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Developmental Pattern of Perineuronal Nets in the Human Prefrontal Cortex and their Deficit in Schizophrenia

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Abstract

Background—Perineuronal nets (PNNs) are extracellular matrix structures that enwrap many neurons in the brain. They regulate the postnatal experience-dependent maturation of brain circuits and maintain their functional integrity in the mature brain by stabilizing their synaptic architecture.

Methods—Eighty-six postmortem human brains were included in this study. We used *Wisteria Floribunda* agglutinin histochemistry to visualize PNNs to investigate whether the densities of PNNs in the prefrontal cortex (PFC) and primary visual cortex were altered in subjects with schizophrenia or bipolar disorder. In addition, we quantified the normal postnatal development of PNNs in the human PFC.

Results—Compared to the normal control subjects, the densities of PNNs were decreased by 70–76% in layers 3 and 5 of the PFC in schizophrenia but not in bipolar disorder. This finding was replicated in a separate group of schizophrenia and normal control subjects. In addition, PNN densities in the primary visual cortex were unaltered in either condition. Finally, the number of PNNs in the PFC increased during postnatal development through the peripubertal period until late adolescence and early adulthood.

Conclusions—These findings suggest that PNN deficit contributes to PFC dysfunction in schizophrenia. The fact that the timing of PNN development overlaps with the period when schizophrenia symptomatology gradually emerges raises the possibility that aberrant PNN formation may contribute to the onset of illness. Thus, characterization of the molecular

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CONFLICTS OF INTEREST AND FINANCIAL DISCLOSURES

The authors report no biomedical financial interests or potential conflicts of interest.

AUTHORS' CONTRIBUTIONS

SAM, KMA, NS and EP processed the tissue. SAM, KMA and HP carried out the WFA histochemical staining. SAM and KMA performed PNN density quantification. SAM and TUWW conducted the data analysis. TUWW conceived of the study, participated in its design and data interpretation and wrote the manuscript.

mechanisms underlying PNN deficit may have important implications for the conceptualization of novel strategies for the diagnosis, treatment, early intervention and prevention of schizophrenia.

Keywords

cerebral cortex; schizophrenia; bipolar disorder; development; GABA; synaptic pruning

INTRODUCTION

Chondroitin sulfate proteoglycans (CSPGs) are the main lectican component of the extracellular matrix (ECM) in the brain (1, 2). CSPGs, together with other ECM components, including hyaluronic acid, link proteins and the glycoprotein tenascin-R, form perineuronal nets (PNNs), which are mesh-like lattice structures that enwrap the cell body and dendrites of neurons, including the inhibitory neurons that contain the calcium-binding protein parvalbumin (PV) and many pyramidal neurons (3–8). PNNs are thought to serve as a buffer for cations in the ECM and, as such, in the case of PV neurons, may facilitate their fast-spiking firing (9). Therefore, deficit of PNNs can lead to the dysfunction of PV neurons, which is thought to be a core pathophysiological mechanism of schizophrenia (10–12). In addition, because PNNs may also play an important role in maintaining the integrity of the connectional architecture of pyramidal cell network by regulating synaptic plasticity (13–15), PNN deficit can destabilize synaptic connectivities and thereby contribute to cortical circuitry dysfunction in schizophrenia (16).

Studies in animals have revealed that PNNs in the cerebral cortex are developmentally regulated. For instance, in the visual cortex, the number of PNNs gradually increases during postnatal development, which temporally parallels the critical period of developmental synaptic plasticity (17–20). In fact, it has been suggested that the maturation of PNNs may play a prominent role in the closure of critical period (21–24), whereas experimental dissolution of PNNs in the adult cortex by tissue plasminogen activator (tPA) has been found to reactivate the molecular machinery of synaptic plasticity (15, 18, 19, 24). Interestingly, during postnatal development, tPA level first rises, and then declines, which signals critical period closure (18, 19, 25). Taken together, it appears that developmental increase in PNNs, together with the gradual reduction in the availability of tPA, stabilizes synaptic connectional architecture by anatomically constraining synaptic plasticity. At present, the postnatal development of PNNs in the cerebral cortex in humans has not been explored.

PNNs have recently been implicated in the pathophysiology of schizophrenia. Specifically, it has been found that the density of PNNs in limbic brain structures, such as the amygdala and the entorhinal cortex, was decreased by as much as 10-fold in subjects with schizophrenia, but it was unchanged in those with bipolar disorder (26). In this context, a goal of the present study is to determine whether PNN deficit in schizophrenia may also occur in the neocortex. In addition, we characterized PNNs in subjects with bipolar disorder in order to establish any possible disease specificity of PNN deficit. In fact, we found that the density of PNNs was decreased by 70–76 % in layers 3 and 5 of the PFC in subjects with schizophrenia, but it was unchanged in any of the layers in the primary visual cortex. Furthermore, PNN density in either the PFC or the primary visual cortex was unaltered in the subjects with bipolar disorder. Finally, we quantified the postnatal development of PNNs in the human PFC and found that their number increased through the peripubertal period until late adolescence and early adulthood, which happens to be the period of time when schizophrenia symptomatology typically begins to gradually emerge. Taken together, these findings suggest that PNN deficit contributes to PFC dysfunction in schizophrenia and raise the possibility that PNN deficit may be a consequence of aberrant peripubertal PNN

formation, which may contribute to the onset of illness by disturbing developmental synaptic reorganization.

