THE CROSSROADS BETWEEN IMMUNOLOGICAL DISORDERS AND NEUROPSYCHIATRIC DISEASES. A CASE FOR SCHIZOPHRENIA

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EDITORIAL
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Editorial: The Crossroads Between Immunological Disorders and Neuropsychiatric Diseases. A Case for Schizophrenia

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Editorial on the Research Topic

The Crossroads Between Immunological Disorders and Neuropsychiatric Diseases. A Case for Schizophrenia

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Schizophrenia (SZ) is a prevalent psychiatric illness with a strong and complex genetic basis as well as wide spectrum of clinical manifestations (Gejman et al., 2010). Environmental inflammatory insults early in life and altered social behaviors have long been recognized to trigger the development of the disease (Zubin and Spring, 1977; Müller, 2018). In this Research Topic, we have compiled complementary articles from scientists working across diverse disciplines: original research studies conducted in human and experimental models, mini-reviews and reviews, as well as hypothesis and opinion articles aimed at clarifying the contribution of neuroimmunological interactions and inflammation to SZ.

In mouse models, Mi et al. explore new concepts of SZ as an acquired channelopathy. They suggest that specific ion channel alterations (voltage-gated sodium and small conductance calcium-activated potassium) induced by maternal immune activation (MIA)—by poly I:C challenge—combined to social isolation may in part account for the altered intrinsic neuronal excitability properties observed within the prefrontal cortex. The role of these channels in SZ-like behaviors warrants further examination. In another poly I:C-induced MIA mouse model of SZ, Ibi et al. focus on the implication of reelin—an extracellular matrix protein involved in neural development and synaptic plasticity—in the cognitive and emotional deficits in SZ. They investigate the potential role of reelin as a new molecular target for the treatment of neurodevelopmental disorders beyond the classical monoamine medication. In human studies, Hughes et al. further address the innate immune dysfunction in a cohort of 25 first episode psychosis patients compared to healthy controls. First episode psychosis patients were further grouped based on their presence or absence of mood disorder. The results suggest that dysfunctional monocyte responses are present in both affective and non-affective psychotic disorder, with a higher proinflammatory profile observed in monocytes/macrophages from patients with an affective psychotic disorder. In the context of

the current COVID-19 pandemics, exploration of the potential relevance for future work studying outcomes in children of mothers who acquired COVID-19 during pregnancy as a putative mechanism for MIA will be of great interest to the scientific community.

Bridging the complex interplay of genetic and environmental etiologies described in SZ, the immune response and inflammatory events offer plausible explanations for the wide spectrum of disease symptoms. Choudhury and Lennox provide a comprehensive review on the epidemiological, genome wide association studies (GWAS), and animal models linking MIA and the complement system activation during specific gestational timings with the increased susceptibility to SZ in exposed offspring. Again very timely study, given the complement activation described in COVID-19 and the potential occurrence of infection during pregnancy (Java et al., 2020). Comer et al. discuss how genetic and environmental risk factors for SZ converge to alter microglial function during development, adolescence and adulthood, in response to systemic and central inflammation; as well as the role of the gut-brain axis. They introduce the microglial sensome as a group of receptors and ligands that enable microglia to sense and react to their changing environment and to modulate multiple cell-types in the brain to push vulnerable individuals above a certain threshold into a disease state (Comer et al.). In this framework, Bordeleau et al. provide a critical overview on the effects of maternal diet on the body's regulatory supersystems of the mother, such as the immune, endocrine, and nervous systems. The authors argue that altered gut microbiome and inadequate nutrient intake can not only affect maternal mental and physical health, but also induce a malabsorptive and inflammatory status that may predispose to neurodevelopmental disorders including SZ. Reale et al. review the key role of cytokines as mediators of immune activation and how imbalances of T helper cell (Th)1/Th2/Th17/regulatory T cell (Treg) influence the dopaminergic, noradrenergic, and serotonergic neurotransmission. They also discuss promising therapeutic strategies aimed at targeting cytokines/cytokine receptors in neurological disorders. A particularly original insight presenting SZ as an inflammatory-triggered neurovascular developmental disease is next given by Carrier et al.. In this mini-review, the potential alterations of the brain vasculature network in early postnatal life in specific regions contributing to the development of SZ are discussed, with key roles of some molecules in the SZ vascular signature, such as defects in claudin-5 and vascular endothelial growth factor signaling. The authors dissect the role played by the different neurovascular unit elements, such as pericytes, endothelial cells, astrocytes, and microglia, to maintain appropriate brain function and behavior.

Two intriguing hypotheses are further presented. Lucchese et al. elucidate the associations between human cytomegalovirus (HCMV) infection, aberrant neuronal migration, and psychosis, building on previous research that had assessed peptide commonality and potential immune cross-reactivity between microbial and human proteins. Numerous human proteins related to neuronal migration are involved in a specific heptapeptide overlap with HCMV. Sánchez-Ramón and Faure also conceptualize SZ as an autophrenic disease, in which autoreactive neurons and engrams interfere with normal discrimination of internal and external signals. They build on an analogy with the immune system based on self-instruction and recognition in the thymus gland to propose specific self-recognition at the cortex subplate during development. Lastly, Maes and Anderson close this Research topic with a thought-provoking opinion article on the "false conceptualizations" of SZ in the pursuit of a holistic view of the disorder and propose a new nomothetic network-model framework based on machine learning methods to track "evidence based" progress through model group-based classification across stages.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Alterations of Electrophysiological Properties and Ion Channel Expression in Prefrontal Cortex of a Mouse Model of Schizophrenia

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Maternal immune activation (MIA) and juvenile social isolation (SI) are two most prevalent and widely accepted environmental insults that could increase the propensity of psychiatric illnesses. Using a two-hit mouse model, we examined the impact of the combination of these two factors on animal behaviors, neuronal excitability and expressions of voltage-gated sodium (Nav) and small conductance calcium-activated potassium (SK) channels in the prefrontal cortex (PFC). We found that MIA-SI induced a number of schizophrenia-related behavioral deficits. Patch clamp recordings revealed alterations in electrophysiological properties of PFC layer-5 pyramidal cells, including hyperpolarized resting membrane potential (RMP), increased input resistance and enhanced medium after-hyperpolarization (mAHP). MIA-SI also increased the ratio of the maximal slope of somatodendritic potential to the peak slope of action potential upstroke, indicating a change in perisomatic Nav availability. Consistently, MIA-SI significantly increased the expression level of Nav1.2 and SK3 channels that contribute to the somatodendritic potential and the mAHP, respectively. Together, these changes may alter neuronal signaling in the PFC and behavioral states, representing a molecular imprint of environmental insults associated with neuropsychiatric illnesses.

Keywords: schizophrenia, ion channel, neuronal excitability, action potential, prefrontal cortex, SK channel, Nav channel

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INTRODUCTION

Schizophrenia is a chronic and disabling psychiatric disorder affecting approximately 1% of the population worldwide (Owen et al., 2016). It is assumed that a single adverse event is rather unlikely to cause schizophrenia. Instead, the two-hit hypothesis suggests that a prenatal genetic or environmental first-hit can prime an individual for an adverse event later (second-hit) in life that ultimately provides substantial triggers for the full clinical syndrome to manifest (Bayer et al., 1999; Maynard et al., 2001; Feigenson et al., 2014). There is ample evidence that maternal immune activation (MIA) can act as a disease primer to increase the risk of neuropsychiatric disorders in offspring (Feigenson et al., 2014; Davis et al., 2016; Estes and McAllister, 2016), while social deprivation during adolescence has been thought to work as a second-hit leading to behavioral and biochemical changes that feature some aspects of schizophrenia (Jones et al., 2011;

Makinodan et al., 2012; Davis et al., 2016; Yamamuro et al., 2017). As two most prevalent and widely accepted environmental insults, MIA and social isolation (SI) can commonly occur together in life. However, research investigating the impact of MIA-SI combination is very limited.

Functions of prefrontal cortex (PFC) primarily rely on electrochemical signals generated by its constituent neurons. Disruptions of these signals have been shown in schizophrenia. Enhanced PFC neuronal excitability was found in Disrupted in schizophrenia 1 (*DISC1*) mutant mouse model of schizophrenia (Crabtree et al., 2017). Two-week SI after weaning results in reductions in intrinsic excitability of a subpopulation of pyramidal cells (PCs) in mouse PFC (Yamamuro et al., 2017). Both NMDA receptor antagonist MK801 and serotonergic agonist DOI are psychotogenic drugs, which cause transient states of psychosis. Interestingly, MK801 increased PFC population activity, whereas DOI decreased population activity (Wood et al., 2012). It remains unclear whether the MIA-SI two-hit would cause any changes in the intrinsic excitability of PCs in PFC.

Given the significance of ion channels in the regulation of neuronal excitability and synaptic plasticity, it's not surprising that altered ion channel expression and function would cause severe disruptions in brain functions, thus contributing to schizophrenia etiology (Smolin et al., 2012). Voltage-gated sodium channels (Nav) are particularly critical for the initiation and propagation of action potentials (APs). Cortical PCs express two Nav subtypes, i.e., low-threshold Nav1.6 and high-threshold Nav1.2 channels, which preferentially accumulate at distal and proximal regions of the axon initial segment (AIS), respectively, (Tian et al., 2014). Distal Nav1.6 triggers AP initiation, whereas proximal Nav1.2 promotes AP backpropagation to the soma (Hu et al., 2009). Polymorphisms of *SCN2A*, the gene encoding Nav1.2, are associated with the occurrence of schizophrenia (Dickinson et al., 2014; Carroll et al., 2016). Mutation of *SCN8A*, which encodes Nav1.6, is also involved in the susceptibility of suicidal behavior among psychiatric disorder patients (Wang et al., 2010). Moreover, Nav channel blockers can be used as mood stabilizers, antidepressants and antipsychotics (Imbrici et al., 2013). Therefore, Nav channels may be a valuable target for effective treatments. However, alterations in Nav channels in animal model of schizophrenia have not been characterized yet.

Previous studies also revealed important roles of potassium channels in schizophrenia (Shepard et al., 2007; Vukadinovic and Rosenzweig, 2012; Georgiev et al., 2014; Yanagi et al., 2014). For example, the small conductance calcium-activated potassium (SK) channels, which mediate the medium after-hyperpolarization (mAHP) (Villalobos et al., 2004), may play a role in the etiology of schizophrenia and related cognitive disorders (Faber and Sah, 2007). The gene *KCNN3* encoding SK3 locates at 1q21, a chromosome closely related to schizophrenia (Gargus, 2006). Polymorphism of *KCNN3,* which reduces SK3 channel function, is associated with enhanced cognitive performance in schizophrenia patients (Grube et al., 2011). In contrast, overexpression of SK3 channel induces hippocampal shrinkage and cognitive deficits (Martin et al., 2017). Inhibition of SK2 channels also improves learning and memory (Lam et al., 2013). It remains unknown whether SK channels change in animal models of schizophrenia.

In this study, we used peripheral administration of immunostimulant Polyinosinic:polycytidylic acid (poly I:C) in pregnant dam combined with juvenile isolation rearing to model schizophrenia in mice. We examined the consequences of MIA and early life SI on animal behaviors and electrophysiological properties of layer 5 (L5) PCs in prelimbic (PL) and infralimbic (IL) regions of PFC, which associates with multiple emotional, cognitive, and mnemonic functions. We also assessed the impacts of MIA-SI on the expression of Nav and SK channels.

MATERIALS AND METHODS

Animals

Protocols of all animal experiments were approved by the Animal Advisory Committee at the State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University. The experiments were conducted in accordance with the guidelines set out by the Animal Advisory Committee. We obtained pregnant C57BL/6 mice from Beijing Vital River Laboratory Animal Technology Co., Ltd., (Beijing, China). Intraperitoneal injection of 5 mg/kg poly I:C (Tocris, Bristol, United Kingdom) or saline were carried out at gestational day 15 (GD15). Their offspring were weaned at postnatal day 21 (P21) and only male littermates were used for the following experiments. Offspring of saline-treated animals were housed in groups of 3–5 animals per cage, whereas the offspring of poly I:C-treated animals were reared individually for a month (**Figure 1A**). In consequence, we obtained two animal groups, control group, i.e., saline treated-social animals, and MIA-SI group, i.e., poly I:C treated-isolated animals. All animals were kept in a controlled environment (22 ± 2°C and 12-h light/darkness cycle). Water and standard pellet chow was available *ad libitum*. Separate cohorts of mice were used for behavioral tests, immunoblots and electrophysiological recordings to avoid the influences of behavioral paradigms on animals.

