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White Matter Neuron Alterations in Schizophrenia and Related Disorders

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Abstract

Increased density and altered spatial distribution of subcortical white matter neurons (WMN) represents one of the more well replicated cellular alterations found in schizophrenia and related disease. In many of the affected cases, the underlying genetic risk architecture for these WMN abnormalities remains unknown. Increased density of neurons immunoreactive for Microtubule-Associated Protein 2 (MAP2) and Neuronal Nuclear Antigen (NeuN) have been reported by independent studies, though there are negative reports as well; additionally, group differences in some of the studies appear to be driven by a small subset of cases. Alterations in markers for inhibitory (GABAergic) neurons have also been described. For example, downregulation of neuropeptide Y (NPY) and nitric oxide synthase (NOS1) in inhibitory WMN positioned at the gray/white matter border, as well as altered spatial distribution, have been reported. While increased density of WMN has been suggested to reflect disturbance of neurodevelopmental processes, including neuronal migration, neurogenesis, and cell death, alternative hypotheses—such as an adaptive response to microglial activation in mature CNS, as has been described in multiple sclerosis—should also be considered. We argue that larger scale studies involving hundreds of postmortem specimens will be necessary in order to clearly establish the subset of subjects affected. Additionally, these larger cohorts could make it feasible to connect the cellular pathology to environmental and genetic factors implicated in schizophrenia and some cases with bipolar disorder or autism. These could include the 22q11 deletion (Velocardiofacial/ DiGeorge) syndrome, which in some cases is associated with neuronal ectopias in white matter.

1. The Neurodevelopmental Hypothesis of Schizophrenia: A Brief Synopsis and Update

Though the clinical symptoms of schizophrenia typically do not emerge until late adolescence and early adulthood, defective brain development at much earlier developmental stages is thought to play a key role in the etiology of disease (Murray et al., 1992, Bearden et al., 2001). Epidemiological studies in support of this hypothesis found an association between pre- and perinatal complications and increased risk for schizophrenia in the offspring (Machon et al., 1997, Buckley, 1998, Tsuang, 2000, McNeil et al., 2000); additionally, longitudinal assessment of children with genetic risk for psychosis were found to demonstrate neurological deficits beginning in early childhood (Fish and Kendler, 2005,

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Fish, 1977). Minor physical anomalies are disproportionally frequent in subjects with schizophrenia, which suggests disturbances during early development (Green et al., 1989). Among the histological findings in brain of schizophrenia patients indicative of aberrant early neurodevelopment is that of disorganized placement of layer II neurons in the entorhinal cortex, suggesting disruption of neuronal migration (Jakob and Beckmann, 1994, Arnold et al., 1991, Falkai et al., 2000, Kovalenko et al., 2003); pyramidal cell disarray has also been observed in hippocampus (Kovelman and Scheibel, 1984, Casanova and Rothberg, 2002). However, it remains unclear whether subjects on the psychosis spectrum show widespread structural changes in the entorhinal cortex, given that even in normal brain the cytoarchitectonic landscape of this cortical region is complex and very heterogeneous across its different subterritories (Beall and Lewis, 1992). In further support of the neurodevelopmental theory, many schizophrenia susceptibility genes, such as Disrupted-in-Schizophrenia 1 (DISC1) (Hennah et al., 2006) and neuregulin 1 (NRG1)-ERBB4 tyrosine kinase (Mei and Xiong, 2008, Jaaro-Peled et al., 2009) among others (O'Donovan et al., 2009, O'Dushlaine et al., 2010), are involved in pre- and postnatal brain development. It remains notoriously difficult, however, to pinpoint the specific stages of brain ontogeny that could have gone awry in affected individuals.

On the other hand, the human brain is notable for its prolonged development, which continues through childhood and adolescence and into early adulthood; some of these later developmental processes have been implicated in schizophrenia as well. For example, the number of synapses declines during childhood and adolescence in human (Huttenlocher and Dabholkar, 1997, Huttenlocher, 1984) and non-human primate (Rakic et al., 1986); synaptic spines and neuropil are decreased in the brains of schizophrenia patients compared to controls, suggesting a possible exaggeration of a normal developmental process (Garey et al., 1998, Selemon et al., 1995, Kolluri et al., 2005). Likewise, gray matter volume declines during adolescence (Giedd et al., 1999) and this process appears to be more pronounced in schizophrenia, particularly in childhood onset cases (Sporn et al., 2003).