METHODS AND MATERIALS

Human Subjects

A total of 86 postmortem human brains were included in this study (Table 1). Sixty-seven of them were adult human brains obtained from the Harvard Brain Tissue Resource Center (HBTRC) at McLean Hospital in Belmont, MA. In 47 of these brains, we compared PNN densities in the PFC (Brodmann's area 9) in subjects with schizophrenia (N=16) or bipolar disorder (N=15) with that of demographically matched normal control subjects (N=16). None of these subjects had a history of substance dependence based on review of medical records and report by the next-of-kin; this was further corroborated by negative toxicology reports. The fact that most of the subjects were free of substance abuse or dependence history is typical for brains that come to the HBTRC, which receives exclusively community-based donations. After it was determined that PNN densities were decreased in the PFC in subjects with schizophrenia, we then attempted to replicate these findings in a separate cohort of brains from 5 schizophrenia and 5 normal control subjects (Table 1). Furthermore, using these 10 brains, together with 5 additional brains for each of the diagnostic groups (Table 1), we quantified PNN densities in the primary visual cortex (Brodmann's area 17) in schizophrenia and demographically matched normal control subjects in order to determine any region specificity of PNN deficit. Finally, we obtained 19 brains from healthy control human subjects at different postnatal ages from the National Institute of Child and Human Development (NICHD) Brain and Tissue Bank at the University of Maryland in Baltimore, MD in order to survey the trajectory of PNN development in the PFC (Table 2). Brain donation and informed consent procedures at the HBTRC and the NICHD Brank and Tissue Bank have been approved by the Partners Human Research Committee and the Institutional Review Board of the University of Maryland, respectively.

Tissue Preparation

For the initial quantification of PNN densities in the PFC in the 47 normal control, bipolar disorder and schizophrenia subjects (cases 1–16, 27–41 and 42–57, respectively; see Table 1), tissue blocks approximately 3 mm-thick containing Brodmann's area 9 of the PFC were post-fixed in 0.1M phosphate buffer (PB) containing 4% paraformaldehyde and 0.1 M Na azide at 4 °C for 3 weeks, cryoprotected at 4 °C for another 3 weeks (30% glycerol, 30% ethylene glycol and 0.1% Na azide in 0.1M PB), embedded in agar, and then sectioned at 40 μm using a freezing microtome, as previously described (26). Sections were then stored in cryoprotectant at −20 °C until use. Due to limited availability of additional paraformaldehyde-fixed tissue, tissue blocks snap-frozen in liquid nitrogen vapor (LNV) were used for the PFC replication experiment (cases 17–21 and 58–62 for the normal control and schizophrenia groups, respectively; see Table 1) and for the quantification of PNNs in Brodmann's area 17 (cases 17–26 and 58–67 for the normal control and schizophrenia groups, respectively; see Table 1). LNV blocks were sectioned to a thickness of 20 μm using a cryostat, mounted on slides and then post-fixed in 4% paraformaldehyde for 20 minutes at room temperature.

Wisteria Floribunda Agglutinin Histochemistry

CSPGs were visualized histochemically using biotinylated *Wisteria Floribunda* agglutinin (WFA), which selectively binds to N-acetyl-galactosamine (6, 27–29), a core component of the long chondroitin sulfate glycosaminoglycan chains characteristic of CSPGs. For each of the subjects, two sections were processed for WFA histochemistry. For the

paraformaldehyde-fixed tissue, antigen retrieval was performed prior to WFA histochemical procedures, as previously described (26). Briefly, free-floating sections were incubated in citric acid buffer (0.1 M citric acid, 0.2 M Na_2HPO_4) overnight. They were then placed in the same buffer heated to 80 °C for 30 minutes, followed by incubation in WFA (1:1,000; Vector Laboratories, CA) in 1% bovine serum albumin for 24 hours at 4 °C, in horseradish peroxidase- conjugated streptavidin for 2 hours (1:5,000, Zymed, CA), and, finally, in nickel-enhanced diaminobenzidine/peroxidase reaction (0.02% diaminobenzidine, 0.08% nickel-sulphate, 0.006% hydrogen peroxide in PB). Sections were then washed and counterstained. For the LNV tissue, sections were incubated in WFA (1:3,000) overnight at room temperature, followed by a 2-hour incubation in horseradish peroxidase conjugated streptavidin (1:5,000, Zymed, CA) at room temperature and subsequent WFA visualization, washing and counterstaining as described above. All of the sections derived from the paraformaldehyde-fixed PFC blocks were processed at the same time and so were those derived from LNV blocks of the PFC and primary visual cortex to ensure that they were subjected to identical experimental conditions.

