

## REVIEW

# Inflammation and the neural diathesis-stress hypothesis of schizophrenia: a reconceptualization

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An interaction between external stressors and intrinsic vulnerability is one of the longest standing pathoetiological explanations for schizophrenia. However, novel lines of evidence from genetics, preclinical studies, epidemiology and imaging have shed new light on the mechanisms that may underlie this, implicating microglia as a key potential mediator. Microglia are the primary immune cells of the central nervous system. They have a central role in the inflammatory response, and are also involved in synaptic pruning and neuronal remodeling. In addition to immune and traumatic stimuli, microglial activation occurs in response to psychosocial stress. Activation of microglia perinatally may make them vulnerable to subsequent overactivation by stressors experienced in later life. Recent advances in genetics have shown that variations in the complement system are associated with schizophrenia, and this system has been shown to regulate microglial synaptic pruning. This suggests a mechanism via which genetic and environmental influences may act synergistically and lead to pathological microglial activation. Microglial overactivation may lead to excessive synaptic pruning and loss of cortical gray matter. Microglial mediated damage to stress-sensitive regions such as the prefrontal cortex and hippocampus may lead directly to cognitive and negative symptoms, and account for a number of the structural brain changes associated with the disorder. Loss of cortical control may also lead to disinhibition of subcortical dopamine—thereby leading to positive psychotic symptoms. We review the preclinical and *in vivo* evidence for this model and consider the implications this has for treatment, and future directions.

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## INTRODUCTION

A relationship between the social environment and mental illness has been recognized throughout the history of medicine, from Hippocrates through to the nineteenth century writings of Philippe Pinel and more recent literature.<sup>1,2</sup> The specific idea that a preexisting vulnerability and external stressors may interact in the pathogenesis of schizophrenia—the ‘diathesis-stress hypothesis’—was suggested over half a century ago.<sup>3</sup> Subsequent refinements have attempted to define how this interaction might occur at a neurobiological level.

Walker and Diforio<sup>4</sup> posited the hippocampus and hypothalamic-pituitary-adrenal (HPA) axis as the mediating pathway between environmental stressors, underlying vulnerability and development of the disorder. Specifically, pre- or perinatal neurodevelopmental insults were suggested to cause aberrant hippocampal function, while psychosocial stress exposure was posited to activate the HPA axis. Furthermore, dysregulation of the hippocampus and HPA axis were hypothesized to act synergistically, and activation of the HPA axis was asserted to stimulate the subcortical dopamine system, leading to the development of psychotic symptoms.

Van Winkel *et al.*<sup>5</sup> extended this model by examining genetic factors underlying the proposed diathesis. Their review highlighted epidemiological studies that have shown a synergism between urbanicity and familial liability for psychosis,<sup>6</sup> and between genetic risk and dysfunctional upbringing.<sup>7</sup>

In this paper, we review findings from the latest neuroimaging, genetic and preclinical work to provide an update of what has proven to be one of the longest standing pathoetiological models for schizophrenia. In particular, we highlight how the immune system, and especially microglial cells, may have a central role.

## THE IMMUNE SYSTEM AND GLIA

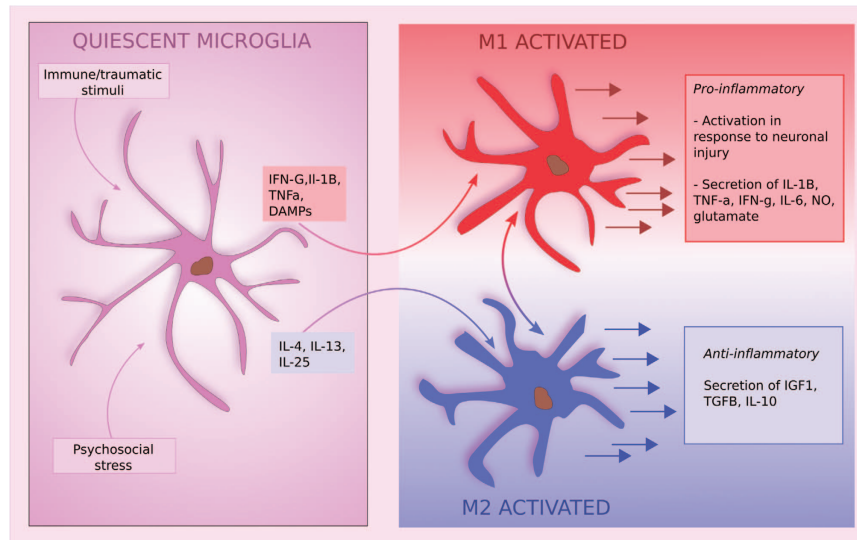
The three primary categories of glial cell are astrocytes, oligodendrocytes and microglia. Astrocytes ensure that the local cellular environment is appropriate for neuronal signaling, whereas oligodendrocytes are involved in the myelination of axons. Although the focus of the current review is on microglia, all three types of glial cell have been suggested as potentially having a pathoetiological role in schizophrenia.<sup>8</sup>

The role of the immune system in the pathoetiology of mental illness has become increasingly recognized.<sup>9,10</sup> As well as the possibility of intrinsic immune abnormalities contributing to illness, the system is also a key pathway via which environmental factors influence central nervous system functioning. Microglia are the primary immune cells of the central nervous system. Quiescent microglial cells have multiple, motile, branch-like protrusions, that continually scan their local environment.<sup>11</sup> Activation of microglia by environmental triggers leads to retraction of these protrusions, and enlargement of the cell body.