Deficits of neural oscillations—synchronous firing of neurons required for higher order cognitive functioning—have been observed in schizophrenia; they emerge during normal development in early childhood and then continue to be refined into early adulthood (Uhlhaas et al., 2010). Abnormalities in cortical neuronal synchronization have been attributed to deficits in GABAergic neurotransmission (Gonzalez-Burgos and Lewis, 2008). Alterations in expression of GABA signaling-related mRNAs and proteins are one of the most consistently replicated findings in schizophrenia postmortem brain (Gonzalez-Burgos and Lewis, 2008, Akbarian and Huang, 2006) and are themselves developmentally regulated during a long period of maturation that starts during prenatal life and continues through late adolescence and early adulthood (Huang et al., 2007, Lewis et al., 2004, Cruz et al., 2009, Hashimoto et al., 2009).

In the present review, we focus on the unresolved mystery of white matter neurons (WMN) that are present in supranormal numbers in schizophrenia. We will describe this type of cellular pathology in more detail, and then discuss whether altered WMN numbers in schizophrenia could reflect perturbed neurodevelopment and abnormal formation of functional connections in the immature brain, manifesting itself as psychosis later in life.

2. WMN: “Out of Place” in Schizophrenia and Bipolar Disorder?

Alterations of subcortical white matter in schizophrenia and bipolar disorder are thought to reflect the neurodevelopmental origins of these diseases. For example, recent neuroimaging studies describe changes in morphology and integrity of white matter tracts that are present from the earliest disease stages (Adler et al., 2006, Szeszko et al., 2005); additionally,

studies have also found alterations in high risk individuals prior to disease onset (Karlskötter et al., 2009, Hoptman et al., 2008). There is also progressive reduction of frontal lobe white matter (Ho et al., 2003) and—in striking contrast to overlying cortex—a relative glucose hypermetabolism (Buchsbaum et al., 2007). Furthermore, schizophrenic patients diagnosed with the 22q11.2 deletion syndrome (22qDS)—one of the strongest genetic risk factors for psychosis and mood and anxiety disorders (Shprintzen, 2008)—are affected by heterotopias, polygyria and/or numerous ectopic neurons scattered throughout the white matter of the frontal lobe (Kiehl et al., 2008). While heterotopias and other unambiguous markers for more severe defects in neuronal migration are not known to be a characteristic of schizophrenia, the 22qDS cases may be indicative of a more subtle developmental defect that is present in a larger proportion of the patient population.

In support of this idea, increased densities, or altered distribution, of WMN—first mentioned in a case report in 1991 (Gertz and Schmidt, 1991)—have been reported in 13 out of 16 studies on schizophrenia postmortem brain (Akbarian et al., 1993a, Akbarian et al., 1996, Akbarian et al., 1993b, Anderson et al., 1996, Beasley et al., 2002, Bertram et al., 2007, Eastwood and Harrison, 2003, Eastwood and Harrison, 2005, Kirkpatrick et al., 1999, Kirkpatrick et al., 2003, Molnar et al., 2003, Nobuhara et al., 2004, Rioux et al., 2003, Ikeda et al., 2004a, Connor et al., 2009b, Beasley et al., 2009) (Fig. 1A). Collectively, these studies reported alterations in frontal, temporal, and parietal lobe white matter (Fig. 1A), suggesting that this neuronal subpopulation may be affected in widespread areas of the cerebral cortex. The majority of studies reported either a generalized increase of neurons in selected compartments of white matter space (Akbarian et al., 1993a, Akbarian et al., 1993b, Anderson et al., 1996, Eastwood and Harrison, 2003, Eastwood and Harrison, 2005, Ikeda et al., 2004a, Kirkpatrick et al., 1999, Kirkpatrick et al., 2003, Connor et al., 2009a), or redistribution towards deeper white matter (Akbarian et al., 1996, Akbarian et al., 1993b, Rioux et al., 2003). As shown in Fig. 1B, the studies explored white matter space located anywhere between the border zone towards the surface (deep layer VI) and up to 6mm deep from overlying gray matter. Because each study focused on a different zone of white matter (as defined by distance from overlying cortex), there is little consensus as to which regions of subcortical white matter are definitively affected. This lack of agreement is further complicated by methodological differences between studies—even those studies which focused on the same brain region vary in terms of sulcogyral position sampled, neuronal markers employed, tissue processing, and characteristics of the clinical cohort. Adding to the confusion, a number of these studies applied terminology such as “superficial” or “deep” white matter without providing specific definition or metric measures, which makes proper comparison across different studies even more difficult (Fig. 1B).

