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Microglial Pruning of Synapses in the Prefrontal Cortex During Adolescence

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Abstract

Exaggerated synaptic elimination in the prefrontal cortex (PFC) during adolescence has been suggested to contribute to the neuropathological changes of schizophrenia. Recent data indicate that microglia (MG) sculpt synapses during early postnatal development. However, it is not known if MG contribute to the structural maturation of the PFC, which has a protracted postnatal development. We determined if MG are involved in developmentally specific synapse elimination in the PFC, focusing on adolescence. Layer 5 PFC pyramidal cells (PCs) were intracellularly filled with Lucifer Yellow for dendritic spine measurements in postnatal day (P) 24, P30, P35, P39, and P50 rats. In the contralateral PFC we evaluated if MG engulfed presynaptic (glutamatergic) and postsynaptic (dendritic spines) elements. Dendritic spine density increased from P24 to P35, when spine density peaked. There was a significant increase in MG engulfment of spines at P39 relative to earlier ages; this subsided by P50. MG also phagocytosed presynaptic glutamatergic terminals. These data indicate that MG transiently prune synapses of PFC PCs during adolescence, when the symptoms of schizophrenia typically first appear. An increase in MG-mediated synaptic remodeling of PFC PCs may contribute to the structural changes observed in schizophrenia.

Key words: dendritic spine, microglia, pyramidal cell, schizophrenia, synaptic pruning

Schizophrenia is usually first diagnosed during late adolescence or early adulthood (Häfner et al. 1994). However, in vivo imaging studies of persons at high risk for developing schizophrenia indicate that structural brain changes antedate the first psychotic break (Borgwardt et al. 2008; Fusar-Poli et al. 2012), suggesting that aberrant neurodevelopmental processes contribute to the pathology of schizophrenia. Though several variants of the neurodevelopmental hypothesis of schizophrenia have been advanced (Murray et al. 2017), most hypothesize an insult during the second or third trimester of pregnancy that remains latent until symptoms emerge in adolescence. An early neurodevelopmental hypothesis that is consistent with the neuropathology of schizophrenia was advanced by Feinberg (1982),

who posited that schizophrenia results “from a defect of synaptic elimination programmed to occur during adolescence.” This hypothesis was based on the temporal pattern of postnatal development of pyramidal cells (PCs) in the frontal cortex (Huttenlocher 1979), with dendritic spines being added during development, and thereafter supernumerary spines being pruned until the PC achieves its final adult spine density (Rakic et al. 1994).

Among the structural alterations in schizophrenia is a reduction in gray matter volume that prominently affects the frontal lobe (Leung et al. 2011; Fusar-Poli et al. 2012). Despite this change, there is no overall neuronal loss in the cortex (Selemon et al. 1998; Thune et al. 2001). The decrease in cortical

volume in the face of a normal complement of neurons led [Selemon and Goldman-Rakic \(1999\)](#) to posit a decrease in cortical neuropil, including axons and dendrites. Consistent with the reduced neuropil hypothesis is a decrease in the density of dendritic spines on PCs in the prefrontal cortex (PFC) ([Glausier and Lewis 2013](#); [Moyer et al. 2015](#)). The mechanisms underlying this spine loss are unclear.

The connections between neurons are achieved by dynamic processes that refine and sculpt synapses. During brain development the number of synapses changes in a relatively systematic manner. In early postnatal development exuberant synaptic connections are formed ([Innocenti and Price 2005](#)), after which some synapses undergo synaptic pruning while remaining synapses are strengthened and maintained ([Katz and Shatz 1996](#); [Hua and Smith 2004](#)). Mounting evidence over the past several years points to the involvement of microglia (MG), the resident immune cells of the central nervous system, in synaptic pruning and remodeling in early postnatal development ([Paolicelli et al. 2011](#); [Schafer et al. 2012](#)). Recently, [Sekar et al. \(2016\)](#) reported that variations in complement component 4 (C4), which is critically involved in MG-mediated synaptic pruning ([Stephan et al. 2012](#)), strongly increase the risk of developing schizophrenia, consistent with Feinberg's hypothesis that enhanced synaptic elimination during adolescence underlies the pathological changes of schizophrenia.