Animal Behavior

Open-Field Test

Spontaneous locomotor activity and general behavior were evaluated using an automated open-field apparatus (Med Associates Inc., Fairfax, VT, United States). The apparatus consists of a 27.31 × 27.31 × 20.32 cm^3 transparent seamless chamber with an open roof, a sound attenuating cubicle with venting and lighting, and a computer. 16-beam infrared arrays located on both the *X* and *Y* axes for positional tracking and *Z* axis for rearing detection. The animals were placed individually in the center of the open-field arena and recorded for 5 min. General locomotor activity was assessed by measuring total distance traveled, as well as total duration of jumps. Total distance traveled is the total Euclidean distance of all ambulatory episodes in centimeters. Jump is when *Z*-axis infrared beam breaks were detected while no *X* or *Y*-axis beam breaks were detected. The jump time is the total duration of the jumping behavior.

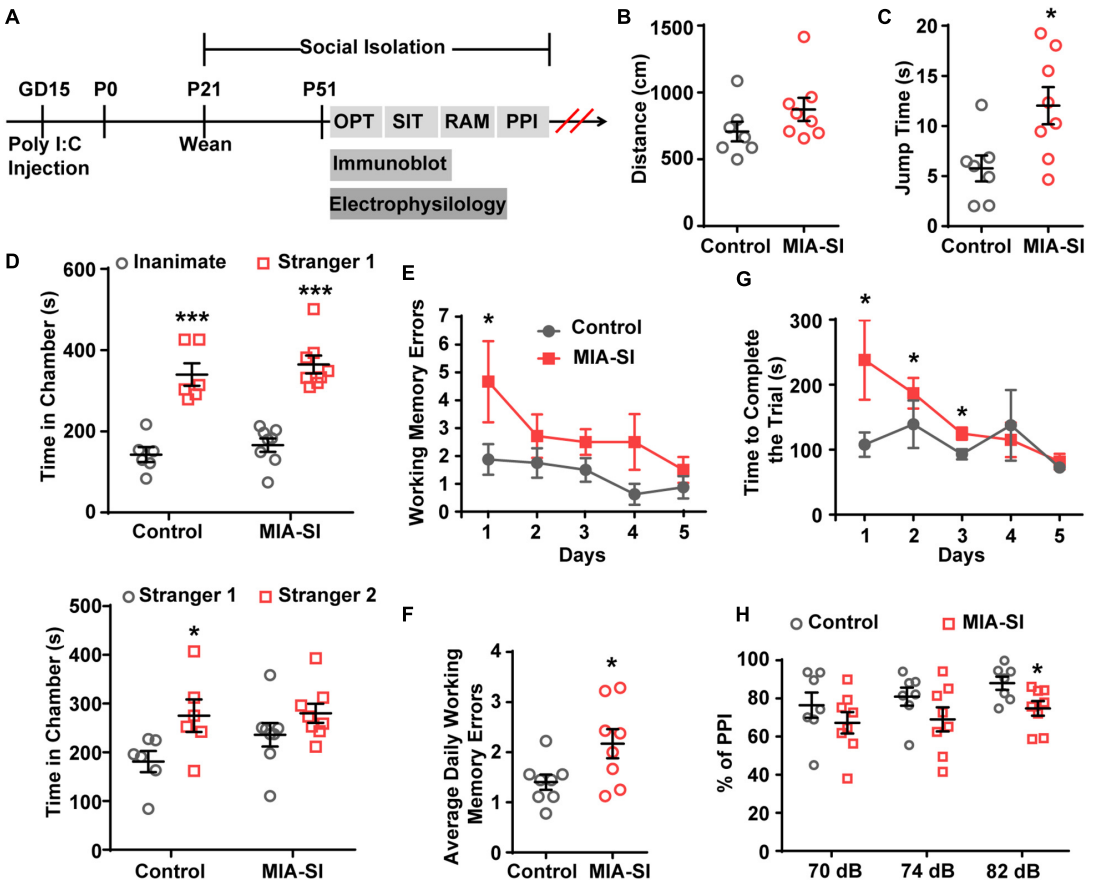

FIGURE 1 | MIA-SI animals develop schizophrenia-like behaviors, including deficits in jumping behavior, social novelty preference, working memory and sensorimotor gating function. **(A)** Overview of the experimental design. OFT, open field test; SIT, social interaction test; RAM, radial arm maze; PPI, prepulse inhibition. **(B)** Total distance traveled was not changed by MIA-SI in OFT. **(C)** Increased jumping time was observed in MIA-SI animals in OFT. **(D)** Top, time spent in the non-social chamber (inanimate) versus the social chamber (stranger 1) during the sociability session in SIT, indicating MIA-SI didn't affect sociability. Bottom, time spent in the chamber with familiar mouse (stranger 1) versus that with novel mouse (stranger 2) during social novelty preference session in SIT, suggesting MIA-SI caused deficit in social novelty preference. **(E)** Working memory errors committed across five test days in RAM test. **(F)** Average daily working memory errors over five test days was increased in MIA-SI animals. **(G)** Time to complete the trial across five test days in RAM test. **(H)** PPI deficits following poly I:C challenge and isolation rearing. $^{*}p < 0.05$; $^{***}p < 0.001$. Control, $n = 7$; MIA-SI, $n = 8$.

Sociability and Social Novelty Preference Test

The apparatus used in this test consists of clear acrylic walls, an opaque gray bottom, and two clear partitions which divide the apparatus into three chambers of same size. Each partitions had one door in the center which could be lifted up to form a passage between chambers. One outer chamber was designated as social chamber randomly, and the other outer chamber was designated novelty chamber. Each outer chamber had a wire cage to entrap one stranger mouse. A video camera was fixed above the apparatus to record the activity of animals and was connected to a monitor and behavioral tracking system (ANY-maze, Stoelting Co., Wood Dale, IL, United States). The sociability and social novelty preference test protocols were adapted from previous study with slight modifications (Moy et al., 2004). The protocol contains three phases that occur in sequence: habituation, sociability, and social novelty preference. The day before the test, all experimental mice and stranger mice were allowed to acclimate to the test room and apparatus. On the test day, the experimental mouse was firstly placed in the center chamber and allowed to freely explore the three chambers for 5 min. Immediately after the habituation phase, the stranger mice 1 was placed in the wire cage in the social chamber, while the wire cage in the other outer chamber still stayed empty. Then the test mouse was allowed to freely access to two wire cages for another 10 min. After this sociability session, stranger mice 2 was introduced into the novelty chamber, and the test mouse was allowed to explore for another 10 min. The sociability and social novelty preference of the test mouse were determined by measuring the time spent in social chamber and novelty chamber during all three phases.

Radial Arm Maze for Working Memory Test

We used an automated eight arm radial maze (Med Associates Inc., United States) consisting of eight runways with dual IR sensors for each arm, pellet receptacles with head entry detector at the end of each runway, and eight automatic guillotine doors

separating the hub area from each of the eight arms. Before experiments, mice were weighed and fasted for 24 h, and 2–3 g pellet chow were given to each animals daily on following test days. Animals were then habituated to the apparatus for three consecutive days. During habituation, mice were placed in the hub and allowed to explore the maze for 10 min per day. Baits were scattered on the arms on the first habituation day, and then gradually reduced and located toward the end of each runway. Following habituation, the animals were tested one session per day for five consecutive days. For each trial, all eight arms were baited with one food reward in food receptacle at the end of the arms. Experimental subjects were first placed in the hub area for 2 min with all guillotine doors closed. Then all eight doors raised, and animals could explore the maze to collect baits. Re-entering a visited arm was defined as one working memory error. Each session ended when all eight arms had been entered or 10 min passed since the door opened. The variables used for the analyzing the performance of the mice are the number of errors in each trial (day), the time taken to complete the trial on each day, and the average daily number of errors throughout 5 days.

Prepulse Inhibition

Prepulse inhibition (PPI) was measured using an acoustic startle reflex system (Med Associates Inc., United States) as described previously (Hadar et al., 2018). Briefly, to measure startle reflex, we set the background noise level to 66 dB. The 100 ms startling pulse (110 dB) was preceded by a 20-ms-long non-startling prepulse (70, 74 or 82 dB in a random order). PPI for a given prepulse intensity was calculated as percent inhibition of the startle response using the following formula:

$$\mathrm{PPI} = 100 - \frac{\text{average startle response for PPI trials}}{\text{average startle response for startle} - \text{only trials}} \times 100$$

Immunoblot

Protein samples were isolated from the PFC tissue blocks and separated on 8% SDS polyacrylamide gels. The primary antibodies are as follows: mouse anti-Nav1.2, clone K69/3, 1:200 (Neuromab, Davis, CA, United States); rabbit anti-Nav1.6, 1:200; rabbit anti-SK1, #APC-039, 1:200; rabbit anti-SK2, #APC-028, 1:200; rabbit anti-SK3 N-term, #APC-025, 1:200 (Alomone Labs, Jerusalem, Israel); mouse anti-α-tubulin, clone DM1A, 1:1000 (Sigma-Aldrich, St. Louis, MO, United States). We used 3 and 4 animals for control and MIA-SI groups, respectively. And experiments were repeated for 3–4 times. The integrated density of samples on blots were analyzed using ImageJ (National Institutes of Health, United States). For each blot, we normalized the integrated density of each sample to β-tubulin and then normalized this ratio to the mean ratio of samples in control groups. Then we averaged the normalized density of each sample, and performed statistical analysis (Student's *t*-test).

Slice Preparation

Coronal slices of PFC were obtained from experimental mice as previously described (Hu et al., 2009). Animals were anesthetized with sodium pentobarbital (50 mg/kg) and perfused with ice-cold sucrose-based artificial cerebrospinal fluid (ACSF) followed by decapitation. The brains were then dissected out and slices with a thickness of 300 μm were cut in sucrose-based ACSF using a vibratome (VT-1200S, Leica, IL, United States). Then brain slices were immediately transferred to an incubation chamber filled with normal ACSF and maintained at 34.5°C for 30 min in water bath. For recording, individual slices were transferred to a recording chamber perfused with normal ACSF at 34.5–35.5°C for whole-cell recording. An infrared-differential interference contrast (IR-DIC) microscope (BX-51WI, Olympus) was used for visualizing individual cells in the slice. The normal ACSF contained (in mM) 126 NaCl, 2.5 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4 and 25 dextrose (315 mOsm, pH 7.4), and was bubbled continuously with carbogen.

Electrophysiological Recordings

We performed whole-cell patch-clamp recordings from L5 PCs in PL and IL regions of PFC as described previously (Hu et al., 2009). Patch pipettes had an impedance of 4–7 MΩ with the normal internal solution contained (in mM) 140 K-Gluconate, 3 KCl, 2 $MgCl_2$, 0.2 EGTA, 10 HEPES, 2 Na_2ATP (pH 7.2–7.25, 285–295 mOsm). Recordings were performed using a Multiclamp 700B amplifier (Molecular Devices, San Jose, CA, United States). We used Micro1401-3 together with Spike2 software (version 8) (Cambridge Electronic Design Limited, Cambridgeshire, United Kingdom) for data acquisition. Voltage signals were filtered at 10 kHz and sampled at 50 kHz. The liquid junction potential (15.2 mV) was not corrected for the data shown in the text and figures. All electrophysiological recordings were performed and analyzed blindly.