It remains to be determined whether or not subjects with schizophrenia show excess amount of WMN in additional white matter spaces, such as the territory formerly occupied by the perireticular nucleus of the thalamus, a diencephalic structure that—similar to the telencephalic subplate—undergoes a dramatic reduction in neuronal densities during late gestation (Tulay et al., 2004). It should be noted that some of the subcortical WMN populations affected in schizophrenia, such as neurons expressing NAPDH-d/NOS1, were reportedly found in excess amounts in the brainstem of schizophrenics (Karson et al., 1991) and showed dysregulated expression in cerebellum (Karson et al., 1996) and striatum (Fritzen et al., 2007, Lauer et al., 2005). These findings could indicate that WMN changes in schizophrenia are, at least in part, reflective of a more widespread brain defect.

Furthermore, there is evidence that increases in WMN density are not a uniform feature in psychosis. Instead, cases with more robust alterations—with WMN densities exceeding the second or third standard deviation of controls—may account for less than 25% of the overall patient population (Akbarian et al., 1996, Connor et al., 2009a). Interestingly, increased

density of WMN in autism and bipolar disorder was recently reported (Simms et al., 2009, Connor et al., 2009a), which is in agreement with the emerging view that there is considerable overlap between these disorders and schizophrenia, in terms of genetic risk factors and molecular pathology (Crespi et al., 2010, Singh et al., 2010), and strengthening the view that psychotic spectrum disorders possess a neurodevelopmental component. Therefore, while a firm conclusion regarding WMN pathology cannot be reached at the present time, the studies—when taken together—strongly suggest that this cell population is affected in patients with a psychosis or autism spectrum disorder.

3. Which subpopulations of WMN are altered in schizophrenia?

Neurons residing in adult white matter (also known as “interstitial neurons,” because they are dispersed among fibers and glia) are—much like their counterparts in the overlying cortex—comprised of a heterogeneous mixture of excitatory (glutamatergic) and inhibitory (GABAergic) neurons. For an update on morphology and phenotypes of the various WMN populations in the human cerebral cortex, and their developmental history, see a recent excellent review (Suarez-Sola et al., 2009). The purpose of the present article is to discuss the potential implications of WMN abnormalities in the pathoetiology of psychosis spectrum disorders, to elucidate methodological and empirical challenges, and outline a practical strategy designed to explore the underlying genetic risk architecture.

In the adult brain, glutamatergic neurons represent a large majority (up to 90%) of WMN expressing immunoreactivity for the classical neuronal marker, microtubule-associated protein 2 (MAP2) (Meyer et al., 1992). As in gray matter, GABAergic WMN are quite heterogeneous, and are classified based upon morphology, differential expression of the Ca^{2+} binding proteins (paralbumin, calbindin, and calretinin), and neuropeptides, such as cholecystokinin (CCK), neuropeptide Y (NPY) and somatostatin (SST) (Suarez-Sola et al., 2009). One particularly interesting type of GABAergic neuron in white matter expresses the enzyme nitric oxide synthase 1 (NOS1), also known as nicotinamide adenine dinucleotide phosphate diaphorase, or NADPH-d (Tao et al., 1999, Oermann et al., 1998). The axons of these cells, in contrast to other GABAergic neurons, extend beyond the local environment to reach distant cortical areas (Tomioka and Rockland, 2007, Meyer et al., 1991, Higo et al., 2007). These neurons innervate blood vessels via plexus-like structures that are thought to produce the potent vasodilator, nitric oxide, and the vasoconstrictor, neuropeptide Y (Suarez-Sola et al., 2009); additionally, this GABAergic neuron subtype also expresses strong immunoreactivity for the alpha splice variant of the schizophrenia risk gene, neuregulin 1 (*NRG1 α*). *NRG1* protein isoforms are classified as either α or β depending on the type of epidermal growth factor (EGF) domain, which is responsible for activation of the ErbB receptor (Tan et al., 2007); interestingly, while fewer neurons in human brain express the α form of *NRG1* compared to β , many WMN are *NRG1 α* -immunoreactive (Bernstein et al., 2006).