Studies of MG-mediated synaptic pruning in rodents have mainly focused on brain areas that mature relatively early in postnatal development, during the first several weeks of life. It is not known if MG sculpt projection neurons in late-maturing structures. Among these sites is the PFC ([Huttenlocher and Dabholkar 1997](#); [Tau and Peterson 2010](#)), an area in which synapse number and maturation is not complete until the third decade of life in humans ([Petanjek et al. 2011](#)). The PFC is critically involved in cognitive processes that are disrupted in schizophrenia ([Berman and Weinberger 1991](#); [Davis et al. 1991](#); [Goldman-Rakic 1999](#); [Lewis and Moghaddam 2006](#); [Keefe and Harvey 2012](#); [Woodward and Heckers 2015](#)). We assessed the role of MG in synaptic remodeling in the PFC, determining if MG engulf both presynaptic and postsynaptic elements during critical periods in postnatal development.

Materials and Methods

Experimental Design

Timed-pregnant Sprague-Dawley dams (Envigo; Indianapolis, IN) were housed on a 12:12 light-dark cycle with food and water freely available. The offspring were weaned on postnatal day (P) 21. Animals were sacrificed at P24, P30, P35, P39, or P50 (± 1 day) by isoflurane overdose and transcardially perfused with 9.25% sucrose followed by 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). The brains were removed and bisected along the midline, with the PFC of one hemisphere used for dendritic spine measurements and the other hemisphere used to assess MG engulfment of synaptic elements. All studies were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and the Vanderbilt University Institutional Animal Care and Use Committee.

Intracellular Fills and Spine Measurements

One hemisphere was postfixed in 4% PFA in PBS for 30 min at 4°C, after which 200 μ M coronal sections through the PFC were cut on a vibrating microtome.

In animals in which we assessed spine density (P24 [$n = 5$; 2 males {m}, 3 females {f}], P30 [$n = 8$; 4m, 4f], P35 [$n = 8$; 3m, 5f], P39 [$n = 9$; 7m, 2f], and P50 [$n = 8$; 3m, 5f]), 5 randomly selected PCs in Layer 5 (L5) of the prelimbic cortex (area 32) in the medial PFC were filled intracellularly with 8% Lucifer Yellow (LY; L0259, Sigma; St. Louis, MO), using a constant negative current of 3–5 nA for 8–10 min. Sections were fixed in 4% PFA in PBS overnight at 4°C, and then mounted and coverslipped using ProLong Antifade (P36970, Thermo-Fisher Scientific Inc.; Waltham, MA) mounting medium.

Dendritic segments were imaged by confocal microscopy (Zeiss LSM 710) under $\times 63$ magnification and a $\times 3$ digital zoom. Images of 3 oblique dendritic segments (located 120–150 μ m distal to the soma) emanating from 3 different primary basal dendrites on each LY-filled PC were acquired. Z stacks were deconvolved (Huygen's Essential, Scientific Volume Imaging; Hilversum, Netherlands) and the projection image of each segment generated. The number of spines/10 μ m of dendritic length was determined. The mean spine densities of the 3 distal basal dendritic segments were collapsed to obtain an average distal basal spine density value for each individual PC, and in turn collapsed across the 5 PCs to yield a "per animal" basal dendrite spine density value.

Changes in spine density reflect the sum of spines pruned from the dendrite and any newly generated spines. Mature and young spines have been suggested to have different shapes (shorter, mushroom-shaped and longer, thin spines, respectively). We therefore measured spine length, maximal spine head diameter, and spine head volume at P24 and P39 to determine if there were changes in the distribution of spine type during PFC development. Spine morphology parameters were measured using Imaris (Bitplane USA; Concord, MA) from 2 randomly selected dendritic segments of 5 PCs/animal. Filopodia (spines ≥ 4.1 μ m in length) made up a very small percentage of the total spines ($\leq 0.4\%$), and were not included in spine morphology analyses.