Quantification of PNN Densities

For each of the subjects, two sections were included for quantification. For each of the two sections, we quantified the density of WFA-labeled PNNs for each of the layers in two 500 μm-wide cortical traverses extending from the pial surface to the white matter border. Thus, for each of the subjects, PNN density for each cortical layer was determined by averaging the density measures derived from the four traverses (i.e. two traverses per section and two sections per case). Cortical layer determination was made in reference to percentages of depth relative to the pial surface measured using neighboring Nissl-stained sections. To avoid the introduction of any systemic biases, all of the cases for each of the experiments (with the exception of the 5 schizophrenia and 5 normal control cases used for the replication study due to the small sample size) were quantified following a stratified random sampling procedure in which cases were stratified into three blocks of equal proportion of schizophrenia, bipolar and normal control cases per block, and cases within each block were quantified in a random sequence. In addition, the investigator performing the quantification was blind to the diagnostic status of the cases.

Statistical Analysis

PNN density for each cortical layer of each cortical region (i.e. Brodmann's areas 9 and 17) was compared separately for subjects with schizophrenia or bipolar disorder relative to the normal control subjects using stepwise regression, as previously described (26). Briefly, we first used correlation analysis to evaluate any potential effect of each of the numerical covariates (i.e. age, PMI, antipsychotic exposure in terms of chlorpromazine equivalent dosage or CED and freezer storage time) on PNN density for each cortical layer. Those variables that were found to have a statistical significant correlation with the density measures were included in the subsequent stepwise linear regression procedure to determine any Bonferroni-corrected differences in PNN densities between diagnostic groups (i.e. between schizophrenia and normal control groups and between bipolar disorder and normal control groups). Finally, linear regression and nonlinear hyperbolic regression were performed to quantitatively survey the course of change in the density of PNNs across development for the entire PFC as a whole and for each of the cortical layers individually. To evaluate the potential influence of PMI or freezer storage on PNN density measures in this developmental cohort of cases, Pearson correlation analysis was performed. We also performed the analysis after removing the female subjects (N=4 per group) to assess if sex might affect the models.

RESULTS

Qualitative Description of PNNs in the Cerebral Cortex

The laminar distribution of PNNs was similar between the PFC and the primary visual cortex. These structures were most prominent in the mid-cortical layers (i.e. layers 3–4), less so in layers 2, 5 and 6, and not present in layer 1 (Figure 1A). Most of the neurons surrounded by PNNs appeared to be interneurons (Figure 1B), although a significant minority of them exhibited clear pyramidal morphology. However, the tissue preparation method utilized in this study does not allow us to accurately and definitively determine the relative proportion of pyramidal versus nonpyramidal cells that were surrounded by PNNs.

Decrease in PNN Density in the Cerebral Cortex in Schizophrenia is Disease-, Layer- and Region-Specific

There was a significant effect of diagnosis on PNN density in layers 3 ($F=6.49$, $p=0.016$) and 5 ($F=5.36$, $p=0.028$) of the PFC in schizophrenia. Specifically, PNN density was decreased by 70% and 76%, respectively, in layers 3 and 5 in the subjects with schizophrenia (0.31 ± 0.48 cells/mm^2, 0.15 ± 0.31 cells/mm^2, respectively; N=16), compared to the normal control subjects (1.02 ± 0.97 cells/mm^2, 0.60 ± 0.70 cells/mm^2, respectively; N=16), with age as a covariate in the model for layer 3 (Figure 1C). When we separated the cases based on medication status, it appears that those who were receiving antipsychotics might have higher PNN densities (Figure 1D); with the caveats that the sample size is very small and we found no statistically significant correlation between CED and PNN densities. In contrast to the finding of decreased PNNs in schizophrenia, no differences in PNN density were observed between subjects with bipolar disorder (N=16) and normal control subjects in any of the cortical layers (Figure 1C).

In an attempt to replicate the finding of PNN deficit in the PFC schizophrenia, we performed PNN quantification in a separate group of schizophrenia (cases 58–62) and normal control (cases 17–21) subjects (see Table 1). As shown in Figure 2, we found that PNN density was significantly decreased by 52% in layer 3 in the subjects with schizophrenia ($F=8.87$, $p=0.018$). As discussed above, LNV tissue was used in this replication study whereas paraformaldehyde-fixed tissue was used in the initial experiment. In addition, the LNV tissue had been, on average, stored in the freezer for a significantly shorter duration than the paraformaldehyde-fixed tissue (see Table 1). Interestingly, it appears that the number of PNNs visualized when LNV tissue was used was about an order of magnitude greater than when paraformaldehyde-fixed tissue was examined across diagnostic groups; hence, tissue processing and/or freezer storage may have an impact on the histochemical visualization of PNNs. However, the fact that decreased PNN density was observed in the schizophrenia subjects regardless of the type of tissue used or the duration of freezer storage strongly suggests that PNN deficit in schizophrenia is a disease-specific finding, rather than an epiphenomenon of the effects of fixation or freezer storage. This argument is further strengthened by the fact that decreased PNNs in schizophrenia was found only in the PFC, but not in the primary visual cortex (Figure 2). In summary, our findings indicate that PNN deficit appears to be present only in the subjects with schizophrenia but not in those with bipolar disorder and that it is present primarily in layer 3 and possibly also in layer 5 of the PFC, but not in any of the layers of the primary visual cortex.