Several studies examined the numbers and distribution of WMN immunoreactive for MAP2 or NeuN in subjects with schizophrenia. As discussed above, the majority of MAP2-immunoreactive neurons in white matter are glutamatergic, while only a small percent (10%) demonstrate nonpyramidal morphology; an even smaller fraction (approximately 3%) express immunoreactivity for NADPH-d (Meyer et al., 1992). Another study found no overlap between MAP2 and NADPH-d immunoreactive neurons (Akbarian et al., 1996). In contrast to MAP2, NeuN is viewed as a more ubiquitously expressed neuronal marker, though evidence does suggest that NeuN may not be expressed by all neurons; for example, it is not expressed by cerebellar Purkinje cells (Wolf et al., 1996) and, in addition, no cellular co-expression was observed in cortical sections double-labeled for *NRG1 α* and NeuN (Connor et al., 2009a). Furthermore, approximately 25% of NADPH-d+ WMN do not

express NeuN (Schahram Akbarian, unpublished observations). Nonetheless, NeuN is still utilized in many studies as a ubiquitous neuronal marker. However, based on the findings above, it appears that at least a subset of WMN expressing NPY and/or NOS1/NADPH-d, and/or NRG α remain NeuN negative (Fig. 1C). While it is known that a subset of WMN and cortical neurons co-express NPY and NOS1/NADPH-d (Unger and Lange, 1992), studies testing for co-expression of NRG α and NPY in WMN are presently missing.

Increased densities of NeuN-immunoreactive neurons in superficial white matter of schizophrenia subjects were reported by three studies (Connor et al., 2009a, Eastwood and Harrison, 2005, Eastwood and Harrison, 2003); additionally, increased densities of MAP2-immunoreactive WMN were reported by four studies (Kirkpatrick et al., 1999, Anderson et al., 1996, Akbarian et al., 1996, Rioux et al., 2003), although negative findings were also published (Beasley et al., 2002). However, there is no consensus on the specific regions of white matter space harboring increased numbers of MAP2+ neurons. Some studies identified the first few millimeters beneath gray matter as the area with excess numbers of MAP2+ cells, while in others the affected space appeared to be several millimeters deeper from overlying cortex (Fig. 1B). One reason for these disparities could be the considerable variability in WMN density with respect to sulcal-gyral position—density is known to peak at the apex of a cortical gyration and is much lower at the sulcal bottom. There is also evidence for regional differences. For example, densities in medial portions of the frontal lobe, including the cingulate, are several-fold lower in comparison to rostral and dorsolateral portions.

There is also evidence that the GABAergic neurons in the subcortical white matter are affected in psychosis, though these alterations differ from those observed for MAP2 and NeuN-immunoreactive neurons; specifically, alterations within the GABAergic cell population appear to be molecular in nature rather than an overt change in cell number or density. For example, the mRNA for NPY, which is expressed by many NOS1/NADPH-d neurons mentioned above (Suarez-Sola et al., 2009), is selectively decreased in neurons within superficial white matter of subjects with psychosis, particularly in cases with prominent mood symptoms (Morris et al., 2009). Similarly, the *RELN* transcript for the glycoprotein Reelin—which is expressed by GABAergic neurons (Guidotti et al., 2000)—was significantly lower in WMN in schizophrenia (Eastwood and Harrison, 2006, Eastwood and Harrison, 2003). These findings are of interest, because dysfunction of the GABAergic system is a core feature of cortical gray matter pathophysiology in schizophrenia and major mood disorders (Akbarian and Huang, 2006, Craddock et al., 2010, Thompson et al., 2009, Benes and Berretta, 2001, Hashimoto et al., 2008, Mellios et al., 2009, Guidotti et al., 2000, Akbarian et al., 1995).

In addition to gene expression changes, pilot studies with small cohorts of case-control pairs reported that NADPH-d+ and NPY+ neurons (presumably inhibitory) may show altered spatial distribution, with increased numbers in deeper white matter in conjunction with a decrease in cortical gray matter (Akbarian et al., 1993b, Akbarian et al., 1993a, Ikeda et al., 2004b); however, subsequent work in larger cohorts did not always support that conclusion (Akbarian et al., 1996), though deficits in the number of NRG1 α + neurons have been reported for gray matter and superficial white matter in schizophrenia and unipolar depression (Bertram et al., 2007). A similar deficit was also observed in the white matter underlying cingulate cortex of schizophrenia and bipolar subjects, although sample variability precluded any statistical significance (Connor et al., 2009a).