Immunohistochemistry

One hemisphere was postfixed in 4% PFA in PBS overnight at 4°C and cryoprotected in 30% sucrose. Sets of coronal sections (42 μ m) through the PFC were cut on a sliding microtome.

An immunofluorescent approach was used to assess MG engulfment of dendritic spines and their presynaptic partners. Sections were blocked in Tris-buffered saline (TBS) containing 4% normal horse serum (16050114, Thermo-Fisher Scientific Inc.) and 0.2% Triton X-100 (TBS⁺⁺) and then incubated for 36–48 h at 4°C in a cocktail of an antibody directed against the MG marker Iba1 (1:6000; 019-19741, Wako; Richmond, VA) and the postsynaptic (spine) marker PSD-95 (1:1500; MAB1596, Millipore; Burlington, MA). Other sets of sections from the same animals were stained with antibodies directed against Iba1 and the presynaptic glutamatergic protein VGluT1 (1:25; 73-066, clone N28/9, RBID:AB_2187693, UC Davis/NIH NeuroMab Facility; Davis, CA). Sections were then placed in the appropriate fluorescently conjugated secondary antibodies from Jackson ImmunoResearch (1:1200; West Grove, PA). Sections were mounted and coverslipped with ProLong Antifade.

An immunoperoxidase method was used to stain MG for determinations of MG soma area and density. Sections were incubated in methanolic peroxide (10% methanol and 0.6% peroxide in TBS) for 10 min, blocked in TBS⁺⁺, and incubated in rabbit anti-Iba1 (1:4000) for 18–24 h at room temperature. Sections were then incubated in the biotinylated secondary

antibody (1:1000; 711-065-152, Jackson ImmunoResearch), followed by HRP-conjugated streptavidin (1:1600; 016-030-084, Jackson ImmunoResearch). Sections were developed in a 0.05% 3,3'-diaminobenzidine (32750, Sigma; St. Louis, MO) in TBS containing 0.009% hydrogen peroxide. Slide-mounted sections were dehydrated through increasing concentrations of ethanol followed by Histo-Clear (HS-200, National Diagnostics; Atlanta, GA) and coverslipped with DPX (44581, Sigma; St. Louis, MO).

Microglial Measurements

MG Engulfment

Z stacks of 8 randomly selected MG cells per animal in L5 of the prelimbic cortex were obtained using a $\times 63$ objective, with a digital $\times 3$ zoom on a Zeiss LSM 880 confocal microscope. The Z stacks were deconvolved, and the projection images generated (Fig. 1C,D). MG engulfment of synaptic elements was assessed by obtaining the ratio of the area of "yellow" pixels (colocalization of MG and synaptic elements), identified by the overlap of defined threshold ranges for the red and green channels, to the area occupied by the red channel (MG), yielding a "colocalization index." Orthogonal views of confocal images were used to verify colocalization (Fig. 1B).

MG Density and Soma Area

Three $15,500\ \mu\text{m}^2$ images of L5 of the prelimbic cortex from rats at P24, P35, P39, and P50 were captured using a Nikon Eclipse Ni-U microscope under a $\times 40$ objective with a $\times 1.5$ digital magnification. MG soma area was determined by measuring the area of 3 microglial cells from each of the images captured, for a total of 9 MG/animal. In addition, the density of MG was determined in each of the 3 images. Soma area and density values were averaged to yield a "per animal" value for each measure.

Data Analysis

All studies were conducted under blind conditions, from acquisition of microscopic images to statistical analysis; all data were coded and maintained by a person not affiliated with the study. Spine density, MG density, and MG soma area were analyzed by one-way ANOVAs, and Bonferroni post hoc tests were used to define significant differences. Frequency distributions of spine length, spine head diameter, and spine head volume were compared using the Kolmogorov-Smirnov test. Measures of MG engulfment of postsynaptic dendritic spines and presynaptic glutamatergic terminals were analyzed with the Kruskal-Wallis nonparametric ANOVA followed by Dunn's multiple comparison test.