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**Figure 1.** Activation of microglia and their subsequent effects. IL, interleukin; TNF, tumor necrosis factor.

Animal models show this occurs in response to immunological and traumatic stimuli, and also in response to psychosocial stress.<sup>12,13</sup> When activated they may release pro-inflammatory cytokines, or conversely have a role in suppressing inflammation.<sup>14</sup>

#### The effects of activated microglia

Activated microglia exist on a continuum between two states, characterized as M1 and M2 activation, each with different molecular triggers.<sup>15</sup> For example, M1 activation is triggered by cytokines such as IFN-G, interleukin (IL)1B, tumor necrosis factor (TNF)-α and damage-associated molecular patterns, whereas M2 activation is induced by cytokines such as IL-4, IL-13 and IL-25 (Figure 1).

The M1 pathway is activated following neuronal injury and leads to the release of a range of pro-inflammatory compounds including NO, IL-1B, TNF-α, IL-6 and glutamate. In a healthy system this is followed by a shift to the M2 state. This is broadly an anti-inflammatory pathway leading to release of IL-10, IGF-1, TGF-β, and various neurotrophic factors (Figure 1). The M2 pathway is involved in debris clearance, extracellular matrix deposition and angiogenesis.<sup>14</sup> Both pathways are required for an appropriate immune response, and the balance between the two is tightly regulated in the healthy system.<sup>14</sup>

Dominance of the M1 pathway, with a prolonged inflammatory response, leads to over-expression of pro-inflammatory cytokines and reactive oxygen species, and thereby to synaptic loss and neuronal death.<sup>16</sup> The possibility that the microglial response might be a cause, rather than solely a consequence of neuronal injury, was first suggested in Alzheimer's disease.<sup>17</sup> Here a self-perpetuating mechanism was discovered whereby neuronal degeneration activates microglia, which then release neurotoxic molecules that cause further neuronal damage.<sup>18</sup> Recently, prenatal immune activation was shown to be associated with a shift towards the M1 pathway in adolescence, and subsequent adult sensory gating deficits.<sup>19</sup>

It is important to note that the M1/M2 dichotomy is likely an over simplification of the various microglial states. Recent research has demonstrated the existence of dark microglia, a phenotype that is rarely seen under normal conditions, but is upregulated under chronic stress and may have a significant role in pathological pruning.<sup>20</sup>

#### The role of microglia in cortical development and pruning

It has recently become clear that the role of microglia extends well beyond the inflammatory response. They promote survival of cortical neurons early in development via IGF-1 secretion,<sup>21</sup> although conversely also demonstrate the ability to phagocytose neural precursor cells.<sup>22</sup> As a result they are vital in regulating the pace and extent of neurogenesis in the developing brain.<sup>23</sup> Moreover, they also have a role in synaptic pruning. This was first observed over 50 years ago,<sup>24</sup> but recently it has become apparent that this is more extensive than originally thought. Microglial cells undertake constant synaptic monitoring; and rodent studies have demonstrated that pathological, and physiological pruning occurs throughout neurodevelopment and adult life.<sup>25,26</sup> There appears to be a fine balance between excessive and insufficient activity in this regard. Pathological reductions in microglial activity during neurodevelopment lead to reduced synaptic pruning, and sustained deficits in synaptic connectivity.<sup>26,27</sup> Conversely, microglial over-activity later in life has been linked to excessive synaptic loss and cognitive decline, and inhibition of microglial activity in this instance reduces the extent of pathological synaptic loss.<sup>28</sup>

#### The effects of stress on microglia

Microglia are affected by a variety of stressors. In particular, ionized calcium binding adaptor molecule 1 (IBA-1) expression, a specific marker of microglial density, is increased in response to a number of stressors, including footshock, restraint, social defeat, maternal separation and social isolation.<sup>29,30</sup> This effect is seen in regions implicated in schizophrenia, including the amygdala, hippocampus, nucleus accumbens and prefrontal cortex. Interestingly, it appears that social defeat has the most marked impact upon IBA-1 expression.<sup>29</sup>

The role of glucocorticoids in the stress response is well established. Glucocorticoids (GCs) affect almost every immune cell type, due to the ubiquitous expression of the glucocorticoid receptor (GR). Within the central nervous system, microglia are a primary target for GCs due to their high level of GR expression.<sup>31</sup> Research involving genetic manipulation of GR expression,<sup>32</sup> and the administration of both GCs<sup>33</sup> and GR antagonists,<sup>34</sup> has demonstrated that GR signaling has a vital role in limiting the duration and amplitude of the microglial response. Paradoxically, animal models of acute and chronic stress (prior to an immune

insult), have described a pro-inflammatory action of GCs.<sup>35,36</sup> Administration of a GR antagonist, or adrenalectomy has been shown to prevent the pro-inflammatory priming effects of stress on microglia.<sup>35,36</sup> GR activation of microglia also seems to be necessary for the expression of pro-inflammatory genes including IL-1B.<sup>37</sup>