Resting membrane potential (RMP) was defined as membrane potential measured when no current injected. Rheobase was defined as the threshold current that elicits a single AP. The AP threshold was defined as the voltage at which the time derivative of membrane potential (dV/dt) reached 20 V/s. AP half width (HW) is the duration of the AP at half amplitude from AP threshold. Amplitude of mAHP is measured as difference between AP threshold and the trough in a time window of 100 ms after individual APs. The sag ratio was measured from the hyperpolarization induced by negative current pulse injections (amplitude: −100 pA). It is the ratio of voltage difference between the peak and the steady-state of hyperpolarization to the peak amplitude. We considered the PCs with sag ratio >5% as those with prominent H-current. The mAHP, threshold, peak amplitude, half width and maximum depolarization and repolarization slope were measured from the single APs elicited by rheobase current, while $Slope_{SD}$ ratio was measured from the first APs in 7-AP trains. The parameters were analyzed using MATLAB R2017b (MathWorks Inc., Natick, MA, United States).

Statistical Analysis

All data in figures and tables are presented as mean ± SEM. In our study, we considered the combination of MIA and SI as a single factor. Therefore, we performed the two-tailed unpaired Student's *t*-test to compare two normally distributed sample groups and Mann–Whitney U test to compare two non-normally distributed

groups. A value of $p < 0.05$ was considered significant. Statistical analysis was carried out using OriginPro-9 (OriginLab Corp., Northampton, MA, United States) and SigmaPlot 14.0 (Systat Software Inc., San Jose, CA, United States).

RESULTS

MIA-SI Causes Deficits in Locomotor Activity, Social Novelty Preference, Working Memory and Sensorimotor Gating Function

We carried out four behavioral paradigms to examine whether the animals develop schizophrenia-like behaviors. Animals was assessed on behavioral tasks in the following sequence: open-field tests, sociability and social novelty preference test, radial arm maze, and PPI (**Figure 1A**). Tasks were separated by 3–7 rest days to ensure minimum interference between tasks.

Hyperactivity has been demonstrated in many different putative animal models of schizophrenia (Powell et al., 2009). We initially examined the locomotor activity in these animals. MIA-SI didn't influence the total distance traveled by these animals (**Figure 1B**). However, we observed substantially longer jump time ($p < 0.05$, Student's *t*-test) (**Figure 1C**) in MIA-SI animals. Jumping behavior has been examined as one of the basic locomotive behaviors in the open field test and homecage monitoring (Choleris et al., 2001; Huang et al., 2007; van Dam et al., 2013; Adamah-Biassi et al., 2014). An increase in jumping behavior has been considered as an indicator of hyperactivity and increased exploratory behavior (Hashimoto et al., 2001; Shintani et al., 2006). Therefore, the results indicate that MIA-SI animals were more hyperactive.

Sociability and social novelty test was often used for assessing the social behavior in animal models of schizophrenia (Powell and Miyakawa, 2006). Within the sociability session, both groups exhibited preference for the stranger mice over the inanimate chamber ($p < 0.001$) (**Figure 1D**, top). In the social novelty preference phase, results varied between groups: control animals appeared to interact more with the novel conspecific stranger ($p < 0.05$), while the MIA-SI group showed no significant preference for the novel stranger versus the familiar conspecific ($p = 0.177$) (**Figure 1D**, bottom). These results indicate that MIA-SI disrupted the animal's preference for social novelty but not animal's sociability.

We next examined the working memory of these animals using a classic eight-arm radial maze paradigm (Powell and Miyakawa, 2006). Working memory errors committed on day 1 was increased in MIA-SI group ($p < 0.05$, **Figure 1E**). MIA-SI also significantly increased the average daily working memory errors over five consecutive days of test ($p < 0.05$, **Figure 1F**). The time taken to complete the trial was significantly longer in MIA-SI animals from day 1 to day 3 ($p < 0.05$, **Figure 1G**). These results suggest that environmental insults jeopardize the working memory of animals.

Prepulse Inhibition deficit is another common symptom of schizophrenia (Powell et al., 2009). We found MIA-SI had no effects on startle reflex at 110 dB (data not shown). PPI at prepulse stimulus intensities of 70 and 74 dB was not affected by MIA-SI (**Figure 1H**). However, there was a significant difference in PPI between the two groups with prepulse stimulus at 82 dB ($p < 0.05$, **Figure 1H**), indicating disrupted sensorimotor gating in this two-hit animal model of schizophrenia. Altogether, our results suggest that MIA-SI two-hit animals exhibit abnormalities in schizophrenia relevant behaviors.

MIA-SI Hyperpolarizes RMP, and Increases Input Resistance and mAHP

Juvenile SI alters intrinsic properties of deep layer PCs in PFC (Yamamuro et al., 2017). We next performed whole-cell recordings from L5 PCs in PL and IL regions of PFC. Analysis showed that MIA-SI caused a significant hyperpolarizing change in the RMP ($p < 0.05$, **Figure 2A**). Cells of the two-hit group also showed a greater input resistance (Rin) in contrast to the control group ($p < 0.05$, **Figures 2B,C**). To examine the effects of MIA-SI on neuronal firing, we injected a series of incremental depolarizing current pulses from 50 pA to 1,500 pA into the recorded cells (example traces shown in **Figure 2D**, top panel) and found no significant difference in the firing frequency between the two groups (**Figure 2D**, bottom panel). We then closely examined the properties of single APs evoked by threshold current (i.e., rheobase). The threshold current and the latency to spike was not affected by MIA-SI (**Table 1**). The peak amplitude of mAHP was significantly increased in the two-hit group ($p < 0.01$, **Figures 2E,F**). Other parameters of the single APs evoked by rheobase were not altered by MIA-SI (**Table 1**). To test the spike accommodation, we analyzed the first and sixth inters-pike interval (ISI_1 and ISI_6) in 7-AP trains (**Figure 2G**). The currents evoking 7 AP were not different between control (median, 230.0 pA) and MIA-SI (median, 250.0 pA) neurons ($p = 0.433$). The mAHP of AP_1 to AP_6 were all increased in MIA-SI group. However, the increase in AHP of AP_1 was larger than other APs (**Supplementary Figure S1**). There is a substantial increase in ISI_1 ($p < 0.01$, Student's *t*-test, **Figure 2H**) but not in ISI_6 ($p = 0.9$, Mann–Whitney test, **Figure 2I**). Such change leaded to a significant decrease in AP accommodation ratio, i.e., $(ISI_6\text{-}ISI_1)/ISI_6$. These results indicate MIA-SI affected spike accommodation of these PCs.

The rising phase of somatic APs contains two components as revealed by the phase plots (**Figure 2I**), the AIS potential and the somatodendritic (SD) potential (Hu et al., 2009). The SD potential is generated mainly by perisomatic Nav channels (i.e., Nav1.2). Second derivative (d^2V/dt^2) of the AP waveforms showed a trough in the AP rising phase, reflecting the breakpoint between the AIS potential and SD potential (Kole, 2011). We defined the contribution of SD potential to the AP rising phase by the ratio of the maximal slope of SD potential (a) to the peak slope of AP upstroke (b) (**Figure 2J**). MIA-SI didn't cause significant alterations in either a or b-a. However, analysis showed that MIA-SI significantly increased the ratio of a to b, suggesting that the perisomatic Nav may be changed ($p < 0.01$, **Figure 2J**).

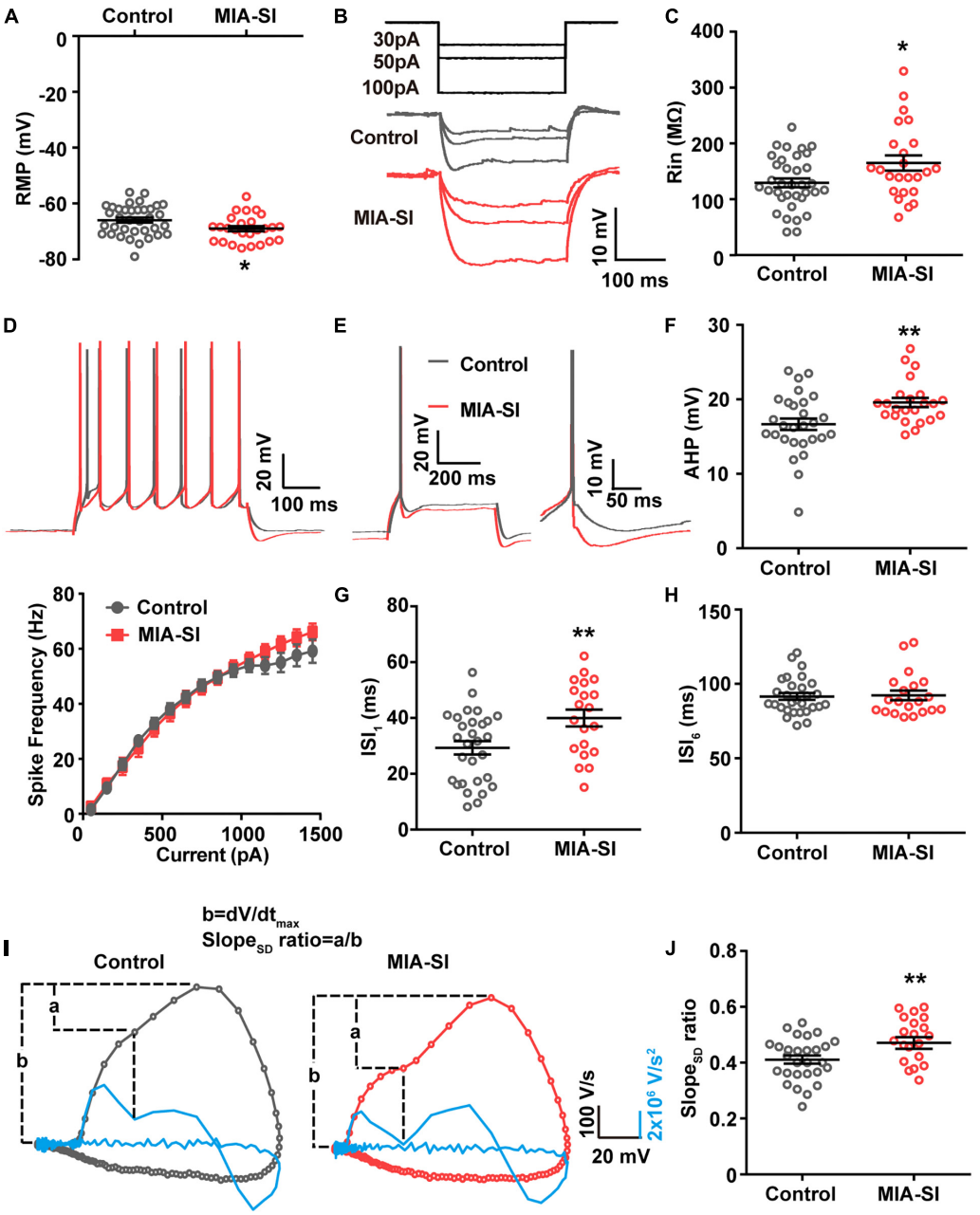

FIGURE 2 | MIA-SI induces alterations in electrophysiological properties in PCs, including hyperpolarized RMP, and increased input resistance, mAHP and AP phase ratio. **(A)** Group data showed that MIA-SI caused hyperpolarization of RMP. **(B)** Example voltage traces of PCs in response to hyperpolarizing current pulses. **(C)** Group data of input resistance (Rin) showed MIA-SI increased Rin in L5 PCs. **(D)** Top, representative 7-AP traces of PCs elicited by current injection in control and MIA-SI groups; Bottom, Input-output (I-F curve) relationships of neurons from two experimental groups were not significantly different. **(E)** Representative full traces (left) and enlarged traces (right) showing changes in mAHP of single AP evoked by threshold current. **(F)** Group analysis showed MIA-SI increased mAHP amplitude. The first inter-spike interval (ISI_1) **(G)** but not the last inter-spike interval (ISI_6) **(H)** of 7-AP trains was increased by MIA-SI. **(I)** Representative phase plots and the second derivatives of the voltage waveforms (shown in blue) for control (left) and MIA-SI (right) PCs. The contribution of SD potential slope to the peak slope is calculated by the ratio of a/b. **(J)** Group data of $Slope_{SD}$ ratio. $^*p < 0.05$; $^{**}p < 0.01$.