The studies discussed above collectively suggest that multiple WMN populations are affected in schizophrenia. Neurons defined by immunoreactivity for MAP2 and NeuN—which include primarily glutamatergic (MAP2) or a mixed (NeuN) population of cells—

appear to be affected by a generalized increase in density in the subcortical white matter. In contrast, NADPH-d+ GABAergic neurons demonstrate molecular deficits and, in some cases, appear to be present in insufficient numbers in cortical gray matter in conjunction with increased numbers in deeper white matter. Because densities of cells expressing GABAergic markers appear not to be overall increased in superficial white matter, we speculate that the increased number of cells expressing MAP2 and NeuN in schizophrenic WM are likely glutamatergic.

4. White matter neuron changes in schizophrenia: What are the underlying mechanisms?

At present, the mechanisms underlying the molecular and cellular changes in WMN in psychosis remain unclear. In earlier reports on WMN changes in schizophrenia, the emphasis was on disruption of early developmental events, including migration of cortical neurons during the second or early third trimester of pregnancy and apoptosis of embryonic subplate neurons, a transient structure beneath the cortical plate in the prenatal and early postnatal brain (Ayoub and Kostovic, 2009), which subsequently is thought to undergo an involution due to a preprogrammed cell death fate (Akbarian et al., 1993a, Akbarian et al., 1996, Anderson et al., 1996, Rioux et al., 2003, Kirkpatrick et al., 2003, Akbarian et al., 1993b, Eastwood and Harrison, 2005). According to these scenarios, increased numbers of WMN in adult subjects with psychosis could be misplaced because they “got stuck” in their path from the proliferative neuroepithelium of the ventricular zone to their destined position in the six-layered cortex (Fig. 2A, panel 1). Alternatively, such neurons could represent the remnants of fetal subplate neurons that escaped apoptosis, and then are subsequently found in the space defined as subcortical white matter of mature brain (Fig. 2A, panel 3) (Akbarian et al., 1996, Kanold, 2004, Bunney and Bunney, 2000). The latter hypothesis adds to the notion of faulty programmed cell death in schizophrenia (Jarskog et al., 2005), but, as with all of the scenarios mentioned here, empirical evidence to validate or refute them is presently lacking.

A new perspective arises from a recent study conducted by the Kriegstein laboratory, reporting that the prenatal human cerebral cortex—in striking contrast to rodents—exhibits a considerable degree of neurogenesis outside of the proliferative neuroepithelium lining the ventricular walls (Hansen et al., 2010). This study describes a previously unidentified class of non-epithelial radial glia-like cell, which is found in the outer subventricular zone during midgestation and generates neuronal precursor cells via asymmetric cell divisions (Hansen et al., 2010, Zecevic et al., 2005). Of note, in human and primate brain, neurons in the outer subventricular zone continue to accumulate throughout mid and late gestation (Smart et al., 2002, Zecevic et al., 2005), in parallel with—or even after—neurons are born and migrate into the overlying cortical plate (Rakic, 1978). It is possible that this continued accumulation of neurons within the future white matter space is generated by this new class of non-epithelial radial glia (Fig. 2A, panel 2); thus, increased numbers of WMN in schizophrenia could be due to dysregulated neurogenesis by these glia during fetal development.

What are some potential molecular mechanisms that could underlie the increased number of WMN observed in schizophrenia? It is worth while to mention again the 22q11 deletion syndrome (the most frequent microdeletion associated with psychosis, mood disorders, and autism), because a study of 22q11 cases diagnosed with schizophrenia revealed nodular heterotopias in deeper white matter—a hallmark for a neuronal migration disorder—or excess amounts of ectopic neurons scattered diffusely throughout the frontal lobe white matter (Kiehl et al., 2008). The latter abnormality is reminiscent of the observed alterations in schizophrenia postmortem studies, although the magnitude of change appears to be larger in subjects diagnosed with the 22q11 deletion syndrome. Mouse models have begun to

delineate the role of the 22q11 locus in neurodevelopment. For example, deletion of the 1.5 megabase minimal critical region in mouse resulted in impaired neurogenesis of basal progenitor cells, decreased projection neurons in cortical layers II-IV, and disrupted tangential migration of interneurons (Meechan et al., 2009).