A key factor in determining whether GCs have a pro- or anti-inflammatory effect is the timing relative to the inflammatory challenge (typically lipopolysaccharide (LPS) in experimental challenges). Administration of GCs prior to LPS has pro-inflammatory effects, whereas GC administration subsequent to LPS has anti-inflammatory effects.<sup>38–40</sup> Interestingly, stress exposure subsequent to LPS administration appears to have anti-inflammatory effects as well.<sup>38</sup>

GC stimulation of neurons has been shown to increase glutamate release, one of the mechanisms potentially underlying stress induced cortical atrophy.<sup>41</sup> This stress induced glutamate release has also been shown to result in microglial proliferation via activation of N-methyl-D-aspartate receptors (NMDAR).<sup>42</sup> In a similar manner to the vicious circle described above in the case of Alzheimer's disease, microglial activation leads to neuronal damage, which causes further glutamate release and ongoing microglial activation.<sup>43</sup>

#### Microglia and the perinatal period

Prenatal infection,<sup>44</sup> neonatal infection,<sup>45</sup> maternal stress<sup>46</sup> and perinatal brain injury<sup>47</sup> activate microglial and increase microglial densities in animal models (Box 1). As discussed below, microglia can have both a protective role (for example, their depletion has been shown to worsen post-hypoxia outcomes<sup>48</sup>), or contribute to pathology. Rats that experience neonatal infection show a blunted corticosterone response to stress in adulthood,<sup>49</sup> which parallels the findings in individuals exposed to childhood trauma,<sup>50</sup> and those with schizophrenia.<sup>51,52</sup>

#### Priming of microglia

'Priming' refers to an exaggerated response to repeated presentations of a stimulus, compared with the initial response to the stimulus. This phenomenon has been observed repeatedly in microglia. Pre and postnatal stress, maternal immune activation, and neonatal infection lead to increased microglial activation and density.<sup>46,53,54</sup> These changes later normalize.<sup>55</sup> However, when subsequently exposed to an inflammatory stimulus in adult life, rats previously exposed to a perinatal hazard show an exaggerated microglial response.

Cross-sensitization of the microglial response has been shown between various stimuli.<sup>46,56</sup> Giovanoli *et al.*<sup>57</sup> demonstrated a synergism in microglial response between perinatal insults, and

adolescent stress. Maternal infection with a viral mimic was followed by five sequential peripubertal stressors. The group exposed to prenatal infection showed a threefold increase in markers of activated microglia in hippocampal and prefrontal areas in response to the peripubertal stress. This was secondary to reduced CD200 expression in the animals that had previously received a prenatal immune challenge (CD200 has a role in attenuating the inflammatory response, and is also downregulated following stress exposure<sup>58</sup>). The microglial response was not significantly different between any group when the stress exposure occurred in adulthood rather than the peripubertal period, suggesting there may be a critical developmental period outside of which the priming response does not occur.

#### Critical developmental periods

Neuronal remodeling leading to an overall decrease in synaptic spine density is mediated by various mechanisms, including microglial pruning.<sup>26</sup> In rodent studies the neonatal period is a period of peak microglia mediated pruning,<sup>27</sup> although microglia have a role in this throughout the lifecourse.<sup>59</sup> Humans may be unique, even among primates, in having a relatively late period of extensive synaptic remodeling during adolescence, that continues into adulthood.<sup>60–63</sup> Although rodent studies show microglia have a key role in synaptic pruning, this remains to be established in humans.

The neurodevelopmental time point at which exposure to a hazard occurs may significantly moderate the effect of that exposure. Bilbo *et al.*<sup>54</sup> showed that neonatal infection at postnatal day (PND) 4 led to increased sensitivity to LPS exposure, but that this did not arise if infection occurred on PND 40. Other work examining later developments of seizures has also highlighted the early postnatal period as a time of particular vulnerability.<sup>64</sup> It has also been demonstrated that changes in microglial density following in utero immune activation become evident in a window corresponding to adolescence, but may not be apparent at earlier or later timepoints.<sup>65</sup> Moreover the Giovanoli *et al.* study discussed above found that a primed response following perinatal immune activation only occurred if the stressor was delivered during adolescence.<sup>57</sup> This indicates that in addition to being a period of extensive neuronal remodeling, adolescence represents a critical period for microglia that are already primed by prior activation to show an increased response to stress.<sup>66</sup>

### SCHIZOPHRENIA AND THE ENVIRONMENT

Chronic and acute stress as a risk factor for schizophrenia  
Epidemiological research has demonstrated associations between a wide range of psychosocial factors and schizophrenia. A history