Previous studies showed that 2-week isolation immediately after weaning reduces the intrinsic excitability only in a subtype of L5 PCs with prominent H-current in PFC (Yamamuro et al., 2017). Therefore, we also analyzed the aforementioned electrophysiological parameters of the neurons showing prominent H-current. Control and MIA-SI groups were not different in sag ratio (**Supplementary Figure S2**). 54.3% PCs in control group (n = 19 out of 35) and 54.2% PCs (n = 13 out of 24) in MIA-SI group exhibit prominent H-current. We obtained similar results in electrophysiological

TABLE 1 | Electrophysiological parameters of L5 PCs in control and MIA-SI animals.

	Control	MIA-SI	*p*-value
RMP (mV)	−65.9 ± 0.8 (*n* = 36)	−69.0 ± 0.9 (*n* = 26)	0.024
Rin (MΩ)	130 ± 8 (*n* = 35)	165 ± 14 (*n* = 24)	0.019
Rheobase (pA)	110 ± 15 (*n* = 36)	133 ± 18 (*n* = 26)	0.91
Spike latency (ms)	180 ± 17 (*n* = 24)	176 ± 19 (*n* = 20)	0.88
Threshold (mV)	−33.5 ± 1.0 (*n* = 28)	−34.4 ± 1.1 (*n* = 23)	0.50
Peak amplitude (mV)	80.3 ± 2.5 (*n* = 28)	78.7 ± 2.8 (*n* = 23)	0.77
Half width (ms)	0.88 ± 0.05 (*n* = 28)	0.85 ± 0.05 (*n* = 23)	0.64
mAHP (mV)	16.7 ± 0.7 (*n* = 28	19.6 ± 0.8 (*n* = 23)	0.009
dV/dt_{max} (mV/ms)	391 ± 27 (*n* = 28)	391 ± 29 (*n* = 23)	0.99
dV/dt_{min} (mV/ms)	−90.5 ± 5.0 (*n* = 28)	−88.5 ± 5.5 (*n* = 23)	0.78

dV/dtmax, maximum depolarization slope; dV/dtmin, maximum repolarization slope; n, number of neurons.

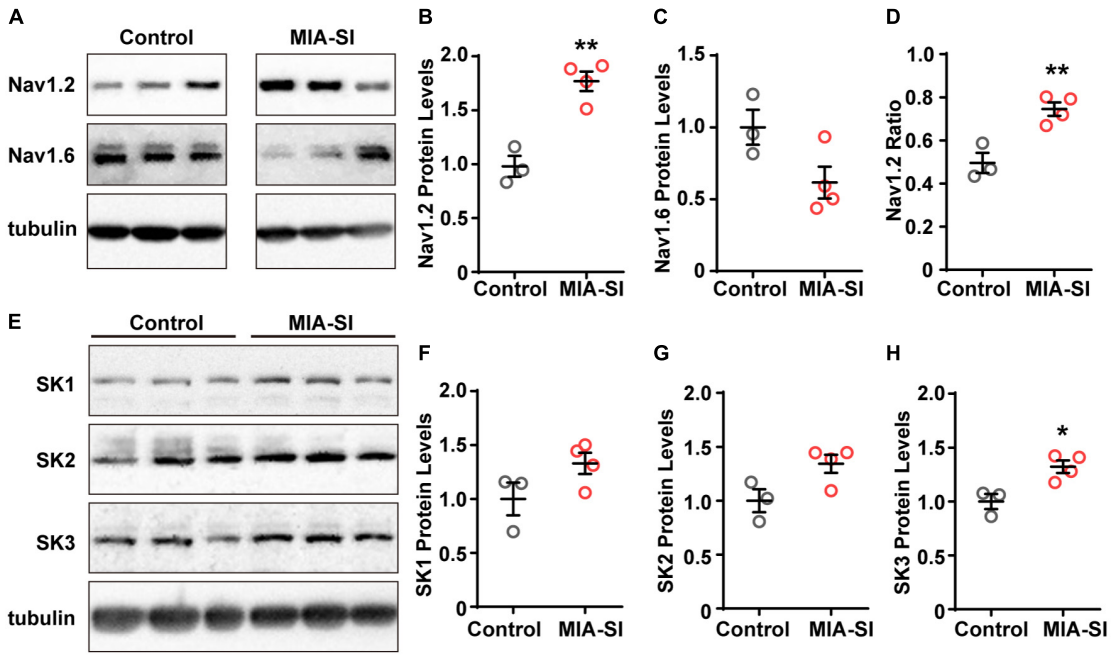

FIGURE 3 | MIA-SI increases the expression levels of Nav1.2 and SK3 channels. **(A)** Representative immunoblots of Nav1.2, Nav1.6 and tubulin, run on a same gel. Each band represents samples from one animal. **(B,C)** Average Nav1.2 and Nav1.6 protein levels in PFC in two experimental groups. **(D)** Averaged ratio of Nav1.2 to the sum of Nav1.2 and Nav1.6 was increased by MIA-SI. **(E)** Representative immunoblots of SK1, SK2, SK3, and tubulin run on a same gel. **(F–H)** Average SK1, SK2, and SK3 protein levels in PFC of experimental animals. For each protein, blots were cropped to retain the important bands at the same size of the same membrane. $*p < 0.05$; $**p < 0.01$.

properties from these cells (**Supplementary Figure S3** and **Supplementary Table S1**). In conclusion, our results revealed that MIA-SI two-hit animal model displayed deficits in neuronal electrophysiological properties.

MIA-SI Increases the Expression Levels of Nav1.2 and SK3 Channels

The channel subtype Nav1.6 triggers AP initiation, whereas Nav1.2 mainly contributes to AP backpropagation to the soma (Hu et al., 2009) and form the SD potential. Therefore, we hypothesized that the alterations in the amount or function of Nav1.2 may cause changes in the SD potential slope ratio. To test this hypothesis, we firstly examined the protein expression of Nav1.2 and Nav1.6. Quantification demonstrated that Nav1.2 protein levels were significantly increased by MIA-SI ($p < 0.01$, **Figures 3A,B**). In contrast, MIA-SI tended to decrease Nav1.6 at an insignificant level (**Figures 3A,C**). We analyzed the ratio of Nav1.2 to the sum of Nav1.2 and Nav1.6, and found Nav1.2 percentages were increased by MIA-SI ($p < 0.01$, **Figure 3D**), indicating that the proportion of Nav1.2 and Nav1.6 was influenced by MIA and SI.

The increased mAHP amplitude in MIA-SI group could be attributable to changes in the expression level of SK channels (Villalobos et al., 2004). To test this assumption, we also

examined the protein levels of SK1, SK2, and SK3 channels. We found that SK1 and SK2 protein levels exhibited an increasing tendency in MIA-SI animals but without statistical significance (**Figures 3E–G**). In contrast, MIA-SI significantly increased SK3 ($p < 0.05$) expression levels in PFC (**Figure 3H**). These results indicate that MIA can cause abnormal ion channel expression.

DISCUSSION

In this work, we investigated behavioral and neurophysiological alterations in animals challenged with two environmental insults, MIA and SI. We found that combining the MIA with SI exacerbates a number of schizophrenia relevant behaviors, including deficits in locomotor activity, social novelty preference, working memory and sensorimotor gating function. Moreover, MIA and SI together also altered neurophysiological properties of L5 PCs in PFC, including RMP, input resistance, mAHP and spike accommodation. The ratio of the maximal slope of SD potential to the peak slope of AP upstroke is also increased by MIA-SI, indicating a change in perisomatic sodium channels. Indeed, immunoblot experiments revealed an increase in Nav1.2 and SK3 channels, consistent with the increases in the somatodendritic potential slope ratio and the amplitude of mAHP, respectively. These results indicate that MIA and SI mediate adverse effects on animal behaviors and neuronal electrophysiological properties.

Immunostimulant challenges and stressors provoke both common and distinct effects, and mediate synergistic effects on some animal behaviors and neurochemistry (Monte et al., 2017). Previous MIA-stress two-hit studies uses different animals (e.g., age, sex, species), dose and timing of poly I:C exposure, as well as behavioral paradigms, thus leading to some discrepant results of behavioral tests (Gandhi et al., 2007; Lukasz et al., 2013; Feigenson et al., 2014; Monte et al., 2017). For example, a study combining the postnatal poly I:C challenge with SI found that MIA-SI induced PPI deficit at 72, 76, 80 and 84 dB (Lukasz et al., 2013), but we only found MIA-SI-induced PPI deficits at 82 dB. This difference may in part results from different dose and timing (P38–46 vs. E15) of poly I:C treatment.

We observed an increase in jumping behavior in MIA-SI mice. We assumed this change was an indicator of hyperactivity and increased exploratory behavior based on previous studies (Hashimoto et al., 2001; Shintani et al., 2006). However, jumping behavior is also a prominent sign of physical dependence during morphine withdrawal in mice (David and Cazala, 2000), which could be related to anxiety, considering that anxiety is one of the common psychological symptoms of morphine withdrawal. However, in mice lacking pituitary adenylate cyclase-acting polypeptide (PACAP), augmented jumping behavior in OFT and lower anxiety level shown in elevated plus maze and novel-object test were discovered simultaneously (Hashimoto et al., 2001). Therefore, we could not conclude that increased jumping behavior was due to anxiety. In the current study, we mainly focused on the core symptoms of schizophrenia. However, early adversities, including both MIA and SI, can induce diverse emotional and behavioral deficits, including anxiety-related behaviors (Babri et al., 2014; Canetta et al., 2016; Rincel et al., 2019). Therefore, it's very likely that one would see changes in MIA-SI animals if their anxiety-like behaviors were tested.

A previous study showed that 2-week SI immediately after weaning reduced the intrinsic excitability of L5 PCs in PFC, reflected by an increase in spike threshold and a decrease in firing frequency (Yamamuro et al., 2017). However, our experiments revealed no changes in spike threshold and the input-output curve (i.e., F-I curve). In our research, we found that the input resistance was substantially increased, which should lead to enhanced neuronal excitability. However, we also observed more hyperpolarized RMP and greater mAHP in the two-hit group, which may decrease the intrinsic excitability. These alterations may counteract with each other and consequently result in no change in firing frequency. For example, we found a prolonged ISI accompanied with increased mAHP, which could offset the increase in the input resistance. This phenomenon could be the mechanism underlying the adaptation to stresses.

An increase in input resistance could be caused by less channels per unit membrane area, which may result from a reduction in total channel amount or an increase in neuron size. We compared the sag ratio of these cells and found no difference, suggesting that HCN channels were not responsible for the changes in input resistance. Considering the major contribution of resting channels (e.g., resting potassium, chloride and sodium channels) to the membrane resistance, we speculate a change in their expression level or channel activity. However, this possibility remains to be examined in future studies.

Nav1.2 plays a pivotal part in mediating AP backpropagation, which is proposed to regulate synaptic plasticity, release of retrograde neurotransmitters and trophic factors, and spike-timing-dependent plasticity. It's likely that an increase in Nav1.2 levels impacts upon these functions, thus disturbing PFC neural circuits and finally causing schizophrenia. Furthermore, compared to Nav1.2, Nav1.6 channels activate with more hyperpolarized membrane potentials and have a higher propensity to generate a non-inactivating persistent sodium current, which contributes to setting membrane potential in a subthreshold range (Astman et al., 2006). Indeed, conditional knockout of Nav1.6 leads to a strong reduction in persistent sodium current (Chen et al., 2018). In our experiments, we found a decrease in Nav1.6 in the MIA-SI group, providing an explanation for the hyperpolarization of the RMP.