Density of PNNs in the PFC Undergoes Postnatal Increase

Linear regression analysis indicates that there was a statistically significant effect of age on PNN density in the entire PFC as a whole ($R^2=0.45$, $p=0.0017$) and specifically in layer 3 ($R^2=0.49$, $p=0.0008$), suggesting that the density of PNNs in the PFC undergoes a prolonged course of progressive increase during postnatal development through adolescence and early

adulthood (Figure 3). However, the nonlinear hyperbolic regression models appear to be a better fit of the data (R^2=0.71 and 0.76 for the entire PFC and layer 3, respectively), consistent with the interpretation that PNN density increases during postnatal development with the most pronounced changes occurring around the peripubertal period (Figure 3). Nevertheless, in order to be able to more precisely map the developmental time course of change in PNN density and to distinguish between these two models, it would be necessary to increase the sample size, in particular the number of subjects that are under the age of 12. Finally, it does not appear that our results were influenced by PMI, freezer storage or sex because Pearson analysis revealed that there was no significant correlation between PMI or freezer storage duration and PNN densities, and removing the four female subjects also did not significantly affect the results.

DISCUSSION

Findings of this study, together with previous observations of decreased PNN densities in the amygdala and entorhinal cortex in subjects with schizophrenia (26), suggest that PNN deficit may be a rather pervasive pathophysiologic feature of this illness, affecting a variety of regions of the brain. In addition, deficit of PNNs appears to be specific for schizophrenia rather than a feature shared by primary psychotic conditions, as decreased PNN density was not found in either of these brain regions in subjects with bipolar disorder. Furthermore, PNN densities appear to be unchanged in the primary visual cortex not only in the subjects with bipolar disorder, but also in the schizophrenia subjects, indicating that, within the neocortex, PNN deficit in schizophrenia is region-specific, although the pathophysiologic significance of such regional dissociation is not immediately clear. Finally, our data suggest that PNNs in the human PFC undergo a protracted course of postnatal maturation, with the density of these structures progressively increasing through the peripubertal period until adolescence and early adulthood, which happens to be the period of time when the symptoms and deficits of schizophrenia typically begin to gradually emerge.

PNN Deficit in the PFC in Schizophrenia

PV-containing inhibitory neurons are thought to play a critical role in the pathophysiology of schizophrenia (10–12). Recent findings suggest that dysfunction of these neurons may contribute to the clinical symptoms and cognitive impairments of the illness by disrupting gamma band synchronized oscillation of pyramidal cell networks in the cerebral cortex (30–35). The etiology of the functional disturbances of PV neurons remains largely unknown, although it has been suggested that oxidative stress and hypofunction of the N-methyl-D-aspartate (NMDA) glutamate receptor, among other factors, may lead to their injury (36–41). Our data, together with findings of a previous study (26), suggest that decreased PNNs, which preferentially (but not selectively) enwrap PV neurons (3, 5, 42, 43), may be an additional pathophysiologic mechanism that contributes to the dysfunction of these neurons. Furthermore, in a recent study, it has been shown that oxidative stress that results from impaired synthesis of glutathione in mice can lead to the disturbances of the functional maturation of PV neurons and the developmental formation of PNNs that normally surround these neurons (44), suggesting that PNN deficit may be a mediator of the pathophysiological effects of oxidative stress. In this context, because PV neurons in the cerebral cortex appear to be relatively intact in bipolar disorder (45, 46), the fact that PNN deficit was not observed in the subjects with bipolar disorder in this study is not unexpected.

Aside from PV neurons, pyramidal neurons in the cerebral cortex, especially those in layer 3, are also known to be disturbed in schizophrenia (47). For instance, the density of dendritic spines on pyramidal neurons in layer 3 of the PFC has been found to be decreased in subjects with schizophrenia (48). Consistent with this observation, the average somal area of these neurons also appears to be decreased (49). PNNs are known to enwrap not only PV

neurons, but also the dendritic trees, somata and axon initial segments of many pyramidal neurons and, as such, they are thought to play an important role in the stabilization and maintenance of the synaptic connectivities of pyramidal neurons (3, 5, 42, 43). Hence, PNN deficit may directly contribute to the dysfunction of pyramidal cell networks in schizophrenia by compromising the integrity and stability of their synaptic architecture, thereby leading to aberrant information processing.

Because the formation of PNNs is known to be activity-dependent (20, 24, 50–52) and because the dysfunction of both PV and pyramidal neurons in schizophrenia is expected to be associated with altered neuronal activities, PNN deficit in schizophrenia may be a consequence, instead of a cause, of the dysfunction of these neurons. Nevertheless, even in this scenario, PNN deficit triggered by the hypofunction of PV and/or pyramidal neurons can further compromise their functional integrity, which in turn may exacerbate PNN disturbances, hence resulting in a malicious, self-sustaining mechanism of neuronal injury. Our data suggest that PNN deficit occurs predominantly in layer 3, which furnishes long-range connections with widespread cortical regions. This observation is consistent with a large body of literature demonstrating that various pathophysiological features of schizophrenia appear to occur primarily in this layer. In addition, our data suggests that PNN deficit may also occur in layer 5, which furnishes subcortical output and provides feedback regulation of layer 3 circuits. Taken together, it appears that a consequence of PNN deficit in the PFC in schizophrenia may involve large-scale information processing disturbances across multiple cortical and subcortical neural networks.