**Box 1** Relationships between microglial cells, schizophrenia, perinatal hazards and stress

	<i>Microglia</i>	<i>Schizophrenia</i>
Perinatal factors	<ul style="list-style-type: none"> <li>• Prenatal infection, neonatal infection, maternal stress, and perinatal brain injury ↑microglial activation/density in animals.</li> <li>• Perinatal hazards 'prime' microglia leading to ↑response to subsequent exposures.</li> </ul>	<ul style="list-style-type: none"> <li>• Prenatal infection, maternal inflammation, maternal prenatal stress, obstetric complications, and childhood infections have been associated with ↑risk of schizophrenia.</li> </ul>
Stress	<ul style="list-style-type: none"> <li>• Microglia activation in rats ↑by wide range of stressors. Adolescent stress exposure leads to ↑microglial activation in rats that have experienced prenatal immune activation.</li> </ul>	<ul style="list-style-type: none"> <li>• Schizophrenia is associated with migration, childhood trauma and urbanicity.</li> <li>• Individuals with schizophrenia display ↑stress sensitivity to acute stress.</li> <li>• Adolescent stress exposure leads to ↑rates of schizophrenia in individuals exposed to prenatal infection.</li> </ul>

of 1st or 2nd generation migration, childhood trauma, and urbanicity have all been associated with schizophrenia with odds ratios of 2–4.<sup>67</sup> For some environmental factors, such as obstetric complications, which may cause neuronal and white matter loss,<sup>68</sup> a relatively direct neurobiological link between exposure and illness may exist. For others, however, such as urbanicity, migration and childhood adversity, the specific component of the exposure is harder to isolate. Nevertheless, studies have shown that social stressors significantly mediate the risk of psychosis associated with migration and urbanicity.<sup>69,70</sup> What these latter factors therefore have in common is that all involve exposure to chronic psychosocial stress.<sup>71</sup>

The role of acute stress in psychosis onset is well recognised clinically, and its role as a potential etiological factor in *acute and transient psychotic disorder* is described in the ICD-10 definition of the syndrome.<sup>72</sup> However, an increase in the number of stressful events prior to psychosis onset has not been consistently demonstrated.<sup>73</sup> This does not, however, rule out a role for acute stress, as the diathesis-stress model proposes that there is increased vulnerability to stress. Thus, there may be no difference in the acute stress exposure, but in vulnerable individuals this may trigger illness.<sup>74</sup> This is supported by work demonstrating an increased incidence of psychosis associated with bombing campaigns during the 1999 Kosovo war,<sup>75</sup> and more recently in refugees compared with non-refugee migrants.<sup>76</sup> These studies have the advantage of investigating a relatively objective exposure, which addresses to some degree the possibility of reverse causality. In addition, in those with an established disorder, longitudinal studies have shown that there is an increase in the frequency of stressful life events prior to psychotic relapses.<sup>77</sup>

#### Perinatal factors and infection

Prenatal infection,<sup>78</sup> maternal inflammation during pregnancy,<sup>79</sup> obstetric complications<sup>80</sup> and childhood infections<sup>81</sup> have all been associated with an increased risk of schizophrenia (Box 1). More recently a weak association between maternal stress during pregnancy and schizophrenia has been demonstrated,<sup>82</sup> and it appears males may be particularly vulnerable.<sup>83</sup> Recent epidemiological research has demonstrated a synergistic effect between prenatal infection and adolescent stress, in increasing schizophrenia risk, with the effect also predominantly in males.<sup>84</sup> The parallels with the microglial findings discussed above are clearly apparent, and also of relevance is the influence of gender on microglial function—with male rats being particularly vulnerable to early-life infection-mediated microglial priming.<sup>85</sup>

Retrospective studies suggest that there is also an increased incidence of infection in adolescence and adulthood in individuals with schizophrenia.<sup>86</sup> A prospective study in a military population demonstrated an association between antibodies to *Toxoplasma Gondii* evident in blood samples and later schizophrenia.<sup>87</sup> Notwithstanding this, a paucity of longitudinal studies investigating infection prior to onset of schizophrenia, makes inferring the direction of causality a challenge.

### SCHIZOPHRENIA AND MICROGLIA

#### Post mortem and *in vivo* imaging studies of microglia

Post-mortem studies in schizophrenia have used a variety of techniques to identify and characterize microglia.<sup>88</sup> IBA-1, the marker elevated by stress exposure in the animal studies discussed above, has been used in two post mortem studies,<sup>89,90</sup> and these showed no difference in density of IBA-1 stained cells, although qualitative assessment of morphology found multiple activated microglia in schizophrenia samples that were not seen in control samples.<sup>89</sup> Studies using other markers have shown increased microglia density, activation, and degeneration compared to controls<sup>89,91–96</sup> (with some exceptions;<sup>97,98</sup>

Supplementary Table 1). There is also evidence from two studies that microglial alterations are linked to the phenotype, with elevations seen in patients with paranoid symptoms but not in patients solely experiencing residual symptoms, suggesting microglial activation may be linked to active phases of the disorder.<sup>99,100</sup>

*In vivo* imaging of microglia has used radioligands that bind to the translocator protein (TSPO), which is expressed on microglia and upregulated when they are activated. TSPO is, however, also expressed by cells other than microglia, such as endothelial cells and astrocytes,<sup>101–103</sup> limiting both its sensitivity and specificity as a marker of microglial activation.