Our results show that Nav1.2 protein levels were significantly increased, while Nav1.6 protein levels tended to be declined by MIA-SI, and the proportion of Nav1.2 was increased in MIA-SI animals (meaning that Nav1.6 proportion was also decreased significantly). The Nav1.6 changes could be a compensatory adaptation subsequent to Nav1.2 elevations. The minimal changes in maximum depolarization slope, which is mainly contributed by Nav, could result from such compensation. Besides, Nav1.2 is the only Nav isoform at AIS and nodes of Ranvier during early development, and replaced by Nav1.6 in the distal AIS subsequently (Ben-Shalom et al., 2017). Therefore, MIA-SI could influence early

developmental transition of Nav1.2 during the course of postnatal maturation.

A growing body of evidence implicates several calcium and potassium channels in the susceptibility of schizophrenia, such as *CACNA1C* (Cav1.2 α subunit), *KCNN3* (SK3), *KCNH3* (Ether-A-Go-Go), and *KCNJ3* (Kir3.1) (Smolin et al., 2012; Imbrici et al., 2013). Among those channels, SK3 channel plays major roles in determining the mAHP amplitude, and represents a potential drug target to improve cognitive functions in schizophrenia. Our data showed a striking increase in mAHP amplitude in the two-hit group. We postulate that the increased expression of SK3 channels could contribute to the change in mAHP. We detected SK channel expression using total PFC tissue samples, which consist of different layers and types of neuronal cells, while we examined the electrophysiological properties only in deep layer PCs in PFC. Neocortical PCs express high levels of SK1 and SK2, and a relative low level of SK3 channels (Pedarzani and Stocker, 2008). Inhibitory neurons, glia cells and cerebral vasculature also contain SK channel expression (Pedarzani and Stocker, 2008; Dolga and Culmsee, 2012). Therefore, the immunoblot results of SK channels could also reflect the changes in other types of cells. Cell-specific expression of SK3 requires a further detection in future studies.

Although the evidence concerning ion channel alterations in the pathophysiology of psychiatric disorders are emerging, knowledge about schizophrenia as channelopathy is still limited. Our data suggests that Nav and SK channels are altered by maternal immune stimulation, and may in part contribute to the changes in intrinsic neuronal excitability properties. However, whether these channels contribute to the changes in electrophysiological properties eventually give rise to schizophrenia-like behaviors remains to be further examined.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Advisory Committee at the State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University.

AUTHOR CONTRIBUTIONS

ZM designed the study, completed the animal behavioral experiment, the whole-cell patch-clamp recordings, and Western blot experiments, managed the literature searches and data analyses, and wrote the first draft of the manuscript. JY and YX completed part of the whole-cell patch-clamp recordings. QH undertook the statistical analysis. XZ conducted part of the animal behavioral experiment. YS oversaw all aspects of study design, protocol development, and data collecting, and edited the manuscript. All authors contributed to and have approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fncel.2019.00554/full#supplementary-material

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A Peptide Link Between Human Cytomegalovirus Infection, Neuronal Migration, and Psychosis

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Alongside biological, psychological, and social risk factors, psychotic syndromes may be related to disturbances of neuronal migration. This highly complex process characterizes the developing brain of the fetus, the early postnatal brain, and the adult brain, as reflected by changes within the subventricular zone and the dentate gyrus of the hippocampus, where neurogenesis persists throughout life. Psychosis also appears to be linked to human cytomegalovirus (HCMV) infection. However, little is known about the connection between psychosis, HCMV infection, and disruption of neuronal migration. The present study addresses the hypothesis that HCMV infection may lead to mental disorders through mechanisms of autoimmune cross-reactivity. Searching for common peptides that underlie immune cross-reactions, the analyses focus on HCMV and human proteins involved in neuronal migration. Results demonstrate a large overlap of viral peptides with human proteins associated with neuronal migration, such as ventral anterior homeobox 1 and cell adhesion molecule 1 implicated in GABAergic and glutamatergic neurotransmission. The present findings support the possibility of immune cross-reactivity between HCMV and human proteins that—when altered, mutated, or improperly functioning—may disrupt normal neuronal migration. In addition, these findings are consistent with a molecular and mechanistic framework for pathological sequences of events, beginning with HCMV infection, followed by immune activation, cross-reactivity, and neuronal protein variations that may ultimately contribute to the emergence of mental disorders, including psychosis.

Keywords: peptide sharing, HCMV, immune response, schizophrenia, cross-reactivity

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INTRODUCTION

Newly generated neurons migrate from their site of origin to specific brain areas and subregions, a process that involves adaptation with different degrees of complexity (1, 2). The cytoskeleton is regulated at the molecular and cellular level to execute neuronal migration (3); polarity in migrating neurons is reached by re-purposing of cytokinetic processes (4, 5); and blood vessels are used as a

physical substrate (6). Cell adhesion, cell cycle, and angiogenesis are implicated in neuronal migration.

Clinically, disruption of this process has been related not only to severe malformations of cortical development (lissencephaly, schizencephaly, neuronal eterotopia, polymicrogyria) (7) but also to psychosis (8–10). However, the relationship between macro- and microscopic structural brain anomalies and psychosis appears to be unclear, and disruption of cellular function has been hypothesized (11, 12). According to current opinion, more subtle alterations starting early during neurodevelopment can alter neural circuits and induce psychotic syndromes during adolescence or young adulthood (13). Indeed, altered migration and development of GABAergic cortical interneurons have been linked not only to schizophrenia but also to depression and anxiety disorders and seem to be strongly dependent on other neurotransmitter networks, such as dopaminergic and glutamatergic systems (14–16).

The present study focuses on HCMV infection as a potential link between neuronal migration and psychosis. On the one hand, it has been shown that herpesvirus infection of the developing brain can disturb migration of neuronal cells in animal models (17–19). On the other hand, HCMV has been discussed in the context of psychosis. Indeed, previous research has demonstrated that maternal HCMV infection and antibodies are associated with psychosis in the offspring (20), that infection during childhood is a risk factor for later psychosis (21), and that concurrent antibody titers are associated with psychosis-related symptoms (22–25). Epidemiological evidence is then not only suggestive of an association between HCMV and psychosis but also points to an influence of the infection on the early development of the central nervous system, possibly on neuronal migration.

Therefore, we here tried to elucidate the associations between HCMV infection, aberrant neuronal migration, and psychosis, building on previous research that had assessed peptide commonality and potential immune cross-reactivity between microbial and human proteins (26–31). More specifically, we investigated the peptide platform shared by HCMV and human proteins involved in neuronal migration.

METHODS

A set formed by primary amino acid (aa) sequences of human proteins involved in neuronal migration was retrieved from the UniProtKB Database (www.uniprot.org) (32). The protein library was obtained by separately searching for "neuron" AND "migration" as well as "neuronal" AND "migration" within the *Homo sapiens* proteins in the reviewed and annotated section of the UniProt database. Duplicates were removed. The procedure yielded 373 protein sequences that are described in **Supplemental Table S1**. Human proteins are expressed as UniProt entry names, if not discussed in detail.

Proteins from HCMV (human herpesvirus 5, Tax Id: 295027; 168 proteins) were dissected into heptapeptides overlapped by six residues (that is, MPATDTN, PATDTNS, ATDTNST, TDTNSTH, and so forth). Then, each viral heptapeptide served as a probe to screen the library for exact matches within the proteins related to neuronal migration.

The viral heptapeptides shared with the neuronal migration-associated proteins were successively analyzed for occurrences in the entire human proteome using the Peptide Match program (https://research.bioinformatics.udel.edu/peptidematch/index.jsp) (33). The 373 human proteins listed in **Supplemental Table 1** were filtered out.

The Immune Epitope Database (IEDB; www.iedb.org) resource (34) was used to investigate the immunological potential of the peptide sharing among HCMV and human proteins related to neuronal migration. Only epitopes that had been experimentally validated as immunopositive in the human host were considered.

RESULTS AND DISCUSSION

Heptapeptide Sharing Between HCMV and Human Proteins Related to Neuronal Migration

Following the procedure described under *Methods*, we found that 41 HCMV heptapeptides are repeatedly distributed among 26 proteins associated with neuronal migration (see **Table 1**). An example of potential neuropathological relevance is the protein expression level in the hippocampus, a brain region where neurogenesis occurs in the adult stage.

The viral versus human peptide sharing displayed in **Table 1** is specific, unexpected, intensive, and endowed with an immunologic potential, as outlined in the following paragraphs.

Specificity: The shared heptapeptides found in this analysis are, in general, scarcely represented in the entire human proteome assumed as a control (see **Table 1**, 1st column). In other words, most of the matches illustrated in **Table 1** do not reflect an unspecific viral heptapeptide over-representation throughout the human proteome. Extreme examples for the specificity of the heptapeptide overlap are the sequences AVENGDS, DRGGGGG, INKRVKR, KPGASAA, LKPGASA, QTVTSTP, SSSSTSH, and YQRFLRE that are uniquely present in proteins related to neuronal migration and absent in the remaining human proteins (see **Table 1**). Actually, the heptapeptides AVENGDS, DRGGGGG, INKRVKR, KPGASAA, LKPGASA, QTVTSTP, SSSSTSH, and YQRFLRE are HCMV molecular signatures of the human proteins associated with neuronal migration SAV1, SHH, MAGI2, SMAD2, SMAD3, ULK1, and ACK1, respectively. Exceptions to such a specific sharing are represented by simple aa repeats such as EEEEEED. GGGGGGG, SSSSSSS, AAAAAAA, and EEEEEEE, known for being common in eukaryotic proteomes (36, 37).

Unexpectedness: The heptapeptide sharing between HCMV and human proteins associated with neuronal migration is largely unexpected in light of the fact that the probability of finding the same heptapeptide fragment in two proteins is 1 out of 20^7.

Intense peptide sharing: The overlap is not just extensive by affecting many of proteins examined, but also intensive, meaning that, in spite of the low probability, many of the proteins

TABLE 1 | Heptapeptide sharing between HCMV and human proteins related to neuronal migration.

HCMV heptapeptide[1]	Occurrences in the human proteome[2]	Occurrences in the set of proteins related to neuronal migration[3]	Human proteins related to neuronal migration[4,5]		
			UniProt Name	Cellular location[6]	Protein expression in the hippocampus[7,8]
AVENGDS	0	1	SAV1	I	I
DRGGGGG	0	1	SHH	I	–
KPGASAA	0	1	MAGI2	I	M
LKPGASA	0	1	MAGI2	I	M
LLLPPPS	0	1	ACK1	I	M
QTVTSTP	0	2	SMAD2	I	h
			SMAD3	I	m
STTAAAA	0	1	BARH2	I	–
YQRFLRE	0	1	ACK1	I	M
AAGPPEA	1	1	CAC1B	M	I
RRERERR	1	1	CAC1B	M	I
SGLGDLS	1	1	AP2A	I	I
TDSSLEA	1	1	MK10	I	M
PPAPRGP	2	1	RTN4	I	h
SGSSASS	2	1	LMNA	I	h
SSGSSAS	3	1	LMNA	I	h
SAVAAAA	4	1	SOX1	I	–
SEEEDDD	5	1	TOP2B	I	h
SGGAGGG	5	1	SMAD2	I	h
DNLTLWT	6	1	1433E	I	h
LAVADLL	11	2	5HT2B	M	nd
			DRD2	I	m
EDDDDDD	21	1	FGFR1	I	M
AAAAASS	24	1	SOX1	I	–
SSGGGGG	26	1	ALK	I	h
EEEDDDD	27	1	APBB1	I	M
AAAAAAP	30	2	CADM1	I	nd
			VAX1	I	–
DDDDDDD	30	1	FGFR1	I	M
QQPPPPP	33	1	BARH2	I	–
GAGGGGG	40	1	SOX1	I	–
AVAAAAA	43	1	SOX1	I	–
EEEEEDD	47	1	APBB1	I	M
VAAAAAA	51	1	SOX1	I	–
AGGGGGG	56	2	ALK	I	h
			SOX1	I	–
GGGGGGA	62	2	ALK	I	h
			SOX1	I	–
AAAAAAS	70	1	SOX1	I	–
QPPPPPP	70	1	BARH2	I	–
SAAAAAA	72	1	VAX1	I	nd
EEEEEED	140	3	ndF4	I	nd
			PAK3	I	–
			RTN4	I	h
GGGGGGG	170	2	ALK	I	h
			SOX1	I	–
SSSSSSS	173	1	ULK1	I	–
AAAAAAA	258	4	BARH2	I	–
			CADM1	I	nd
			SOX1	I	–
			VAX1	I	nd
EEEEEEE	301	4	CELR2	M	M
			NDF4	I	nd
			PAK3	I	–
			RTN4	I	h

[1]*HCMV heptapeptide sequences in 1-letter aa code.*

[2]*HCMV heptapeptide occurrences in the human proteome, with proteins related to neuronal migration (**Table S1**) filtered out.*

[3]*HCMV heptapeptide occurrences in human proteins related to neuronal migration.*

[4]*Human proteins related to neuronal migration and sharing HCMV heptapeptide(s). Proteins indicated according to UniProtKB entry name.*

[5]*Data from the Human Protein Atlas (35).*

[6]*I, intracellular; M, membrane.*

[7]*Expression level: nd, not detected; l, low; m, medium; h, high.*

[8]*Data pending.*

associated with neuronal migration share more than one HCMV heptapeptide. An example is the human transcription factor SOX1 that shares 10 heptapeptides with HCMV (see **Table 1**). Of note, the 10 viral heptapeptide matches that are disseminated along the SOX1 primary amino acid consecutively overlap to form long peptide stretches which may be targeted by anti-HCMV immune responses (see **Figure 1**).

