified a few decades later and categorised around 1920 by the same histopathologist who classified oligodendrocytes in 1922, Rio Hortega, using silver carbonate staining [79]. They typically exist in a quiescent state in the healthy CNS, becoming activated immune cells upon injury or infection [110]. Microglia also play a role in synapse refinement by cell–cell interaction, notably in synapse function by means of ligand–receptor groups already well characterised in immune function (reviewed in [111]), including key complement receptors CR1, CR3 and CR4 [112]. Unfortunately, it is particularly difficult to interpret the role of microglia morphology and activity in psychiatric disorders in neuropathology as they have been shown to remain active and remodel their process for up to 10 h after death, long before the majority of human post-mortem samples are taken [113–115].

Blood-resident monocytes infiltrate the brain parenchyma and differentiate into monocyte-derived macrophages which express receptors to detect exogenous risk chemical signals for pathogens, as well as endogenous inflammatory signals, such as cytokines, ATP and glucocorticoids. In many neurological diseases, binding and activation of these receptors lead to an altered phenotype and the induction of inflammatory responses ([116–124].

Monocyte-derived macrophages generated from 15 schizophrenia patients and 15 healthy controls exposed to pro-inflammatory and anti-inflammatory stimuli (LPS, R848, IL-4 and dexamethasone). One genes of interest, P2RX7, belongs to a family of purinoceptors for ATP (location: 12q24.31), and is implicated as a potential treatment for schizophrenia via two antipsychotics drugs, prochlorperazine and trifluoperazine, which may inhibit human P2X7 receptor function [125]. P2X7 is significantly reduced in expression in schizophrenia [126], but no other measured changes in monocyte-derived macrophages were reported [111].

The decreased synapse density in post-mortem cortical tissue discussed in previous chapters in schizophrenia has been suggested to be a result of increased synapse elimination. Controlled study of this process using a reprogrammed in vitro model of microglia-mediated synapse engulfment demonstrates increased synapse elimination in patient-derived neural cultures and isolated synaptosomes. This excessive synaptic pruning reflects abnormalities in both microglia-like cells and synaptic structures. Additionally, schizophrenia risk-associated variants within the human complement component 4 locus are associated with increased neuronal complement deposition and synapse uptake. As useful as these findings are, they do not fully explain the observed increase in synapse uptake [127].

The density of cells expressing immune markers as well as expression of some pro-inflammatory cytokines is increased in post-mortem brain tissue in schizophrenia [128, 129]. Immunological pathways have repeatedly been implicated in schizophrenia. Single-nucleotide polymorphisms (SNPs) in immune genes have been linked with the illness [130, 131], and the prevalence of immune-related disorders is higher in patients and their family members [132]. As discussed in Chap. 3, immunological markers have been found to be altered in blood, cerebrospinal fluid and post-mortem brain tissue in schizophrenia [129, 133].

One recent study investigated possible association between SNPs and the expression levels of 190 serum proteins in 149 schizophrenia patients and 198 matched controls. The results suggested that the effect of these SNPs on the expression of the respective proteins varies with diagnosis with a total of 21 SNPs showing significant interactions for 19 proteins. This also demonstrated that in both schizophrenia and the control group there were seven SNPs and seven proteins statistically linked with the diagnosis (Factor-VII [rs555212], Alpha-1-Antitrypsin [rs11846959], Interferon-Gamma Induced Protein 10 [rs4256246] and von-Willebrand-Factor [rs12829220] in the control group; Chromogranin-A [rs9658644], Cystatin-C [rs2424577] and Vitamin K-Dependent Protein S[rs6123]), as well as two SNPs associated with two proteins in both the control and schizophrenia groups [134].

The interleukin-6 receptor (IL-6r) gene has been investigated extensively, with a reported significant association of rs2228145 C-allele with schizophrenia [135], and although the Ala allele of Asp358Ala was significantly associated with higher levels

of both IL-6 and sIL-6, whilst IL-6 levels were significantly elevated in schizophrenic patients, the specific association of rs2228145 was not replicated [136]. However, schizophrenia patients homozygous for this SNP allele had significantly higher levels of IL-6r protein compared to controls homozygous for the wild-type allele, suggesting a possibility of differential regulation of protein expression in schizophrenia patients based on allele copy number of rs7553796 [134].

Also studied in this area is Chromogranin-A (CgA), a protein widely expressed in secretory granules in the CNS which is co-released with several neurotransmitters, notably catecholamines. CgA acts as a neuromodulator via calcium binding and has a key role in the regulation of microglial activity, neurotoxicity mediated through the secretion of glutamate, TNFα and NO, inducing mitochondrial stress and apoptosis. CgA has been found decreased in the PFC cortical layer III–V in schizophrenia, and plasma CgA and derived peptides are now commonly used as diagnostic and prognostic markers or to monitor the response to pharmacotherapeutic intervention [137–142].

For some time there was hope that an immune-marker would allow a blood test for schizophrenia. The peripheral benzodiazepine receptor (PBR) was targeted by a probe known as PK11195 and was reported to have decreased platelet-binding in schizophrenia [143–146], as well as increased PBR levels associated with the degree of demyelination and temporal activation of glial cell types in different anatomical regions [147]. Despite some discussion over PBR playing a significant role in the pathogenesis of several neurodegenerative disorders, such as Alzheimer's disease, multiple sclerosis, Parkinson's disease and HIV-associated dementia, brain injury and neuroinflammation [148–150], follow-up studies have not validated this as an accurate predictor of schizophrenia diagnosis, and so this has largely been abandoned as a potential diagnostic route.

With greater understanding of the roles and biology of glial cells, it is gratifying to see the increasing focus on their role in neurodevelopment, normal brain function and illnesses such as schizophrenia. However, as a field we still have a way to go to integrate measures of glial morphology, density and clustering as part of routine studies, as well as the deficits in including neuropathology research alongside molecular, biochemical and imaging studies, where different approaches often yield results that complement one another and so yield increased biologically useful information generated from research.

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