Table 1 summarizes the studies using this approach to index microglia in schizophrenia. The earliest two studies showed increased binding potentials in whole brain gray matter,<sup>104</sup> and hippocampus,<sup>105</sup> in individuals with schizophrenia. Later studies, however, have not consistently demonstrated an increase in binding.<sup>106–110</sup> Meta-analysis has shown that there is a moderate effect size elevation in schizophrenia when binding potential is used as the outcome, but no effect when volume of distribution is used (Reis-Marques *et al.*, in submission). Methodological differences may account for this inconsistency between outcome measures.<sup>111</sup> There is also preclinical evidence that antipsychotics may dampen microglia activity, raising the possibility that this could mask group differences in the studies of treated patients.<sup>112,113</sup> However, one preclinical study has found evidence antipsychotics increase microglial activity.<sup>114</sup> An issue for the preclinical studies is that the dosing of antipsychotics does not reflect that used in patients, which limits translation. Further work using doses and modes of administration that reflect those used in patients is thus needed to determine the potential influence of antipsychotics for microglial activity in patients. Nevertheless, the only study to date in individuals at ultra-high risk for psychosis, who were all antipsychotic naïve, found increased relative binding in total gray matter, and in frontal and temporal regions.<sup>115</sup>

A number of studies have found associations between the magnitude of ligand binding and symptom severity. In ultra-high risk individuals, relative binding was directly correlated with symptom severity, and highest in the subject who subsequently developed a psychotic illness.<sup>115</sup> Takano *et al.*<sup>106</sup> found greater cortical binding potential was directly correlated with higher symptoms scores in schizophrenia, and Holmes *et al.* found that in a frontal cortical region it directly correlated with the PANSS-negative subscale, whereas, potentially paradoxically, Hafzi *et al.*<sup>110</sup> found greater hippocampal binding correlated with better cognitive function. Although these findings suggest a link to symptoms, caution is warranted as not all correlations were corrected for multiple comparisons so there is a risk of false positives.

#### Peripheral markers of inflammation

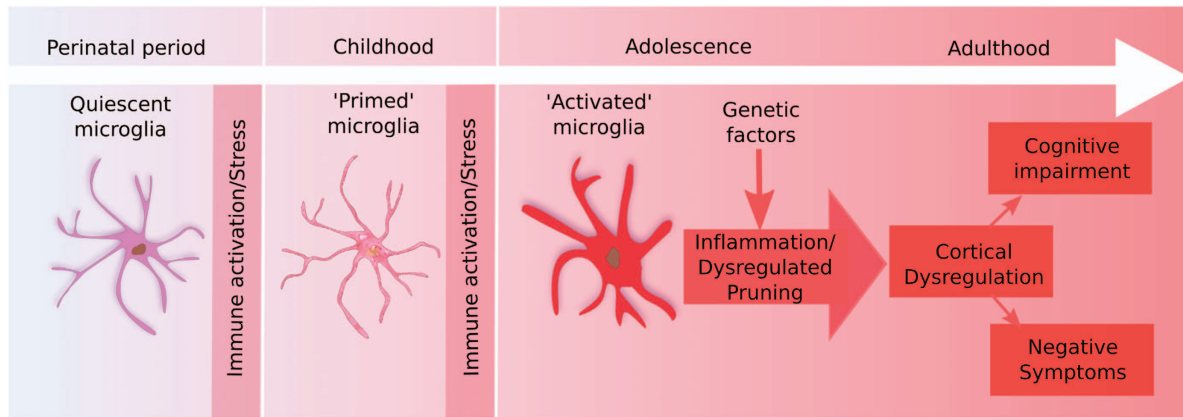
As described above microglial activation can have pro- or anti-inflammatory effects. Determining which pathway predominates in psychotic disorders *in vivo* is currently not possible as the available radioligands do not distinguish between M1 and M2 states.<sup>117</sup> Nevertheless, evidence that there may be an imbalance in favor of the M1 pathway comes from studies examining peripheral cytokine levels.<sup>118</sup> This suggests that medication-naïve first-episode psychosis patients have increased expression of the M1 associated pro-inflammatory cytokines: IL-1B, IL-6 and TNF $\alpha$ .<sup>119,120</sup> Moreover, one of the triggers of M1 activation, S100B, is present at higher levels in individuals with schizophrenia.<sup>121</sup> A parallel is seen here with childhood trauma in which raised levels of pro-inflammatory IL-6 and TNF $\alpha$ ,<sup>122</sup> and reductions in brain-derived neurotrophic factor expression (a product of the M2 pathway) have been observed.<sup>118,120</sup>

There is also evidence that alterations in inflammatory markers may exist well before the onset of psychosis, and may predict