Immunological potential: Finally, many of the heptapeptides shared between HCMV and the 26 human proteins related to neuronal migration are endowed with an immunologic potential by being part of epitopes that have been experimentally validated as immunopositive in humans (see **Table 2**).

Immunological Relevance of the Heptapeptide Sharing Between HCMV and Human Proteins Associated With Neuronal Migration

Tables 1 and **2** support the possibility that immune responses against HCMV may cross-react with brain proteins involved in neuronal connectivity, synaptogenesis, and transmitter networks. Although the protein cell location is mainly intracellular (see **Table 1**), proteins involved in the viral overlap nonetheless remain fully accessible to immune cross-reactions, given the availability of intracellular antigens to the immune system, which is a well-known phenomenon (38, 39). Immune cross-reactions with these proteins can (1) impair brain development, structure, and function; (2) alter cognitive processes and behavior; and (3) be involved in complex mental disorders: in particular, disorders from the psychotic spectrum.

Indeed, examples are, *inter alia*:

1. BarH-like 2 homeobox protein (BARH2) and sonic hedgehog protein (SHH) contribute to establish the positional identities of progenitor cells in the diencephalon (40), while alterations of BARH2 and SHH can affect cerebellum development (41, 42). Notably, reduced cerebellar volume has been reported in first-time psychotic episodes (43).
2. Ventral anterior homeobox 1 (VAX1) is a transcription factor, and its deficit causes severe depletion of GABAergic neurons in the neocortex (44), thus possibly

MYSMMMETDLHSPGGAQAPTNLSGPA**GAGGGGGGGGGGGGGGA**KA
NQDRVKRPMNAFMVWSRGQRRKMAQENPKMHNSEISKRLGAEWKVM
SEAEKRPFIDEAKRLRALHMKEHPDYKYRPRRKTKTLLKKDKYSLA
GGLLAAGAGGGGAAVAMGVGVGVGAAAVGQRLESPGGAAGGGYAHV
NGWANGAYPGS**VAAAAAAAA**MMQEAQLAYGQHPGAGGAHPHAHPAH
PHPHHPHAHPHNPQPMHRYDMGALQYSPISNSQGYMSASPSGYGGL
PYG**AAAAAAAAA**GGAHQN**SAVAAAAAAAAASS**GALGALGSLVKSEP
SGSPPAPAHSRAPCPGDLREMISMYLPAGEGGDP**AAAAAAAA**QSRL
HSLPQHYQGAGAGVNGTVPLTHI

FIGURE 1 | Distribution of overlapping HCMV heptapeptides through SOX1 primary aa sequence. HCMV peptide sequences are highlighted.

TABLE 2 | Immunopositive epitopes containing heptapeptides shared between HCMV and human proteins associated with neuronal migration.

IEDB ID[1]	Epitopes[2,3]	IEDB ID[1]	Epitopes[2,3]
71055	vsnappvaspsiLKPGASAA	512030	asggAAAAAAAPaap
424109	AVENGDSgsryyy	515004	epAAAAASSacaapsq
429240	aSAAAAAAAAlly	516191	gAAAAAAAPaapaapr
432006	qtdprAGGGGGGdy	516566	GGGGGGAaaagray
433931	fvrepedEEEEEEEEED	517250	gptGGGGGGGfntvgr
440752	srevftSSSSSSS	518048	hqpsasggAAAAAAAPa
440782	sSSGGGGGGGrfssssgy	519007	ipSAAAAAAAAgria
441180	tSSSSSSSrqtrpilk	519995	kkwenEEEEEEEEqppp
456753	mAAAAAAAPs	521695	lppkpgtmEEEEEDDdy
457859	QPPPPPPpm	525008	rlAAAAAAAqsvy
465590	glAAGPPEA	525963	sggAAAAAAAPaapa
466037	gprpAAAAAAAtpav	530324	ypdppgtmEEEEEDDd
474480	AAAAAAAqsvy	541856	esnGGGGGGGAgsgggp
483230	qeSAAAAAA	542212	gaavVAAAAAASm
510536	AAAAAAAAPaaaat	542215	GAGGGGGeagagggaaava
510982	AGGGGGGAaaagray	544474	pQPPPPPPp

[1]*Epitope IEDB IDs are listed according to numerical order. Further details and references are reported in http://www.iedb.org/.*

[2]*Epitope peptides are given in one-letter codes.*

[3]*Epitope fragments shared between HMCV and human proteins associated with neuronal migration are indicated in capital letters.*

triggering the emergence of disorders within the psychotic spectrum. Indeed, a deficit in GABAergic system is one of the predominant pathophysiological features in psychotic disorders (45–47).

3. Fibroblast growth factor receptor 1 (FGFR1) may be involved in aberrant dopaminergic firing in psychotic disorders. Altered FGFR1 affects development and function of dopamine neurons, resulting in psychotic disorders in transgenic mice (48).
4. Cell adhesion molecule 1 (CADM1) expression has been detected in glutamatergic neurons, including the granule cells of the dentate gyrus, the pyramidal cells of the CA1 and CA3 regions (namely, in parvalbumin-positive neurons in the CA3 region), and in a subset of GABAergic neurons in the hippocampus (49, 50).
5. The 1433E epsilon protein (1433E or tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein [YWHAE]); 5-hydroxytryptamine receptor 2B (5HT2B or serotonin receptor 2B); and dopamine D2 receptor (DRD2) are three proteins, that—when altered—appear to be involved in the genesis of psychotic disorders. Actually, theories on potential causes of psychotic disorders assign a causal role to altered serotonin and dopamine neurotransmission (51–58). Specifically, the HCMV peptide sharing with 5HT2B and DRD2 consists of the heptapeptide LAVADLL (**Table 1**). The HCMV LAVADLL peptide is present in the transmembrane domain 2 (TMD2) of 5-HT2B and is involved in the interaction with TMD7 that allows the human 5-HT2B to adopt a conformation able to bind the neurotransmitter serotonine (59). Moreover, the LAVADLL sequence is endowed with an immunogenic potential by being part of the epitope KLAVADLEK (IEDB ID: 213202), derived from human centromere protein F (aa pos 557–565) (60). Therefore, cross-reactions targeting LAVADLL may hit multiple proteins involved in neurotransmission as well as centriolar proteins involved in brain malformations (microcephaly and ocular anomalies) (61).
6. The transcription factor Sex-determining Region Y-related HMG-box 1 (SOX1) is uniquely expressed at a high level in the majority of telencephalic neurons that constitute the ventral striatum (62), a brain area closely associated with decision making and belonging to the reward-salience circuitry (i.e., ventral striatum, dorsal caudate, and anterior cingulate cortex) (63–65). SOX1 regulates the neural primordia and promotes neurogenesis not only by acting as a transcription factor but also by forming protein-protein interactions through its COOH-terminus (66). Of note, the HCMV *versus* SOX1 peptide overlap is mainly allocated in the COOH-terminus (**Figure 1**). Consequently, cross-reactions targeting the SOX1 C-terminus may have multiple pathologic consequences, from disruption of the molecular network underlying neurodevelopment to alteration of specific neural circuits that produce complex behavior.
7. The anaplastic lymphoma kinase (ALK) protein is a tyrosine kinase receptor that, when altered, is involved in psychotic disorders (67, 68) and in neuroblastoma, a common neoplasm of early childhood that arises from cells of the primitive neural crest, giving rise to the adrenal medulla and the sympathetic nervous system (69).
8. The serine/threonine-protein kinase (PAK3) (also known as oligophrenin-3) plays a role in dendrite spine morphogenesis as well as synapse formation and plasticity, and its dysregulation may lead to synaptic deficits in psychotic disorders (70–72).
9. The reticulon-4 (RTN4) protein is implicated in the stabilization of wiring and restriction of plasticity in the adult central nervous system (73). RTN4 is differentially expressed in the dorsolateral prefrontal cortex from individuals with psychotic disorders (74).
10. MAGI2 is a membrane-associated guanylate kinase that acts as a scaffold molecule at synaptic junctions by assembling neurotransmitter receptors and cell adhesion proteins. MAGI2 seems to be involved in psychotic disorders (75–77).
11. The brain calcium channel III or voltage-dependent N-type calcium channel subunit alpha-1B (CAC1B) may have a key role in etiology of bipolar disorder and psychosis (78).

The variety of proteins involved in peptide sharing with HCMV presented here is consistent with the complex multifactorial nature of mental disorders in general, and psychosis in particular. These proteins were examined in the present study in light of their involvement with neuronal migration, while it is highly likely that any alteration of their function or structure may affect higher cognitive processes through impairment of different mechanisms above and beyond migration (i.e., axon guidance, neurotransmission, excitatory-inhibitory balance, oscillatory neuronal firing, and others). Notably, these mechanisms can also be directly affected by cross-reactive targeting of proteins allowing membrane excitability (26–30), in a complex endotypical scenario that mirrors the phenotypical complexity of mental disorders without the need for a biunivocal match between them.

CONCLUSIONS

The present study demonstrates that numerous human proteins related to neuronal migration are involved in a specific heptapeptide overlap with HCMV. Such a wide peptide sharing supports the possibility that, following HCMV active infection, anti-HCMV human immune responses may cross-react with proteins involved in peptide sharing with the herpesvirus. In the case of cross-reactions, neuropathological consequences might include the development of mental disorders, such as psychotic syndromes. In fact, the 26 human proteins listed in **Table 1** hold the key to specifying brain processes, such as neuronal connectivity, synaptogenesis, and neurotransmission in a prolonged temporal window that runs

from fetal-early postnatal neurodevelopment to adult neurogenesis. In the context of peptide sharing described here, GABAergic and glutamatergic circuitry might play a central role, with disturbances potentially leading to psychotic syndromes by altering excitatory-inhibitory balance in oscillating brain networks underpinning higher cognitive functions (79–84). Different strategies could allow to test this hypothesis *in vivo*. Observationally, sera from human patients suffering from psychotic disorders might be examined for immunoreactivity against the sequences analyzed here. Causally, animal models of neuropsychiatric disorders might be obtained by immunizing pregnant and young animals with the same sequences.

DATA AVAILABILITY STATEMENT

All datasets analyzed for this study were retrieved from publicly accessible curated databases: UniProtKB (http://www.uniprot.org/), The Immune Epitope Database (IEDB; http://www.iedb.org/), and the Human Protein Atlas (https://www.proteinatlas.org/).

AUTHOR CONTRIBUTIONS

GL formulated the hypothesis, analyzed the data, and wrote the manuscript. GL, AF, and BS interpreted the data and revised and finalized the manuscript.