Results

Spine Density and Morphology Changes in the PFC Through Development

An ANOVA uncovered an overall age effect in L5 PFC PC spine density ($F[4,32] = 5.85$, $P = 0.0012$; Fig. 2). Bonferroni post hoc tests revealed that spine density was significantly increased at all ages at or after P35 relative to P24. Because we used both male and female animals, we also examined if there was a sex difference in spine density at various ages. Although the overall trend appeared grossly similar in males and females, with spine density changing in parallel across the sexes, no significant effects were uncovered; the study was underpowered to detect a sex difference.

Comparison of the frequency distributions of spine length at P24 and P39 showed that spine length distribution was shifted to the left at P39 relative to P24 ($D = 0.1686$, $P \leq 0.0001$), that is, dendritic spines on PFC PCs of adolescent rats were shorter than those on PCs from preadolescent animals (Fig. 3A). The

Figure 1. (A) Representative projection image of a basal dendritic segment from a L5 PFC PC intracellularly filled with Lucifer Yellow. Scale bar, $5\ \mu\text{m}$. (B) Orthogonal views showing the microglia and the dendritic spine in the same plane (white arrows) were used to verify colocalization. (C) and (D) Show examples of projection images of microglia with low (P24) and high (P39) levels, respectively, of colocalization (yellow puncta) of Iba1-labeled microglia (red) and PSD-95-ir dendritic spines (green). PSD-95-ir puncta were visible in the soma and in the processes (white arrows). Scale bars, $5\ \mu\text{m}$.

Figure 2. Spine density changes in L5 prefrontal cortical pyramidal cells as a function of age. Mean ($\pm$ SEM) basal spine density differed significantly as a function of age. Spine density peaked at P35, and remained significantly higher than P24 at all ages thereafter. * $P \leq 0.01$.

frequency distributions of spine head diameter ($D = 0.0953$, $P \leq 0.01$; Fig. 3B) and spine head volume ($D = 0.1134$, $P = 0.0001$; Fig. 3C) were both significantly shifted to the right at P39 compared with P24, that is, the spine heads were larger in the adolescent rats.

MG Soma Area and Density

An ANOVA uncovered age-dependent significant differences in soma area ($F[3,19] = 4.273$, $P = 0.0182$; Fig. 4A). Post hoc tests revealed a single significant difference, with larger mean MG soma area at P50 than P39 ($P \leq 0.05$). MG density did not differ across age ($F[3,18] = 0.839$, NS; Fig. 4B).

Microglial Engulfment of Dendritic Spines

A Kruskal–Wallis nonparametric ANOVA uncovered a significant age difference in MG engulfment of dendritic spines ($H = 36.9$, $P \leq 0.0001$; Fig. 5). Post hoc tests showed a significant increase in MG phagocytosis of spines at P39 relative to P24 ($P \leq 0.05$), which subsided by and was reversed at P50 compared with both P24 ($P \leq 0.01$) and P39 ($P \leq 0.0001$).

Microglial Engulfment of Presynaptic Elements

The nonparametric ANOVA showed an overall age effect on MG phagocytosis of presynaptic glutamatergic elements in the PFC ($H = 30.1$, $P \leq 0.0001$; Fig. 6). Post hoc tests found an increase in MG engulfment of VGluT1-immunoreactive (ir) glutamatergic terminals at both P39 ($P \leq 0.05$) and P50 ($P \leq 0.0001$) relative to P24.