**Table 1.** Imaging studies of translocator protein density in individuals with psychotic disorders

Study	Population	Patient age, mean (s.d.)	Medication	Methods	Findings
Van Berckel <i>et al.</i> (2008) <sup>104</sup>	10 Scz within 5 years of disease onset. 10 HC	24 (2)	All patients antipsychotic treated	Ligand: (R)-[11C] PK11195 TSPO genotype: not measured	Significantly greater whole brain gray matter BP in patients ( $d=0.87$ )
Doorduyn <i>et al.</i> (2009) <sup>105</sup>	7 Scz. (Mean PANSS 74) 8 HC	31 (7)	All patients antipsychotic treated	Ligand: (R)-[11C] PK11195 TSPO genotype: not measured	Hippocampal BP significantly greater in patients ( $d=1.92$ ) Whole brain gray matter non-significantly greater ( $d=0.84$ )
Takano <i>et al.</i> (2010) <sup>106</sup>	14 Scz (Mean PANSS 78.6) 14 HC	43.9 (7.4)	All patients antipsychotic treated	Ligand: [11C] DAA1106 TSPO genotype: not measured	No significant differences in BP <sub>ND</sub> between groups. BP <sub>ND</sub> directly correlated with symptoms score.
Bloomfield <i>et al.</i> (2015) <sup>115</sup>	14 UHR (Mean CAARMS 49.5) 14 HC	24	No antipsychotic exposure	Ligand: [11C] PBR28 TSPO genotype: controlled for	Vtr elevated for UHR for total GM ( $d=1.2$ ), frontal lobe ( $d=0.89$ ) and temporal lobe ( $d=0.83$ ). No difference between groups in Vt.
Kenk <i>et al.</i> (2015) <sup>107</sup>	14 Scz (Mean PANSS 63.7) 14 HC	47	Antipsychotic treated	Ligand: [18F]-FEPPA TSPO genotype: controlled for	Vtr elevated for Scz for total GM ( $d=1.77$ ), frontal lobe ( $d=1.25$ ) and temporal lobe ( $d=1.43$ ). No difference between groups in terms of Vt.
Coughlin <i>et al.</i> (2016) <sup>108</sup>	16 Scz (Mean PANSS 70.2) 27 HC	43 (14.0)	All patients antipsychotic treated	Ligand: [11C] DPA-713 TSPO genotype: controlled for	No significant differences in whole brain or ROIS white or gray matter Vt.
van der Doef <i>et al.</i> (2016) <sup>109</sup>	12 Scz (Mean SAPS 3.8) 14 HC	24.1 (3.1)	All patients antipsychotic treated.	Ligand: (R)-[11C] PK11195 TSPO genotype: not measured	No significant differences in whole brain or ROIS white or gray matter Vt.
Hafizi <i>et al.</i> (2016) <sup>110</sup>	19 Psychotic disorder (Mean PANSS 53) 17 HC	26 (4)	15/19 antipsychotic treated	Ligand: [18F]-FEPPA TSPO genotype: not measured	No significant differences in whole brain or ROIS BP <sub>ND</sub>
Holmes <i>et al.</i> (2016) <sup>116</sup>	19 FEP (Mean PANSS 68.6) 20 HC	27.5 (6.7)	All < 4 weeks lifetime antipsychotic exposure and 14 antipsychotic naïve	Ligand: [11C] DPA-713 TSPO genotype: controlled for	No significant differences in whole brain or ROIS Vt.
	16 Scz 16 HC	32.5	8 antipsychotic free 8 antipsychotic treated	Ligand: (R)-[11C] PK11195 TSPO genotype: not measured	Cortical BP <sub>ND</sub> significantly higher in medicated patients than in controls. No difference between unmedicated patients and controls.

Abbreviations: BP, binding potential; CAARMS, Comprehensive Assessment of the At-Risk Mental States; FEP, first-episode psychosis; HC, healthy control; PANSS, Positive and Negative Syndrome Scale; SAPS, Scale for the Assessment of Positive Symptoms; UHR, ultra-high risk; Vt, volume of distribution; Vtr, ratio of Vt in the region of interest to the Vt of whole brain.



**Figure 2.** The ‘two hit’ model. Perinatal activation of microglia leads to a primed state. Subsequent stress in adolescence triggers pathological overactivation, leading to cortical loss and the development of symptoms.

progression to psychosis.<sup>123</sup> Post-mortem and neuroimaging studies in individuals with schizophrenia provide support for a link between immune activation and damage to both gray and white matter.<sup>94,118,124–126</sup> In individuals with schizophrenia, an increase in peripheral cytokines associated with the M1 pathway has been shown to correlate with reductions in both hippocampal,<sup>118</sup> and prefrontal cortex volumes.<sup>124,126</sup> A link between cytokine levels and TSPO binding, however, has not been demonstrated,<sup>108</sup> which could be because cytokine levels fluctuate.