Many questions remain unanswered. For instance, without colocalizing WFA histochemical signal with specific neuronal markers, the relative proportion of PV versus pyramidal neurons that were associated with decreased PNNs is not known. Furthermore, WFA is only one of the many markers that recognize some but not all PNNs, as various markers recognize chemically distinct populations of these structures (2, 51, 53–57). The relationships, if any, between specific PNN populations recognized by these various markers and the different neuronal types (e.g. PV versus pyramidal neurons) are unknown. Determining such relationships and how these relationships might be altered in schizophrenia is likely to deepen our insight into the molecular abnormalities that underlie cortical circuitry disturbances.

PNNs and Schizophrenia Onset

Studies in the mammalian cerebral cortex suggest that PNNs are developmentally regulated; hence, the number of PNNs gradually increases during postnatal development, which temporally parallels the time course of the critical period of developmental synaptic plasticity (17–20). In fact, it has been shown that the maturation of PNNs may play a crucial role in determining the timing of the closure of critical period, whereas proteolytic treatment in the adult brain (e.g. by tPA) reactivates the molecular machinery of this process (15, 18, 19, 24). Interestingly, during postnatal development, tPA level first rises, and then declines, which appears to signal the endpoint of developmental experience-dependent synaptic remodeling (18, 19, 25). In other words, developmental increase in PNNs, together with the decrease in tPA, stabilizes experience-dependent formation of synapses by preventing them from undergoing further structural modification. If PNNs also regulate the extensive synaptic refinement process that occurs in the human PFC during the periadolescent period (58–61), similar to their important role in determining the time course of critical period in other cortical regions in animals, then it can be expected that deficient developmental PNN formation may compromise the experience-dependent consolidation of synaptic connectivities. As a result, the synaptic architecture of the PFC may remain in an excessively plastic, permanently juvenile state where synapses and thus functional cortical circuits fail to be stabilized, compromising the integrity, stability and fidelity of PFC circuits

that are necessary for reliable and predictable information processing and may thereby contribute to the onset of schizophrenia and the persistent symptomatic and cognitive deficits that characterize the course of this chronic debilitating illness. This scenario may, at least in part, explain the previous postmortem findings of decreased dendritic spines and neuropil in subjects with schizophrenia (48, 62, 63). Consistent with this hypothesis, using a novel free-water imaging technique, it has recently been shown that the extracellular space in the cerebral cortex, of which PNNs are a component, was significantly decreased in first-episode schizophrenia patients (64).

Clinical Implications and Future Direction

Given the presumably critical role of PNNs in the normal functioning of PV and pyramidal neurons, the maturation of cortical circuits involving these neurons, and the maintenance of cortical circuit stability, one can speculate that effective therapeutic and preventative strategies may involve restoring the structural and developmental integrity of PNNs. Our findings may also inform the development of novel diagnostic techniques for schizophrenia, using PNNs as a biomarker. For instance, ligands that recognize specific molecular domains that make up PNNs can be developed to detect and quantify these structures in the living human brain, much like imaging amyloid protein in Alzheimer's disease. In summary, the finding of decreased PNNs in the PFC in schizophrenia suggests that detailed characterization of the molecular and pathogenetic basis of PNN deficit has the potential of leading to breakthroughs in the diagnosis, treatment, early intervention and prevention of this devastating illness.

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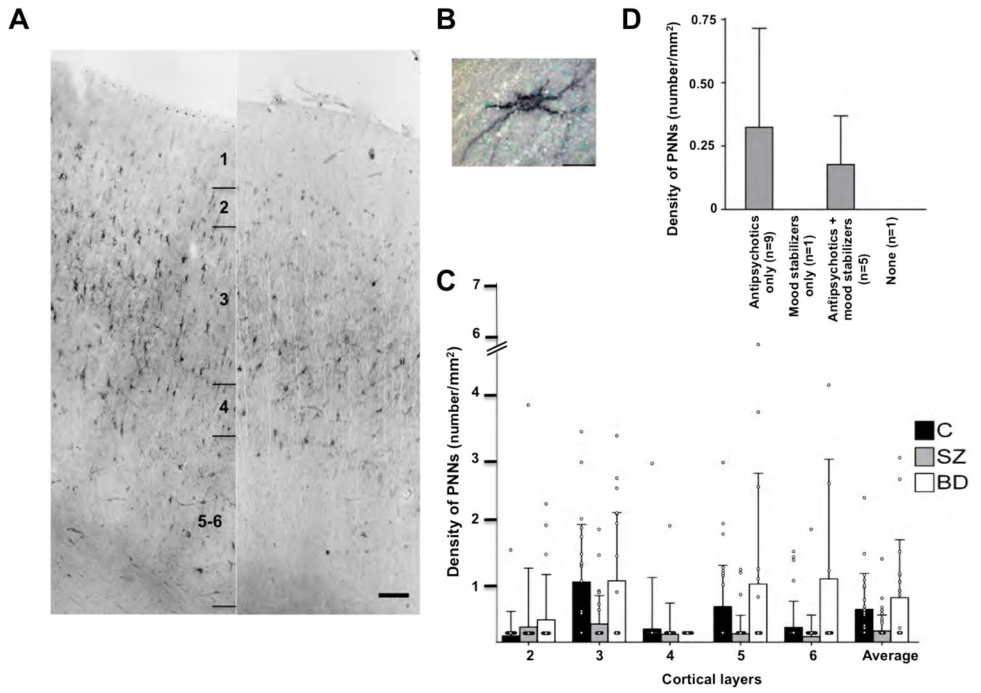