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SUPPLEMENTARY MATERIAL

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REVIEW
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The Inflamed Brain in Schizophrenia: The Convergence of Genetic and Environmental Risk Factors That Lead to Uncontrolled Neuroinflammation

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Schizophrenia is a disorder with a heterogeneous etiology involving complex interplay between genetic and environmental risk factors. The immune system is now known to play vital roles in nervous system function and pathology through regulating neuronal and glial development, synaptic plasticity, and behavior. In this regard, the immune system is positioned as a common link between the seemingly diverse genetic and environmental risk factors for schizophrenia. Synthesizing information about how the immune-brain axis is affected by multiple factors and how these factors might interact in schizophrenia is necessary to better understand the pathogenesis of this disease. Such knowledge will aid in the development of more translatable animal models that may lead to effective therapeutic interventions. Here, we provide an overview of the genetic risk factors for schizophrenia that modulate immune function. We also explore environmental factors for schizophrenia including exposure to pollution, gut dysbiosis, maternal immune activation and early-life stress, and how the consequences of these risk factors are linked to microglial function and dysfunction. We also propose that morphological and signaling deficits of the blood-brain barrier, as observed in some individuals with schizophrenia, can act as a gateway between peripheral and central nervous system inflammation, thus affecting microglia in their essential functions. Finally, we describe the diverse roles that microglia play in response to neuroinflammation and their impact on brain development and homeostasis, as well as schizophrenia pathophysiology.

Keywords: neuroinflammation, microglia, schizophrenia, genes, Environment, risk factors, brain development, neurodevelopmental

INTRODUCTION

Schizophrenia (SCZ) is a prevalent mental illness without satisfactory treatment options. Approximately 20 million people worldwide are afflicted by this chronic and debilitating mental disorder (American Psychiatric Association, 2013; Whiteford et al., 2013). SCZ is characterized by a broad range of clinical manifestations including hallucinations, social and cognitive impairments, as well as disordered thinking and behavior that impair daily functioning (American Psychiatric Association, 2013). Current treatment options do not improve cognitive or negative symptoms, both of which contribute more significantly to the long-term prognosis of SCZ than positive symptoms (Lieberman et al., 2005; Green, 2006). More effective therapies for SCZ have lagged due to a lack of understanding of its underlying mechanisms.

Genome-wide association studies (GWAS) have identified novel susceptibility loci that confer greater risk for SCZ (Ripke et al., 2013; Li et al., 2017). These breakthroughs have enabled the characterization of genes that may shed light on the pathophysiology of SCZ. In addition, much progress has been made in preclinical studies focusing on environmental risk factors for SCZ and other neurodevelopmental disorders (NDD) that alter brain development such as psychosocial stress, maternal immune activation (MIA), and exposure to pollution (Bergdolt and Dunaevsky, 2019; Gomes et al., 2019; Horsdal et al., 2019). Although there are a multitude of genetic and environmental factors conferring increased risk for SCZ, recent work suggests that these factors converge by altering immune processes, which are known to play an essential role in shaping brain development (Müller and Schwarz, 2010; Stephan et al., 2012; Kroken et al., 2018). Indeed, elevated immune function and chemokine responses are found in SCZ and therapeutics that target immune function have shown some success in symptom reduction (Sommer et al., 2014; Frydecka et al., 2018; Kroken et al., 2018). Importantly, subclinical inflammation correlates with cognitive deficits in SCZ (Misiak et al., 2018), which are a critical determinant for the long-term prognosis of this disease. It is unclear how immune molecules regulate synaptic wiring during normal brain development and contribute to synaptic pathology in neuropsychiatric disorders. Causal links between specific immune molecules and altered synaptic connectivity within circuits implicated in neuropsychiatric disorders are currently lacking (Elmer and McAllister, 2012).

Microglia are central nervous system (CNS) phagocytes that, among their other roles, orchestrate innate immunity in the brain. Microglia have well-described roles in rapidly responding to inflammatory insults through dynamic surveillance of the CNS parenchyma (Nimmerjahn et al., 2005; Liu Y. U. et al., 2019) and clearing debris and apoptotic cells through phagocytosis (Ayata et al., 2018; Galloway et al., 2019). Recent studies have begun to uncover the diversity of microglia, which can have significantly different gene expression patterns across brain regions, in health and in pathological states, and at different developmental time points (Tay et al., 2017a; Hammond et al., 2019; Sankowski et al., 2019; Tan et al., 2020). These complex cells contribute to normal brain development and function by supporting the neuronal circuitry through synapse addition, elimination, maintenance, and plasticity (Hammond et al., 2018; Bohlen et al., 2019). Despite variability in the findings of several studies, there is evidence of microglial dysfunction in SCZ (Bayer et al., 1999; Hercher et al., 2014; Bloomfield et al., 2016; Trépanier et al., 2016; De Picker et al., 2017; Sellgren et al., 2019; Uranova et al., 2020). A key element to understand the pathogenesis of SCZ is to discern how genetic and environmental risk factors intersect to alter microglial function given. Furthermore, outstanding questions that remain to be answered are at what stage(s) of disease progression microglial function ameliorates or contributes to the pathology of SCZ, and what are the particular subtypes or phenotypes of microglia that could be targeted for therapeutic intervention.

In this review, we discuss the genetic and environmental risk factors for SCZ and how they converge to alter microglial function in response to systemic and central inflammation. Additionally, we highlight how these risk factors alter the indispensable functions of microglia during development, adolescence and adulthood. Limitations of the current knowledge are also addressed, and key future experiments are proposed. Understanding how the heterogeneous genetic and environmental risk factors for SCZ interact to reach a disease threshold and determine its progression is necessary for the development of more effective therapeutics.

GENETIC RISK FACTORS THAT INTERPLAY WITH IMMUNOLOGICAL RESPONSES

Schizophrenia is driven by genetic factors, as the risk for developing this disorder increases from 1% in the general population to 50% in individuals with a diagnosed twin (Cardno and Gottesman, 2000; Stefansson et al., 2009). Recent ground-breaking genome-wide association studies (GWAS) have made progress in discovering loci throughout the genome that are associated with SCZ (Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011; Ripke et al., 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Li et al., 2017; Dennison et al., 2019). These studies reveal that SCZ has a heterogeneous etiology, with genes likely conferring risk across the entire genome. This heterogeneity, in combination with environmental factors, has made it difficult to pinpoint which genes contribute to the disease pathology. Although the genetic determinants for SCZ are not well understood, evidence suggests that immune dysfunction and inflammation contribute to its pathophysiology (Trépanier et al., 2016; van Kesteren et al., 2017).

The major histocompatibility (MHC) locus is located on chromosome 6 and has the highest association to SCZ compared to any other loci across the genome (Shi et al., 2009; Stefansson et al., 2009; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). This region encodes genes that are involved in innate immunity. For instance, complement component 4A (*C4A*), located in the MHC locus, is highly associated with SCZ: specific structural variants and regulatory regions that increase the expression of *C4A* confer a greater risk

for SCZ (Sekar et al., 2016). The complement cascade is part of the innate immune system that recognizes foreign pathogens and apoptotic cells, and tags them for destruction, such as through phagocytosis by macrophages (Veerhuis et al., 2011). Besides their established role in immune defense, complement proteins play a role in various stages of brain development including neurogenesis, cellular migration and synaptic development (Veerhuis et al., 2011; Lee et al., 2019). Ground-breaking work in the last 5-10 years have linked complement proteins to microglia-mediated pruning of synapses, suggesting that *C4A* could directly contribute to SCZ pathology (Stevens et al., 2007; Schafer et al., 2012; Hong et al., 2016).

In line with this, it was recently shown that increased expression of the mouse homologue of *C4A, C4b,* in medial prefrontal cortex (mPFC) layer (L) 2/3 pyramidal neurons led to a marked reduction in connectivity and decreased sociability in juvenile and adult mice, both of which mirrored the deficits seen in SCZ (Comer et al., 2020). These results suggest that *C4A* might contribute directly to pathology in SCZ. Although, the molecular mechanisms that link increased *C4* expression to synaptic loss remain unclear, overexpressing this neuroimmune gene led to increased localization of the postsynaptic protein PSD-95 to microglial lysosomes, suggesting upregulated microglia-dependent synaptic engulfment (Comer et al., 2020). Additionally, variation in *C4* structural alleles increases risk for autoimmune diseases and indicate that sex-differences in the *C4* gene might explain greater vulnerability to SCZ in males (Kamitaki et al., 2020). In another study, C4 serum levels were assessed at baseline and in a 1-year follow-up in a cohort of twenty-five patients with first episode psychosis that were taking either olanzapine or risperidone (Mondelli et al., 2020). Compared with responders to antipsychotic medication, non-responders showed significantly higher baseline C4 levels, suggesting that baseline expression of this immune gene can predict clinical outcome (Mondelli et al., 2020). Since this study focused on a limited number of markers, it is not clear however how psychosis progression correlates with levels of other immune genes. Lastly, the gene 'CUB and sushi multiple domains 1' (*CSMD1*) is an important regulator of C4 that is expressed during early postnatal development (Kraus et al., 2006). Genetic variants located in the *CSMD1* and *CSMD2* genes have been linked to SCZ (Håvik et al., 2011) and their dysregulation led to deficits in general cognitive ability and executive function (Athanasiu et al., 2017), both of which are affected in SCZ. Conversely, a recent study showed that CSMD1 levels in the blood are decreased in SCZ, while antipsychotic treatment resulted in up-regulation of CSMD1 and improved cognitive symptoms (Liu Y. et al., 2019).

Transcriptomic and genomic studies have implicated alterations in key cytokines with SCZ, including increases in interferon regulatory factor 3 (*IRF3*) (Li et al., 2015), which is a major transcription factor in viral infection, and interferon gamma (*IFN-γ*), an important regulator of viral propagation (Paul-Samojedny et al., 2011). In support of neuroimmune genes altered in SCZ, other studies have found changes in pro-inflammatory interleukin 1 (*IL)-1α* (Katila et al., 1999), *IL-1β* (Katila et al., 1999; Sasayama et al., 2011), *IL-6* (Kalmady et al., 2014; Frydecka et al., 2015) and anti-inflammatory *IL-10* [reviewed in Gao et al. (2014)]. Several studies also investigated circulating C-reactive protein (CRP), IL-6, IL-1β, TNF-β, and TGF-β, which are also elevated at the mRNA level in people with SCZ, to determine their reliability as peripheral biomarkers (Kroken et al., 2018). However, other studies reported limited immune gene enrichment in SCZ (Pouget et al., 2016), highlighting the genetic complexity of the disease, in addition to possible variability between cohorts and confounding factors such as medication, among other challenges with GWAS.

Several GWAS have revealed that multiple immune receptors are associated with SCZ including the MHC receptors and Toll-like receptors (TLRs) (Purcell et al., 2009; Shi et al., 2009; Stefansson et al., 2009; Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). TLRs play a role in the recognition of microbe-derived molecular signals by innate immune cells including microglia [reviewed in Wright et al. (2001) and Lehner (2012)]. In addition to their established role in innate immunity, TLRs regulate early brain development (Mallard, 2012; Chen et al., 2019) via their effects on synaptic plasticity and neurogenesis (Barak et al., 2014). Other groups have shown alterations in TLR2 (Kang et al., 2013) and TLR4 (García-Bueno et al., 2016; MacDowell et al., 2017) in either the blood or post-mortem brain tissue of people with SCZ. Overall, these data have linked MHC signaling and other immune receptors pathway with the pathology of SCZ, However, the molecular underpinnings of their contribution to SCZ are not yet clear. It also still not understood how disruption in particular immune pathways contributes to specific cellular and behavioral hallmarks of this disorder, such as decreased gray matter volume.