#### Genetic findings

The largest genome-wide genetic association study (GWAS) to date identified multiple loci linked to the immune system among the strongest associations in the over 100 loci associated with schizophrenia.<sup>127</sup> Although the potential impact of many of these has yet to be determined, one locus identified implicated the complement component 4 (C4).<sup>128</sup> Alleles of this gene were subsequently shown by Sekar *et al.*<sup>128</sup> to associate with schizophrenia in proportion to the amount of C4A that they generate, and greater expression of C4 in brains of individuals with schizophrenia was related to genotype. C4 activates complement component 3 (C3), allowing it to attach to a synapse. This marks the synapse for phagocytosis,<sup>129</sup> and the complement receptor 3 (CR3) drives synaptic pruning by microglia.<sup>27</sup> Sekar *et al.* went on to find that mice with the C4 alleles associated with greater C4 production showed elevated synaptic pruning during neurodevelopment.<sup>128</sup> They also demonstrated that the C4 allele linked to schizophrenia determined the extent of C3 immunostaining thereby identifying an important genetic influence on the extent of microglial synaptic pruning.<sup>128</sup> These results are an exciting development, and the first to provide a clear mechanistic pathway linked to the GWAS findings. However, for their full significance to be accepted, replication will be required.

#### THE POTENTIAL ROLE OF MICROGLIA IN AN INTEGRATED MODEL OF THE DEVELOPMENT OF SCHIZOPHRENIA

Meta-analyses provide robust support for both dopamine dysfunction and reduced cortical gray matter in schizophrenia, including in medication-naïve patients.<sup>130,131</sup> Although dysregulation of the dopaminergic system is thought to be central to the development of psychotic symptoms in schizophrenia,<sup>67</sup> it is unclear what accounts for the loss of cortical synapses and cortical volume seen in schizophrenia. The lines of evidence we have reviewed suggest that microglia could explain this. First, in addition to affecting the development of dopaminergic neurons; perinatal insults, and early-life stress prime microglia to act in a

hyper-responsive manner to later stress and encourage a shift to a pro-inflammatory M1 phenotype. Second, microglia have a significant role in pruning cortical synapses. Third, genetic variants in the complement pathway linked to schizophrenia have been shown to moderate microglial pruning.<sup>128</sup> Thus in people with these genetic risk factors—subsequent stress, or immune activation could act on primed microglia, leading to overactivation and aberrant synaptic pruning.

Microglial overactivation secondary to these ‘two hits’ may then lead to spine loss via excessive pruning of stress-sensitive areas such as the prefrontal cortex and hippocampus (Figure 2). The loss of synapses due to this could account for the structural brain changes associated with schizophrenia and the development of negative and cognitive symptoms.<sup>132</sup> This is supported by findings that lower gray matter volume is correlated with greater cognitive symptoms<sup>133,134</sup> and at least partially secondary to a reduction in the density of synapses.<sup>135</sup>

Furthermore, disrupted cortical development could exacerbate the disinhibition of subcortical dopamine neurons, which is thought to underlie the development of positive symptoms.<sup>136,137</sup> This would also have the effect of sensitizing the dopaminergic response to acute stress, creating a system unable to respond appropriately to acute stress, leading to further dysregulation. Interactions with genotype are also likely to occur at this point, for example, a polymorphism within the dopamine receptor 2 gene was also implicated in schizophrenia GWAS, and has been shown to moderate the dopaminergic response to stress.<sup>138</sup>

Although the model we present is wide ranging, we do not intend to suggest that microglia are the sole architects of the neurobiological abnormalities associated with schizophrenia and it is important to note the variability seen in the disorder. For example, although cognitive impairments and lower cortical gray matter volumes are consistent findings in schizophrenia, a proportion of patients show evidence of neither. Thus, it is likely that the schizophrenia syndrome encompasses several pathoae-tiological pathways, which may co-occur in some but not all individuals. For example, stress<sup>139</sup> and perinatal hazards<sup>140</sup> may both directly act on the dopamine system to disinhibit it without involving microglia. This could lead to psychosis without marked cognitive impairments or gray matter reductions, although the involvement of the inflammatory system as well could account for the cortical volume loss, and negative and cognitive symptoms seen in other patients.

#### IMPLICATIONS FOR TREATMENT

Currently licensed treatments for schizophrenia all operate by blocking dopamine neurotransmission, and, while effective in

**Box 2** Directions for future research

Preclinical research

- Determine effects of antipsychotic on microglia using doses and modes of administration that appropriately reflect human use.
- Conduct studies using translational approaches (such as MR and PET imaging and peripheral blood measures) that can inform interpretation of human studies

General

- Develop and validate approaches to the analysis of current PET tracers for TSPO that address methodological issues
- Develop novel radioligands with greater specificity for microglia and that will distinguish between M1 and M2 states.

Clinical Research

- Multimodal longitudinal studies to determine the relationship between microglia and other brain changes as well as the effect of moderating factors (such as sex hormone levels)
- Comparative studies across disorders to determine common and specific mechanisms
- *In vivo* testing of how risk factors for psychosis (such as stress, cannabis use, migration) impact microglial function
- Determine the effects of antipsychotic treatment upon microglial activity in longitudinal studies
- Experimental medicine studies of the effects of modulating microglial function in schizophrenia on gray matter changes and symptoms

controlling positive symptoms for some patients, they have little impact on cognitive or negative symptoms. There is a pressing need for novel treatment mechanisms, and in this regard microglia and the inflammatory response presents an attractive target, with the potential for modification of disease course, as opposed to solely symptomatic improvement.