Figure 1. Densities of PNNs are decreased in the PFC in subjects with schizophrenia

A. Representative photomicrographs showing the distribution of PNNs in the PFC in a schizophrenia (right) and a normal control (left) subjects. Scale bar=100μm. **B.** Photomicrograph showing a WFA-labeled PNN. Scale bar=20μm. **C.** WFA-labeled PNNs are significantly decreased in layers 3 (70%) and 5 (76%) in subjects with schizophrenia (SZ; N=16). Bar graphs represent the mean and upper 95% confidence interval by cortical layer. Layer 1 is not shown because no PNNs were found in that layer. There are no significant differences in PNN densities between subjects with bipolar disorder (N=15) and normal control (N=16) subjects. p value, F ratio: *(0.016, 6.49); **(0.028, 5.36); ***(0.042, 4.51). **D.** PNN densities in relation to medications in the schizophrenia subjects. Note that in the two subjects who were on no medications (N=1) at the time of death or on a mood stabilizer only (N=1), PNNs were essentially undetectable in the PFC. C=control, SZ=schizophrenia, BD=bipolar disorder.

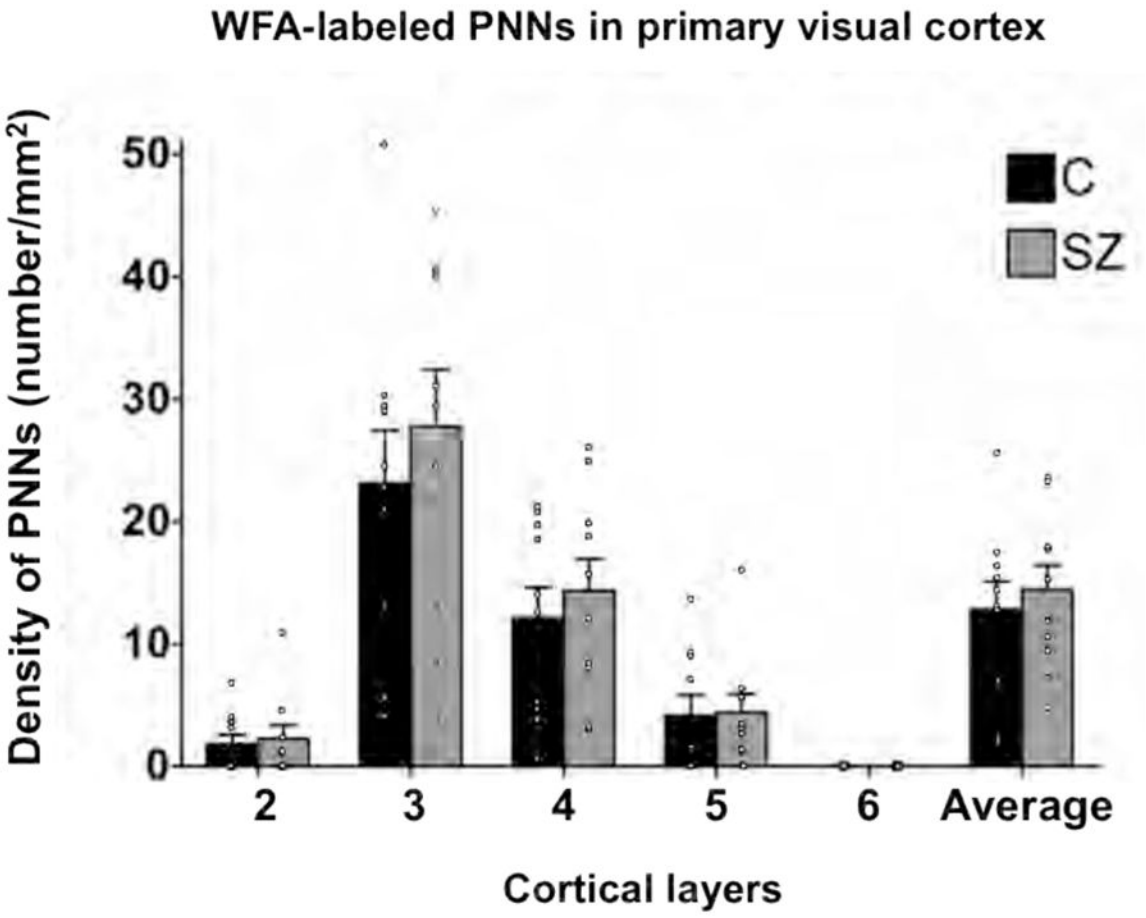

Figure 2. Densities of PNNs are unaltered in the primary visual cortex in subjects with schizophrenia

There are no significant differences in PNN densities between subjects with schizophrenia (SZ; N=10) and normal control (C; N=10) subjects.