In addition, a number of schizophrenia susceptibility genes at other loci, including *DISC1*, *NRG1*, and *RELN*, are known to play a critical role in neuronal migration and positioning in developing brain and could contribute to the observed increase of WMN observed in schizophrenia (Duan et al., 2007, Ogawa et al., 1995, Lopez-Bendito et al., 2006, Flames et al., 2004). Indeed, an increase in NADPH-d+ neurons was observed in the subcortical white matter of the heterozygous *reeler* mouse (Tuetting et al., 1999). And in mutants for Apolipoprotein E Receptor 2 (*ApoER2*)—one of the receptors for Reelin—only a portion of neurons reach their normal destination in the cortical plate and remain displaced beneath layer V and VI neurons (Hack et al., 2007). In addition to disrupted neuronal migration, altered neurogenesis could potentially increase the numbers of neurons in subcortical white matter; cytokines, growth factors, and their receptors known to modulate neurogenesis, such as TGF β and p75NTR, have also been implicated in schizophrenia (Battista et al., 2006, Young et al., 2007). Alterations in extracellular matrix-glia interaction may also play a role (Pantazopoulos et al., 2010).

While explanations for increased WMN in schizophrenia traditionally emphasize perturbed prenatal brain development, the possibility that these increased cell numbers in white matter arise during later, postnatal developmental stages should also be considered. Alternative theories include transdifferentiation from microglia (Fig. 2B, panel 1), or neurogenesis from an unknown stem cell-like source (Fig. 2B, panel 2) or other differentiated cell type (Boucherie et al., 2009, Cogle et al., 2004), or increased expression of a neuronal marker in otherwise “dormant” adult WMN (Fig. 2B, panel 3). While conclusive evidence for neurogenesis in postnatal human cerebral cortex is currently lacking (Spalding et al., 2005), a recent study described a highly localized increase in NeuN+ neurons in close proximity to white matter lesions containing activated microglia in multiple sclerosis (Chang et al., 2008), suggesting that adult neurogenesis in regions outside of the subventricular zone is indeed possible. Furthermore, there is evidence that activated microglia, via TGF β or some other growth factor, promotes neurogenesis and differentiation (Battista et al., 2006). Whether the increased number of NeuN-immunoreactive cells observed in schizophrenia arises from any of the above mechanisms will require further investigation.

One question that arises is whether there is microglial activation in schizophrenia subjects who also demonstrate increased WMN density. At present, this hypothesis neither can be firmly rejected nor confirmed. Only one study explored NeuN+ neuronal densities and microglial activation in the white matter of subjects with schizophrenia and bipolar disorder (Connor et al., 2009a). Cases with elevated NeuN+ density showed no clear association with microglial activation, but at least one case was positive for both increased NeuN+ density and increased immunoreactivity for the microglia marker, Iba1 (Connor et al., 2009a). However, study of microglial activation in postmortem human brain is complicated by the fact that this process is highly dynamic (Nimmerjahn et al., 2005), and, in addition, microglial activation in schizophrenia—as described by two in vivo studies (Doorduyn et al., 2009, van Berckel et al., 2008) and one postmortem study (Steiner et al., 2008)—was found only in those patients experiencing, or immediately recovering from, a psychotic episode. Thus, assessment of microglial activation in postmortem human brain in schizophrenia, and any potential association with adult neurogenesis, must consider these findings.

As mentioned above, GABAergic neurons in white matter appear to be differentially affected as compared to glutamatergic neurons; specifically, they seem to be affected by

molecular changes or alterations in spatial distribution rather than by overt changes in cell number or density. A possible explanation for the observed decreases in *RELN* (Eastwood and Harrison, 2003) and *NPY* (Morris et al., 2009) mRNA expression by inhibitory WMN could be reflective of a more extensive defect in the cortical transcriptome, particularly of its GABAergic components (Kim and Webster, 2010, Altar et al., 2009, Hashimoto et al., 2008). This defect, in turn, could be secondary to decreased cortical neuronal activity, leading to activity-dependent downregulation in gene expression for inhibitory neurotransmission (Akbarian and Huang, 2006, Akbarian et al., 1995). In addition, GABAergic circuitry may be affected by a complex interaction of genetic and epigenetic mechanisms, affecting genes regulating GABA metabolism (Huang et al., 2007, Huang and Akbarian, 2007) or Reelin glycoprotein (Grayson et al., 2005), the Neuregulin-ERBB4 signaling cascade (Vullhorst et al., 2009, Wen et al., 2010), and others.