Discussion

We found that MG age-dependently phagocytose both presynaptic and postsynaptic elements of excitatory synapses on L5 PFC PCs. MG actively engulf dendritic spines of PFC PCs shortly after peak spine density is reached. Similarly, MG phagocytose presynaptic excitatory terminals. Exaggerated pruning of synapses during adolescence has been posited to be a central pathophysiological feature of schizophrenia (Feinberg 1982), contributing to the reduction in spine density reported in post-mortem studies (Glausier and Lewis 2013; Moyer et al. 2015) and the resultant cognitive disturbances in schizophrenia. Our data

Figure 3. Dendritic spine morphology in prefrontal cortical L5 pyramidal cells across development. The frequency distributions of (A) spine length, (B) maximal spine head diameter, and (C) spine head volume were compared at P24, when microglial engulfment of spines was low, and P39, the age at which microglial engulfment of spines was high. The distribution of spine length at P39 was significantly different from that at P24, and was shifted to the left, indicating that spine length was shorter at P39. The frequency distributions of spine head diameter and volume were shifted to the right, signifying that shorter spines that have larger heads are more numerous at P39. * $P \leq 0.01$; ** $P \leq 0.0001$.

suggest that MG are an effector of excessive PC synaptic pruning during adolescence in schizophrenia.

Dendritic Spine Density During Postnatal Development

We found that L5 PCs in the prelimbic cortex reached peak spine density at P35; there was no decrease in spine density over the next 2 weeks. Although the same temporal pattern was seen in male and female subjects, when analyzing the data with sex as a factor in the ANOVA, our study was underpowered to detect a

Figure 4. Microglia soma size and density in the prefrontal cortex through adolescence. (A) The mean ($\pm$ SEM) area of PFC microglial somata at P39 was significantly less than at P50. * $P \leq 0.05$. (B) Microglial density was unchanged across development.

Figure 5. Microglial engulfment of dendritic spines during development in the prefrontal cortex. A sharp increase in microglial phagocytosis of dendritic spines was observed at P39 relative to earlier time points. By P50 microglial engulfment had subsided and was significantly lower than observed at P24. * $P \leq 0.05$; ** $P \leq 0.0001$.

significant effect. The current data warrant a focused investigation to determine if there are significant differences between males and females.

Surprisingly, there have been few previous studies of the ontogeny of dendritic spines on PFC PCs. Consistent with our observations, Koss et al. (2014), using Golgi impregnation, reported that basal spine density on L5 PCs of the rat PFC increase from P20 to P35. Markham et al. (2013), studying L3 PCs in the rat prelimbic cortex, found that basal and apical

Figure 6. Microglia engulf excitatory presynaptic elements in the prefrontal cortex during adolescence. The engulfment of glutamatergic (VGLUT1-ir) presynaptic elements by microglia was significantly increased at both P39 and P50 relative to P24. * $P \leq 0.05$; ** $P \leq 0.0001$.

spine density increased from P20 to P30, and decreased from P30 to P56 in the basilar tree of female but not male rats. Examination of synapse number in the PFC of rats revealed a similar pattern, with females but not males showing an increase from P25 to P35, and a decrease from P35 to P45 (Drzewiecki et al. 2016). Gourley et al. (2012), using mice in which GFP was expressed in PCs under a Thy1 promoter, concluded that spine density in the orbitofrontal cortex peaked at P31 and declined by P56–60. A decrease in apical dendritic spine density of L5 PFC PCs between P31 and P42 has also recently been reported (Shapiro et al. 2017).

Our data and these reports agree that spine density on frontal cortical PCs peaks between P30 and P35, but the results of the studies on subsequent changes in spine density somewhat differ. We did not observe a decrease in spine density at the latest point in our study. In contrast, earlier studies reporting a decrease in spine density were performed in animals 56 days of age or older (Markham and Juraska 2002; Gourley et al. 2012; Markham et al. 2013; Koss et al. 2014), except for Shapiro et al. (2017), which monitored spine density in the apical dendrites. While a number of the factors discussed above probably contribute to this, the most likely explanation is simply that the rats in our oldest group of animals were sacrificed at 50 days of age.

The lack of consistency across studies is not surprising because PC dendritic spine density varies across brain regions and laminar position, and is also influenced by technical factors, such as the methods used to reveal and analyze spines (Wang et al. 2006; Hattox and Nelson 2007; Van Aerde and Feldmeyer 2015). We examined PCs in L5 of the prelimbic PFC for several reasons: (1) the prelimbic PFC has been suggested to be homologous to the dorsolateral PFC of primates, including humans (see Uylings et al. 2003; however, also see Preuss 1995); (2) L5 of the prelimbic cortex in the rat contains the highest density of dopaminergic terminals in the medial PFC (Descarries et al. 1987; Van Eden et al. 1987); and (3) dopamine depletion of the PFC induces dendritic spine loss in L5 but not L2/3 PCs in the prelimbic cortex (Wang and Deutch 2008).