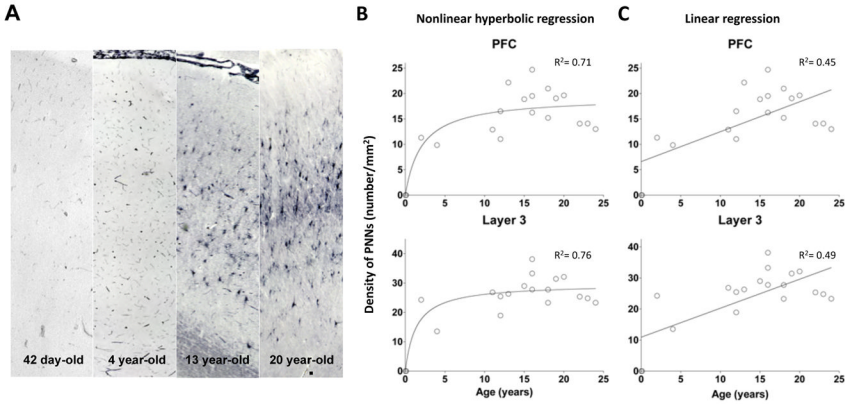

Figure 3. Postnatal development of PNNs in the human PFC

A. Photomicrographs demonstrating the increase in PNNs in the PFC during postnatal development. **B.** Nonlinear hyperbolic regression model of PNN densities in layer 3 and in the entire PFC. **C.** Linear regression model of PNN densities in layer 3 and in the entire the PFC.

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Table 1

Case[1]	Group	Sex	Age (years)	PMI[2] (hours)	Freezer Storage Time (days)	Cause of Death	Antipsychotics	Anticonvulsants/Mood Stabilizers	Area 9[3]
1	Control	M	69	15.3	5258	Chronic obstructive pulmonary disease	None	None	+
2	Control	F	78	14.1	5088	Myocardial infarction	None	None	+
3	Control	M	36	24.5	4684	Myocardial infarction	None	None	+
4[#]	Control	M	37	18.7	4426	Electrocution	None	None	+
5	Control	M	49	24.6	4410	Myocardial infarction	None	None	+
6	Control	M	40	16.6	4246	Cardiac arrest	None	None	+
7	Control	F	74	12.5	4244	Renal failure	None	None	+
8	Control	M	67	22.3	4028	Cardiac arrest	None	None	+
9	Control	M	79	20.9	3874	Pancreatic cancer	None	None	+
10[#]	Control	F	78	23.9	3865	Breast cancer	None	None	+
11	Control	F	65	24.2	3831	Lung cancer	None	None	+
12	Control	F	66	7.4	3844	Lung cancer	None	None	+
13	Control	M	89	7.4	3724	Cancer	None	None	+
14	Control	M	50	24.1	3422	Myocardial infarction	None	None	+
15	Control	M	73	20.5	3334	Cardiac arrest	None	None	+
16	Control	M	62	26.1	4957	Myocardial infarction	None	None	+
Average±SD (cases 1–16)			63.0±16.0	18.9±6.1	4152±554				
17	Control	M	76	23.9	1181	Pancreatic cancer	None	None	+*
18	Control	F	47	25.8	1142	Cardiopulmonary arrest	None	None	+*
19	Control	M	46	28.8	1029	Myocardial infarction	None	None	+*
20	Control	F	53	34.5	656	Chronic obstructive pulmonary disease	None	None	+*
21	Control	M	55	23.9	773	Cardiopulmonary arrest	None	None	+*
Average±SD (cases 17–21)			55.0±12.0	27.4±4.5	1049±232				
22	Control	F	51	30.6	1171	Pulmonary embolism	None	None	
23	Control	M	65	20.9	1236	Myocardial infarction	None	None	
24	Control	M	57	35.5	686	Myocardial infarction	None	None	
25	Control	F	65	27.2	654	Unknown	None	None	