It should be noted that, to date, no information exists with regard to the potential role of antipsychotic or other types of medication, or environmental influences on WMN. To examine the impact of these variables on WMN in schizophrenia would require extraordinarily well-characterized cohorts, which remains a challenge for brain banks as medical records are often incomplete or not provided by donor families. However, it remains entirely possible that environmental factors, including prenatal malnutrition, maternal infection during pregnancy, or obstetric complications, could be involved in alterations of WMN in psychosis by affecting neuronal migration or neurogenesis. While it is not known whether the WMN changes in subjects with schizophrenia precede the onset of psychosis, the fact that many schizophrenia susceptibility genes are involved in early neurodevelopmental processes, in addition to the observation that environmental factors associated with schizophrenia are typically pre- or perinatal, suggest that WMN alterations do indeed precede disease onset.

5. Modeling WMN Alterations in the Animal

Typically, discovery of cellular or molecular pathology in human is followed by translational work in animal models or cell culture systems, in order to better understand the potential implications for the pathophysiology of the underlying disease. To date, only one group has studied WMN in a mouse model of schizophrenia, reporting increased densities of NADPH-d+ cells in white matter of the heterozygous *reeler* mouse (Tueting et al., 1999). While intriguing, such studies are limited based on the fact that the developmental history and origins of WMN in human brain varies considerably from those of rodents (Suarez-Sola et al., 2009) (Hansen et al., 2010). One has to conclude then that rodents—including the mouse, the only mammal for which genetic engineering tools are readily available—offer only a very limited means for the study of WMN, as the sources of this cell population is far less developed in mice, with regards to the fetal subplate, or is absent entirely (neurons derived from interstitial zone radial glia). Furthermore, rodents—with their much more limited vertical extension of subcortical white matter space—are not likely to provide suitable models for these milder phenotypes of the putative migration defect.

6. Implications of white matter neuron changes for the pathophysiology of schizophrenia

The final chapter of this Review will discuss some of the implications of WMN changes for the neurobiology of schizophrenia.

(i) GABAergic neurons in superficial white matter

These neurons potentially co-express NOS1/NADPH-diaphorase, NPY, and NRG1 α , as discussed above. Some of these neurons may project to distant cortical areas, which is an unusual characteristic for GABAergic interneurons, whose efferents are typically confined to a few hundred microns of surrounding cortex (Meyer et al., 1991, Tomioka and Rockland, 2007, Higo et al., 2007). Like their cortical counterparts, these neurons are thought to play an important role in the coupling of neuronal activity to the microvasculature and fine-tuning of hemodynamic parameters (Suarez-Sola et al., 2009, Cauli et al., 2004, Estrada and DeFelipe, 1998). While nitric oxide, produced by NOS1, facilitates blood flow through the cortical microvasculature, NPY mediates microvessel constriction (Cauli et al., 2004). Therefore, any alterations in gene expression or spatial distribution of these subpopulations of WMN (Akbarian et al., 1993a, Eastwood and Harrison, 2003, Morris et al., 2009) may seriously impair the proper regulation of cerebral blood flow and metabolism. This hypothesis is consistent with *in vivo* neuroimaging studies reporting functional hypoactivity in cerebral cortex in schizophrenia, as assessed by Blood-Oxygen-Level-Dependent (BOLD) and functional magnetic resonance imaging (fMRI) (Potkin and Ford, 2009, Fu and McGuire, 1999, Sava and Yurgelun-Todd, 2008).

(ii) NeuN+ and MAP2+ white matter neurons

As discussed above, there is increasing evidence that a subset of subjects on the psychosis spectrum is affected by an approximately 2-4 fold increase in WMN immunoreactive for NeuN or MAP2. Because these neuronal markers are likely to be expressed by a neurochemically and functionally heterogeneous cell population. It remains unclear whether these alterations in the clinical cases are driven by specific subpopulations of GABAergic or glutamatergic WMN; however as discussed above we speculate that it is the glutamatergic WMN that are increased in schizophrenia. Regardless of the specific neuronal subpopulation, one question that arises is if the numbers of MAP2+ or NeuN+ WMN could impair orderly function of neuronal networks in the overlying cortex. Presently, this question is left unanswered. While the neurological literature presents ample evidence that cases with neuronal ectopias in white matter are at increased risk for seizure disorders, these are often associated with nodular heterotopias and “gray matter”-like micro-structures embedded within white matter in association with dysplasia of overlying cortex. In contrast, the WMN changes of subjects on the psychosis spectrum are more subtle and occur without any gross alterations in cortical morphology or other “hard” signs indicative of altered development. While it may seem unlikely that such a small increase in WMN could seriously interfere with cortical circuitry and neurotransmission, this remains a distinct possibility given that these cells maintain functional connectivity with the overlying cortex (Torres-Reveron and Friedlander, 2007) and thalamus (Giguere and Goldman-Rakic, 1988). Of note, subplate neurons are pivotal for orderly development of GABAergic circuitry and thalamic innervation patterns in overlying cortex (Kanold and Shatz, 2006, Kanold et al., 2003). Therefore, excess of WMN in adult brain of some subjects with schizophrenia could indicate a developmental defect of the fetal subplate. This could be associated with compromised thalamo-cortical connections and changes in local circuit (GABA) neurons, which are increasingly recognized as a critical cellular substrate in the pathophysiology of schizophrenia (Uhlhaas and Singer, 2010, Endoh-Yamagami et al., 2010, Sohal et al., 2009, Eggen et al., 2008, Akbarian and Huang, 2006).