Exogenous factors, such as housing conditions and other environmental conditions, can also influence spine density (Rosenzweig 2003). Because of the large number of endogenous and exogenous factors that can modify spine density and morphology, it is necessary that each study attempting to relate developmental changes in spine density or other dendritic

parameters define the ontogeny of dendritic changes experimentally, rather than rely on published data.

Dendritic spines have been categorized into several different classes based on morphological features (Jones and Powell 1969; Peters and Kaiserman-Abramof 1970). This spurred attempts to relate different morphological types of spines to different functions, although measures of spine structure occur along a continuum and do not map as non-overlapping distributions (Arellano 2007). We examined spine morphology, including spine length, maximal spine head diameter, and spine head volume, as a proxy for spine maturity. Our data indicate that the overall distribution of spine length is shifted to the left and the distributions of spine head diameter and spine head volume are shifted to the right. In other words, spines at P39 relative to P24 are shorter and have larger heads, and thus are presumably more representative of stable, mushroom-shaped spines, suggesting that MG preferentially target longer, immature spines for elimination. This suggestion is consistent with a large body of data indicating that spine maturation and stability is activity dependent, operating under a use-it-or-lose-it rule (Zuo et al. 2005; Holtmaat and Svoboda 2009; Xu et al. 2009).

Most studies of dendritic maturation on PFC PCs lack the temporal resolution necessary to discern structural changes during the periadolescent and adolescent period, when PFC neurons undergo relatively rapid changes in morphology and function during adolescence. We examined spines at a number of different ages through this period. Nonetheless, our data represent static snapshots of dendritic trees over this period of active change, including both pruning of spines and the potential contribution of spinogenesis. Longitudinal multiphoton microscopy studies in cortical window preparations will be required to unravel the relative contribution of spinogenesis and spine pruning during periadolescence and adolescence.

There is no universally accepted definition of adolescence in rodents or humans. The periadolescent period in rats has been defined by Spear and Brake (1983) as the time between the onset of diurnal gonadotropin cycling (~P28) and the age at which reproductive capacity is achieved (~P38–P42). A variety of data suggest that adolescence then extends until ~P50. For example, based on recordings of PFC interneurons, Tseng and O'Donnell (2007) suggest that the physiological transition from adolescence to young adulthood in the PFC occurs at ~P50. Consistent with this suggestion, dopamine D₁ and D₂ receptor binding in the rat PFC peaks at P40 and declines significantly by P60 (Andersen et al. 2000). Moreover, the dopamine innervation of the rat PFC doesn't achieve its stable adult density until ~P60 (Kalsbeek et al. 1988). These considerations collectively lead us to refer to the period between P35 and P50 as adolescence.

Microglial Pruning of Dendritic Spines

Over the past several years there has been a major reconsideration of the role of MG in shaping the mature architecture of neurons in cortical and subcortical sites. Most of these studies of MG engulfment of synapses during postnatal development have focused on brain regions that mature relatively early in postnatal life (Paolicelli et al. 2011; Schafer et al. 2012). We are not aware of previous studies of developmentally mediated MG engulfment in the PFC, a late-maturing structure (Petanjek et al. 2011).

We found a sharp increase in MG engulfment of (PSD-95-ir) dendritic spines in P39 rats, shortly after peak spine density was achieved. PSD-95 is a well-validated marker of dendritic

spines that is localized to the postjunctional spine head (Hunt et al. 1996; Harris and Weinberg 2012).