A wide range of pharmacological agents have the ability to modify microglial function, including many existing psychotropics,<sup>141–143</sup> and treatments originally developed for non-psychiatric indications such as statins, non-steroidal anti-inflammatories, N-acetyl cysteine, minocycline, and natalizumab.<sup>144–146</sup> It is also possible that psychological interventions addressing stress reactivity could conceivably indirectly affect microglial function. There are a number of challenges, however, in developing interventions to modify microglial function. First, microglia have a vital physiological role, and attempts to inhibit their activity may potentially have deleterious effects.<sup>26,147</sup> Second, it appears that pathological overactivation of microglia occurs early in the course of the illness.<sup>19,57,65</sup> Thus treatment may need to be given early, potentially during a prodromal period, to be effective and to prevent the secondary loss of synapses.

**LIMITATIONS AND UNANSWERED QUESTIONS**

A general issue for the field is that it is not clear how specific the findings discussed above are to psychotic disorders. The HPA axis, psychosocial stress, dopaminergic dysfunction, and microglial activation have been implicated in a wide range of mental illnesses. An interaction with genetic risk factors could explain different trajectories, and is supported by the findings linking genes in the complement pathway and the dopamine D2 receptor to schizophrenia. However, the interaction between these genetic

and developmental risk factors, and alterations in microglial function has yet to be tested.

Moreover, although there is an extensive animal literature showing the impact of stress on microglia, research methods to investigate whether this corresponds to findings in humans are only starting to be developed, and remain limited by the specificity and resolution of the techniques available. In addition, strong evidence linking stress and microglial activity to negative and cognitive symptoms is lacking. Although we hypothesize that excessive pruning of cortical gray matter could lead to these symptoms, this requires testing. Although the finding that elevation in pro-inflammatory cytokines is associated with gray matter loss provides some support for a link,<sup>124</sup> a longitudinal multimodal approach will be required to determine whether microglia are causally implicated in the gray matter changes observed in schizophrenia.

In the current review, we have focused upon stress and infection as risk factors for schizophrenia. A number of other factors, however, have relevance both in terms of their relationship with microglia functioning, and the pathoetiology of schizophrenia. Estradiol is thought to contribute to the gender differences in schizophrenia incidence, and is known to have anti-inflammatory effects.<sup>148,149</sup> Cannabis, meanwhile, has become increasingly accepted as having a causal role in increasing schizophrenia risk, and has been shown to activate microglia.<sup>150,151</sup> In addition, a wide range of non-dopamine neurotransmitter systems may be involved in the development of psychosis.<sup>152,153</sup> Box 2 highlights future directions to address the issues discussed above.

Current human imaging and post-mortem studies of microglia show inconsistency. Several reasons may underlie this. First, schizophrenia is a heterogeneous concept that likely encompasses various aetiologies, this is highlighted by the recent finding that peripheral markers of inflammation show marked differences between responders and non-responders to antipsychotic treatment.<sup>154</sup> In addition to this inter-individual variability, intra-individual temporal variability is suggested by the findings of Giovanoli *et al.* that microglial changes may only be present at specific time points (for example, during adolescence).<sup>57</sup>

**CONCLUSIONS**

The importance of environmental stressors in the development of schizophrenia has been recognized for longer than our current classifications of mental illness. Over recent years, studies have shown the impact of these risk factors on the immune system. In the present review we draw on these lines of evidence to suggest how microglial cells in particular may have a role in the pathoetiology of schizophrenia.

Evidence shows that microglial cells may become primed early in life, making them vulnerable to subsequent chronic over-activation following further stimulation. This may then cause gray matter loss in regions such as the prefrontal cortex and hippocampus, leading to negative and cognitive symptoms, and potentially contributing to the dopaminergic dysregulation of subcortical structures. The wealth of evidence supporting the link between perinatal and later life risk factors and schizophrenia, the elevation in pro-inflammatory cytokines including those associated with M1-activated microglia in schizophrenia, and the elevation in microglia seen with stress mean we can be fairly confident about these aspects of the model. Nevertheless, it is important to recognize that the role of microglia in the disorder, and their link to other elements of its pathology, requires further testing.

Although we have concentrated on psychotic disorders, it is clear that many of the mechanisms described above do not segregate according to traditional diagnostic boundaries. The mechanisms we describe present a wealth of targets for potential

therapeutic intervention, for many mental illnesses. However, their complexity and wide ranging effects means producing targeted interventions will be a significant challenge.

### CONFLICT OF INTEREST

ODH has received investigator-initiated research funding from and/or participated in advisory/speaker meetings organized by Astra-Zeneca, Autifony, BMS, Eli Lilly, Heptares, Janssen, Lundbeck, Lyden-Delta, Otsuka, Servier, Sunovion, Rand and Roche. Neither ODH nor his family have been employed by or have holdings/a financial stake in any biomedical company. The remaining author declares no conflicts of interest.

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