7. Outlook

While there is little doubt that a subset of subjects with psychosis and autism harbor supranormal numbers of neurons in their subcortical white matter, the implications for the neurobiology of schizophrenia and the related diseases remain unclear. Future studies, using

hundreds of samples, will be necessary in order to firmly establish the portion of diseased subjects affected by excess WMN, and to explore the underlying genetics, including a potential role for microdeletions at 22q11. However, because this would require collection from multiple, independently operated brain banks each of which employs different procedures for the collection and storage of tissues, a postmortem project involving hundreds of samples faces considerable logistical and technical hurdles. It should also be possible to assess alterations of specific subtypes of WMN, including the subset of NPY+ and NOS1+ GABAergic neurons closely associated with the microvasculature, and the subpopulation of MAP2+ and NeuN+ WMN that appear to be increased in clinical cases. We hypothesize that NPY+ and NOS1+ neurons show a range of molecular alterations, are part of the GABAergic deficit affecting widespread areas of the cerebral cortex in schizophrenia, and may impact cortical circuitry via a dysregulated hemodynamic response to neuronal activity. We further speculate that excess numbers of MAP2+ and NeuN+ WMN in adult subjects with schizophrenia may reflect an early neurodevelopmental defect ultimately resulting in disordered cortical connectivity. Presently, it is very difficult to test these and other hypotheses (such as the possible link to microglial activation, as discussed above) and it will be necessary to develop novel animal models that mimic these conditions. We predict that the study of subcortical WMN—and perhaps other neuronal populations affected by abnormal positioning in schizophrenic brain (Kovalenko et al., 2003, Parlapani et al., 2009, Arnold et al., 1997)—could uncover novel disease-associated molecular and cellular mechanisms, thereby significantly advancing current knowledge on the neurobiology of schizophrenia.

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Figure 1. Overview of white matter neuron changes in schizophrenia

(A) Highly schematic presentation of the various regions-of-interest in frontal, parietal and temporal lobe for which WMN numbers or densities were assessed in schizophrenia postmortem brain. (B) Summary presentation of significant changes (+, increase; -, decrease) of WMN densities in schizophrenia, grouped by neuronal marker (NeuN, MAP2, or the GABAergic markers NPY, NADPH-d, NRGα) and depth of white matter space in relation to overlying six-layered cortex, as indicated. The n specifies the total number of specimens for each study, as indicated. (C) Highly schematic overview of the complex subtype composition of WMN. The figure shows the proportion of WMN expressing NeuN and MAP-2, and of those that are NeuN negative, including subsets of NPY, NADPH-d, NRGα expressing neurons in cortical gray and white matter of human cerebral cortex. Notice that NPY/NADPH-d/ NRGα expressing neurons show peak density in superficial white matter.

Figure 2. Potential mechanisms for increased white matter neuron densities in schizophrenia (A) Altered neurodevelopment, including defective neuronal migration or aberrant neurogenesis from various stem cells or radial glia residing in ventricular or subventricular zone (VZ and SVZ), (panel 1) or in interstitial zone (panel 2) or defective apoptosis in the subplate (SP) (panel 3), could play a role. (B) Highly speculative scenarios involving activated microglia in mature brain, including direct transdifferentiation into neuronal phenotype (1), or growth factor-induced increase in neurogenesis from a neuronal progenitor cell (2) or increased expression of NeuN or MAP-2 neuronal markers in preexisting but 'dormant' WMN (with previously undetectable levels of NeuN or MAP2 expression) (3). See text for further details.