PSD-95-ir puncta were not present in all MG analyzed in the PFC of P39 animals. In some cases puncta were seen throughout the soma and processes of MG cells, but in other MG no PSD-95 spine head particles were observed; this suggests that MG are functionally heterogeneous. Lawson et al. (1990) noted subtle differences in the distribution of MG across brain regions, suggesting different populations of MG. Recently, De Biase et al. (2017) reported that local cues determine divergent structural and functional properties of MG. It is not clear if these local cues include eat-me and find-me signals (and their negative counterparts), as described in apoptotic cells (Hochreiter-Hufford and Ravichandran 2013).

Classically, highly ramified MG with extensively branched processes and smaller somata are thought to surveil the neuropil for injury or pathogens. Conversely, MG with large soma and little to no branching are thought to be in an activated state (Hanisch and Kettenmann 2007; Ransohoff and Perry 2009). However, we did not detect a significant change in MG soma area except at P50 relative to earlier (P39) ages. Because MG soma area at P39 was not increased relative to earlier time points, yet there was extensive MG engulfment of dendritic spines at this age, there is seemingly no consistent relationship between this measure of MG activation and synapse pruning. Moreover, we observed that many MG that engulfed synaptic elements had extensively branched processes, consistent with the findings of Sierra et al. (2010) and Schafer et al. (2012). These data suggest that the functional processes MG use to find and engulf synapses during normal (physiological) development may differ from those used by MG in the removal of pathological (nonphysiological) debris.

We have interpreted the presence of PSD-95 puncta in MG as a consequence of active phagocytosis of spiny protuberances attached to the dendritic shaft. It is possible that MG instead phagocytose synaptic compartments, such as spines, that have already been severed from the dendrites. Lending support for the possibility that MG strip spines and other synaptic material from dendrites or presynaptic axons themselves are in vivo and light and electron microscopic studies that have shown direct contact between MG and both presynaptic and postsynaptic elements (Wake et al. 2009; Tremblay et al. 2010). MG have been shown to contain phagocytic structures under normal conditions, with smaller dendritic spines being more frequently contacted by MG (Tremblay et al. 2010). Furthermore, CX₃CR1 knockout mice exhibit a transient increase in spine density during the second and third postnatal weeks (Paolicelli et al. 2011). These data suggest that MG remove spines and presynaptic boutons from their structural foundations (dendritic shafts and presynaptic axons).

Microglial Pruning of Presynaptic Glutamatergic Elements

Microglial engulfment of glutamatergic presynaptic elements, as marked by VGluT1-ir, was increased at P39, when MG phagocytosis of spines occurred, and at P50, when spine pruning by MG had ceased. This is consistent with MG developmentally pruning both presynaptic and postsynaptic elements of axodendritic PC synapses, as has been reported in the hippocampus (Paolicelli et al. 2011). The observation that MG engulfment of presynaptic elements began at the same time as MG engulfment of spines but persisted until spine pruning had ceased agrees with the model of synapse development in which spine

growth precedes synapse formation (Miller and Peters 1981; Yuste and Bonhoeffer 2004; Knott et al. 2006).

Because most axons that synapse onto dendrites are glutamatergic, we used VGLUT1 to mark the presynaptic glutamatergic element. Postmortem studies of vesicular glutamatergic transporter levels in schizophrenia have yielded mixed results. In part this appears to reflect differences in the transporter examined. Several studies have reported decreased levels of VGLUT1 in samples from patients with schizophrenia (Eastwood and Harrison 2005; Oni-Orisan et al. 2008; Bitanirwe et al. 2009), but 2 different studies measuring cortical VGLUT2 reported unchanged levels of the transporter (Oni-Orisan et al. 2008; Shan et al. 2013). Because VGLUT1-ir axon terminals predominantly are expressed on cortical neurons, with VGLUT2-ir localized to the axons of subcortical neurons (Fremeau et al. 2001; Kaneko and Fujiyama 2002), it will be of interest to determine if there is selective MG pruning of synapses of specific cortical circuits.