Case[1]	Group	Sex	Age (years)	PMI[2] (hours)	Freezer Storage Time (days)	Cause of Death	Antipsychotics	Anticonvulsants/Mood Stabilizers	Area 9[3]
26	Control	M	62	26.1	674	Myocardial infarction	None	None	
Average±SD (cases 17–26)			58.0±9.0	27.7±4.7	920±252				
27	Bipolar	F	80	11.6	4921	Stroke	None	Valproic acid	+
28#	Bipolar	F	42	15.8	4613	Suicide by overdose	Perphenazine	Valproic acid, lithium	+
29	Bipolar	F	76	22.8	4552	Myocardial infarction	Olanzapine	None	+
30	Bipolar	M	50	30.5	4551	Cardiopulmonary arrest	None	Lithium	+
31	Bipolar	M	74	24.8	4472	Pneumonia	Quetiapine	Valproic acid	+
32#	Bipolar	M	73	7.18	4032	Pneumonia	Olanzapine	Gabapentin	+
33#	Bipolar	F	73	20.8	3985	Sepsis	Olanzapine	None	+
34#	Bipolar	M	74	14.3	3898	Pneumonia	Quetiapine	Carbamazepine, valproic acid, lithium	+
35#	Bipolar	F	73	17.0	3851	Cardiopulmonary arrest	Olanzapine	Valproic acid	+
36	Bipolar	M	72	27.7	4793	Respiratory failure	None	Lithium	+
37	Bipolar	M	78	30.2	3675	Cardiopulmonary arrest	Fluphenazine, chlorpromazine	Valproic acid, lithium	+
38	Bipolar	M	41	30.7	3613	Suicide by hanging	Risperidone, ziprasidone	Topiramate, gabapentin	+
39#	Bipolar	M	83	17.5	3401	Unknown	Unknown	Unknown	+
40	Bipolar	M	83	5.0	3376	Cardiopulmonary arrest	Unknown	Unknown	+
41	Bipolar	M	38	22.0	4921	Suicide by carbon monoxide poisoning	Olanzapine	Valproic acid	+
Average±SD (cases 27–41)			67.0±16.0	19.9±8.2	4179±544				
42	Schizophrenia	F	83	23.2	5086	Unknown	Haloperidol, fluphenazine	None	+
43	Schizophrenia	F	84	25.8	5054	Congestive heart failure	Risperidone	Valproic acid	+
44	Schizophrenia	M	44	19.0	4881	Pneumonia	Clozapine	None	+
45	Schizophrenia	M	35	28.0	4882	Cardiopulmonary arrest	None	Valproic acid	+
46	Schizophrenia	M	42	18.1	4881	Suicide by carbon monoxide poisoning	Olanzapine	None	+
47	Schizophrenia	F	78	13.4	4848	Sick sinus syndrome	Haloperidol	Lithium	+
48	Schizophrenia	M	46	18.5	4840	Sepsis	Olanzapine	Valproic acid	+
49	Schizophrenia	F	72	21.7	3849	Cardiac arrest	Risperidone	None	+
50	Schizophrenia	M	26	16.0	4793	Suicide by hanging	Fluphenazine	None	+
51	Schizophrenia	F	42	27.1	4791	Cancer	Unknown	Unknown	+

Case[1]	Group	Sex	Age (years)	PMI[2] (hours)	Freezer Storage Time (days)	Cause of Death	Antipsychotics	Anticonvulsants/Mood Stabilizers	Area 9[3]
52	Schizophrenia	F	47	19.2	4776	Cancer	Unknown	Unknown	+
53	Schizophrenia	M	31	29.0	4649	Unknown	Olanzapine, risperidone	None	+
54	Schizophrenia	F	80	10.9	4547	Cardiopulmonary arrest	Thioridazine	None	+
55	Schizophrenia	M	49	19.1	4527	Suicide by hanging	Thiothixene	Valproic acid	+
56	Schizophrenia	F	73	24.1	4062	Lung cancer	Risperidone	None	+
57	Schizophrenia	M	60	22.2	5086	Cardiopulmonary arrest	Quetiapine, olanzapine	None	+
Average±SD (cases 42–57)			56.0±20.0	20.9±5.2	4698±341				
58	Schizophrenia	M	77	25.3	1246	Pneumonia	Clozapine	Valproic acid	+*
59	Schizophrenia	F	48	29.9	1177	Uterine cancer	Olanzapine	None	+*
60#	Schizophrenia	M	32	38.4	1130	Suicide by hanging	None	None	+*
61	Schizophrenia	F	56	26.5	1081	Colon cancer	Olanzapine, Chlorpromazine,	None	+*
62	Schizophrenia	M	58	25.3	947	Coronary artery disease	risperidone	None	+*
Average±SD (cases 58–62)			54.0±16.0	29.1±5.5	1077±193				
63	Schizophrenia	F	47	31.8	492	Dilated cardiomyopathy	Risperidone	None	
64	Schizophrenia	M	65	21.1	750	Congestive heart failure	Thioridazine	Valproic acid	
65	Schizophrenia	M	59	29.7	939	Colon cancer	Clozapine	Valproic acid	
66#	Schizophrenia	F	69	23.1	325	Cardiopulmonary arrest	Olanzapine	None	
67#	Schizophrenia	M	60	22.2	454	Cardiopulmonary arrest	Quetiapine, olanzapine	None	
Average±SD (cases 58–67)			57.0±13.0	27.3±5.2	854±331				

Table 2

Human subjects included for PNN quantification during PFC development.

Age[1]	Race[2]	Sex	PMI (hours)	Freezer Storage Time (days)
2 days	B	M	6	1731
42 days	B	M	2	5238
2	C	M	16	1098
4	B	F	15	1599
11	C	M	20	4853
12	B	M	15	1046
12	B	M	15	2189
13	C	F	17	3253
15	C	M	9	1216
16	C	M	16	2494
16	C	F	13	1688
16	C	F	13	1744
18	C	M	17	2231
18	B	M	14	1300
19	B	M	5	1924
20	B	M	5	2349
22	B	M	13	2186
23	C	M	18	2830
24	C	M	14	1945

[1] Age in years except for the first two subjects.

[2] B=black, C=Caucasian; M=male;

[3] PMI=postmortem interval.