Relation to Schizophrenia

Structural and functional alterations in the PFC are suggested to explain the cognitive deficits seen in the illness (Sawaguchi and Goldman-Rakic 1994; Yamamuro et al. 1994; Goldman-Rakic 1995; Lewis and Moghaddam 2006). The loss of dendritic spines on PFC PCs is among the most replicated postmortem finding in schizophrenia (Glausier and Lewis 2013; Moyer et al. 2015). Because cortical volume loss, which is not attributable to a decrease in neurons (Selemon and Goldman-Rakic 1999), may presage the diagnosis of schizophrenia (Borgwardt et al. 2008; Fusar-Poli et al. 2012) and is present at the time of the first psychotic episode (Borgwardt et al. 2008; Leung et al. 2011), some event involving the neuropil that occurs prior to diagnosis is thought to be a primary factor in the pathogenesis of schizophrenia.

Feinberg (1982) proposed that schizophrenia may be the result of a disturbance in synaptic elimination that occurs in adolescence. This hypothesis has long intrigued investigators, but the mechanism that accounted for excess spine pruning during adolescence remained unknown. A variety of recent data have pointed to changes in immune function as being involved. Genome-wide association studies of schizophrenia have consistently observed an association of genes in the major histocompatibility locus and schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Recent data have specifically pointed to genetic variation in C4 (Sekar et al. 2016), a critical component in the complement cascade which mediates MG pruning (Stephan et al. 2012).

Postmortem studies of the number of MG in schizophrenia have produced mixed results. Both increases in PFC MG density (Radewicz et al. 2000; Fillman et al. 2013) and no change in PFC MG density (Steiner et al. 2006, 2008; Hercher et al. 2014) have been reported. However, these studies used different markers to define and visualize MG, different methods to count MG, and there were differences across patient characteristics, such as medication status and agonal state. Moreover, the samples were obtained in most cases from subjects who died long after adolescence.

Positron emission tomography studies using radioligands for the translocator protein (TSPO), which was advanced as a selective radioligand for activated MG (Banati 2002), have also led to conflicting data. Some studies report no alterations in TSPO binding potential in the brain in populations at various stages in the illness (Takano et al. 2010; Coughlin et al. 2016; van der Doef et al. 2016; Di Biase et al. 2017; Hafizi et al. 2017), with other groups reporting increases in TSPO binding potential

(van Berckel et al. 2008; Doorduyn et al. 2009; Bloomfield et al. 2016). Unfortunately, TSPO is not a selective marker for MG, but also binds to astrocytes and endothelial cells (Cosenza-Nashat et al. 2009; Lavis et al. 2012; Notter et al. 2018), and perhaps neurons as well (Varga et al. 2009). Moreover, TSPO protein expression increases in rodent-derived MG, but not in human primary MG, in response to a proinflammatory challenge (Owen et al. 2017). Together these considerations render interpretation of TSPO imaging data suspect (O'Donnell 2017).

We focused our attention on determining the role of MG in synaptic pruning during development. However, the synaptic stripping that accompanies postnatal maturation of the cortex may not be solely attributable to microglia. Although it has been known for over a century that there is not an overt astrogliosis in schizophrenia (Falkai et al. 1999; Garey 2010), it is possible that astrocytic function is altered in psychosis. Astrocytes may contribute to synapse elimination both directly (via recognition of an “eat-me” signal and subsequent phagocytosis of synaptic elements) and indirectly (by inducing the deposition of complement proteins at synapses, which are then recognized and eliminated by MG) (Chung et al. 2015). In addition, both autophagy (Tang et al. 2014) and spine retraction (Woods et al. 2011) may contribute to synapse elimination. Future studies will be required to determine if astrocytes contribute to PFC PC spine pruning across development, and to assess autophagic processes during these periods.

In summary, our data suggest that MG may be an effector of the excess synaptic pruning during adolescence in schizophrenia postulated by Feinberg (1982). Similar MG involvement at other postnatal ages could contribute to the “spinopathies” of autism spectrum disorder and even Parkinson's disease (Penzes et al. 2011). Understanding the processes subserving synaptic remodeling, including dendritic spine loss, may lead to the development of new MG-based pharmacotherapies to mitigate structural and functional cortical changes in schizophrenia.

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Notes

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