To identify robust peripheral biomarkers that can predict SCZ pathology, researchers have compiled an architecture of genes observed in patients from multiple GWAS. A subset of overlapping genes from these studies identified candidates including *CD14, CLU, DPP4, EGR1, HSPD1, MHC* and *C4* genes (Pouget et al., 2016). Despite the identification of these candidate biomarkers, other studies highlight that the current literature does not provide sufficient evidence that increased inflammation is a hallmark of all SCZ cases (Kroken et al., 2018). Some studies have identified markers that are related to antigen presentation and immune activity (Pouget et al., 2016), whereas others have revealed changes in inflammatory cytokines (Hudson and Miller, 2018; Kroken et al., 2018). These studies together indicate that some cases or stages of SCZ may involve the innate and/or adaptive immune system. However, genetics only explains part of the susceptibility and pathophysiology of SCZ, which provides further support that environmental risk factors are also required to trigger the disease in most cases (Knuesel et al., 2014).

Lastly, SCZ-associated genes with diverse functions in the brain have also been implicated in inflammation (Brandon et al., 2009). For example, the gene Disrupted-in-Schizophrenia 1 (*DISC1*) was first found in a Scottish family with SCZ (St Clair et al., 1990) and subsequently in other populations worldwide (Chubb et al., 2008). Interestingly, the disruption of DISC1 protein in mice led to dysregulation of an immune-related network of genes that are perturbed in SCZ (Trossbach et al., 2019), suggesting that non-immune genes can modulate

the expression of inflammatory gene networks. In support of this, in a dual-hit genetic-environmental mouse model of SCZ, where *DISC1* mutation was combined with MIA, transient administration of minocycline, an anti-inflammatory antibiotic drug, rescued electrophysiological and structural deficits during early postnatal development, as well as cognitive abilities in juvenile mice (Chini et al., 2020). It is clear that the expression of hundreds of genes is altered in SCZ, although it remains to be determined how the interaction between immune and non-immune pathways is implicated in this disorder. Overall, growing evidence suggests that immune gene dysfunction and inflammation both contribute to the pathophysiology of SCZ (Trépanier et al., 2016; van Kesteren et al., 2017).

EXPOSURE TO POLLUTION CAUSES NEUROINFLAMMATION

The environment is becoming increasingly polluted from multiple sources. Traffic-related air pollution (TRAP), such as diesel exhaust (Inoue et al., 2006; Hartz et al., 2008; Block and Calderón-Garcidueñas, 2009; Bolton et al., 2017), is the result of the combustion of fossil fuels and can be modeled in the lab using elemental carbon (Newman et al., 2013) or by taking the finest particles (<200 nm) from TRAP and re-aerosolizing them into nanoparticulate matter (nPM). nPM is the most toxic component of TRAP, in terms of its impact on the brain (Davis et al., 2013). By-products of TRAP, such as ozone (O_3), which can be generated from nitrogen oxide, can also be changed photochemically after their release from motor vehicles (Mumaw et al., 2016). Altogether, multiple paradigms are currently used in animal models to study the effects of air pollution on brain development (Davis et al., 2013; Newman et al., 2013; Woodward et al., 2017; **Table 1**). This work is particularly relevant when considering the epidemiological studies that link air pollution to SCZ pathogenesis (Horsdal et al., 2019). Indeed, many of the genes altered in SCZ overlap with genes that are affected by exposure to air pollution (**Figure 1**). Interestingly, immune genes, including those expressed by microglia, are at the center of this interaction (Peters et al., 2006; Genc et al., 2012).

While the mechanisms involved in SCZ pathogenesis are still unclear, exposure to air pollution has been found to increase the expression of multiple inflammatory genes in humans and mouse models. Children exposed to TRAP have elevated circulating levels of pro-inflammatory cytokines, including IL-6, IL-1ß, CD14, and TNF-α, compared to children living in less-polluted cities (Calderón-Garcidueñas et al., 2008, 2015; Gruzieva et al., 2017). Additionally, nPM from air pollution induced a similar inflammatory cytokine signature in the circulation of healthy young adults, characterized by an elevation of IL-6, together with an increased density of inflammatory cells and microparticles, suggesting the occurrence of endothelial injury (Pope et al., 2016). In line with this, TRAP exposure in rodents increased IL-1α, IL-6 and TLR4 expression in the brain (Bos et al., 2012; Bolton et al., 2017). Pollution exposure especially impacted microglial TLR4 signaling in multiple mouse models involving TRAP (Woodward et al., 2017, 2018), O_3 (Mumaw et al., 2016), or diesel exhaust particle (Bolton et al., 2017; Bai et al., 2019) exposure, by upregulating TLR4 in a MyD88-dependent pathway (Woodward et al., 2017). Male offspring were especially susceptible to these deleterious effects, showing greater changes in microglial TLR4 signaling that were accompanied by behavioral deficits in anxiety-like behavior, contextual and auditory cue fear conditioning and the forced swim test (Bolton et al., 2012, 2013; Bolton et al., 2017). Prolonged exposure to inflammatory molecules, such as IL-6, additionally led to neuroadaptive effects such as altered synaptic plasticity (Gruol, 2015). Therefore, exposure to pollution could alter brain development and function by causing increased expression of pro-inflammatory markers, a feature on which MIA models of SCZ rely on (Girgis et al., 2014).

TRAP alters brain development and increases the risk for SCZ (Pedersen et al., 2004; Woodward et al., 2015), however it is unclear if pollution-mediated changes in brain development or inflammatory signaling directly contribute to pathology. Recent work has studied the effects of chronic nPM exposure using a double-hit model where cortical neuronal cultures from exposed mice were re-exposed in culture. A double exposure to nPM reduced neurite outgrowth (Davis et al., 2013) while resulting in an inflammatory transcriptomic profile (Solaimani et al., 2017) in neuronal cultures. Another group showed that TRAP can reduce hippocampal neurogenesis by 70% in rats, which correlated with behavior deficits in object recognition, food-seeking behavior, and in the forced swim test (Woodward et al., 2018). These phenotypes were reproduced in mice using elemental carbon exposure (Morris-Schaffer et al., 2019). In the mouse brain, nPM exposure induced neuroinflammation evident through a microglia-mediated increase in TNF-α (Cheng et al., 2016). Furthermore, exposure to nPM led to altered microglia morphology and elevated levels of C5, C5a, and CD68 proteins, indicative of increased phagocytic activity, in the corpus callosum (Babadjouni et al., 2018) a region that is particularly reduced in volume in SCZ patients (Kubicki et al., 2005). Other work has highlighted the neurotoxicity of ultrafine particles (UFP), which induced pro-inflammatory signaling and lead to a long-lasting reduction of corpus callosum volume (Allen et al., 2017). Overall, neuroinflammation induced by pollution appears to have a substantial impact on the brain by altering axonal myelination (Cole et al., 2016). However, it is still unclear to what extent pollution-driven inflammation, compared to other risk factors, drives myelination deficits in SCZ. Taken together, the inflammatory state caused by exposure to air pollution has been shown to alter microglial function and neuronal development, as well as axonal myelination, thus affecting several processes of neurodevelopment that have been linked to SCZ pathogenesis.

THE GUT-BRAIN AXIS IN SCZ

The CNS communicates bi-directionally with the gastrointestinal (GI) system to maintain homeostasis, for instance by regulating hunger and digestion processes at steady state (Konturek et al., 2004). There has been extensive study of the reciprocal gut and CNS interactions, which communicate through the enteric nervous system and vagus nerve, and via alternative pathways

TABLE 1 | Overview of the effects of different pollutants on neuroinflammation.

Pollutant Expourse	Species	Age	Sample measurements	Phenotype	Articles
NO2 and PM	humans	8 years old (exposure in infancy)	serum levels	increase in IL-6 and IL-10 2017	Gruzieva et al., 2017
CO, NOx, NO2, and benzene	humans	longitudinal- from childhood to adulthood	SCZ diagnosis	increased risk of developing SCZ (only for exourse to benzene and CO	Pedersen et al., 2004
NOx, NO2 and PM	humans	longitudinal- from childhood to adulthood	Presense of psychosis	increased odds of psychotic experiences (only for exposure to NO2 and NOx)	Newbury et al., 2019
PM (2.5 um)	humans	young adults	plasma levelsapoptosis	apoptosis of endothelial cells, increased levels of circulating monocytes and T-cells, increased proinflammatory cytokines (IL-6, IL-1β, MCP-1, and MIP-1)	Pope et al., 2016
DEP	mice	embryonic (E18) to young adulthood (P30) (prenatal exposure)	cytokine ELISAs and IHC from hippocampus and parietal cortex	increased cytokines and altered morphology of microglia in male mice dependent on TLR4 signaling; altered cortical volume; increased microglia-neuron interactions in males	Bolton et al., 2012, 2017
DEP	mice	adults	olfactory bulb and hippocampus protein levels	increased lipid peroxidation and pro-inflammatory cytokines (IL-1α, IL-1β, IL-3, IL-6, and TNF-α). Increased expression of Iba1 and TSPO	Cole et al., 2016
DEP	mice	adult (prenatal exposure)	behavioral, brain protein and mRNA levels from PCF, HPC, hypothalamus and parietal cortexi	increased anxiety behaviors; Increased IL-1β and TLR4 in males and decreased IL-10	Bolton et al., 2013
nanoscale PM (<0.2 um)	mice	adults	neonatal cortical neurons	impaired neurnal differentiation; increased depressive behaviors	Davis et al., 2013
nanoscale PM (<0.2 um)	mice	adults	corpus callosum protein levels	increased complement protein deposition (C5, C5a and CD88) in brain but not serum; altered microglial morphology	Babadjouni et al., 2018
PM	mice	juvenile mice (postnatal day 11-15) (prenatal exposure)	cerebellum myelin density, cerebellum iron levels, RNAseq of cerebellum	increased inflammation signaling; increased iron inclusions; myelin sheath damage	Klocke et al., 2018
PM (2.5 um)	mice	adults (exposure in utero)	western blot, ELISA and IHC in temporal cortex	deficits in spatial memory; increase in COX2 and Arg1 protein, increase in GFAP reactivity, decreased cytokines levels in temporal cortex (IL-1α, IL-2, IL-4 IL-6, IL-10, IFN-γ, GM-CSF and TNF-α) and spleen (IL-2,IL-6, IL-10 and TNF-α)	Kulas et al., 2018
ultrafine elemental carbon	mice	adults (neonatal exposure)	behavioral assays, protein expression in the corpus callosum and ventricles	no changes observed in locomotion, learning, memory, impulsivity or anxiety behaviors, no changes in GFAP or MBP	Morris-Schaffer et al., 2019
ultrafine PM	mice	juvenile and early adulthood	hippocampus and amygdala transcript and protein, corpus callosum IHC, behavioral measures	lateral ventricle dilation; changes in cytokines, neurotransmitters and microglia activation markers in sex-dependent manner, hypomyelination, elevated glutamate, increased repetitive and impulsive behaviors	Allen et al., 2014, 2017
nanoscale PM (<0.2 um)	rats	gestation to adulthood	behavioral, protein levels in the adult hippocampus	70% decrease in adult hippocampal neurogenesis, 35% increase in Iba1 in the dentate gyrus; 75% decrease in tight junction protein of the BBB; impaired contextual memory, food-seeking and depressive-like behaviors	Woodward et al., 2018

(Continued)

TABLE 1 | Continued

Pollutant Expourse	Species	Age	Sample measurements	Phenotype	Articles
nanoscale PM (<0.2 um)	rats/mice	*in vitro*: postnatal day 3, *in vivo*: adult	glial transcript measurements in culture (rat mized glial cultures) and hippocampus (mice)	TLR4-mediated increase in 2000 transcripts related to neuroinflammation and stress	Woodward et al., 2017
carbon black and DEP	rats/mice	adults	cultured microglia (mice BV-2 cells) and hippocampus (rat) protein levels	increased IL-6, TNF-a, and Iba1, increased caspase-3 mediated autophagy in microglia	Bai et al., 2019
ozone (O3), mixed vehicle exhaust	rats/mice	young and aged adults	serum levels	increased microglial proinflammatory response especially pronounced in aging mice	Mumaw et al., 2016

PM: particulate matter, SCZ: schizophrenia, ELISA: enzyme-linked immunosorbent assay, IHC: immunohistochemistry, PFC: prefrontal cortex, HPC: hippocampus, mRNA: messenger RNA, IL: interleukin, TSPO: translocator protein, Iba: Ionized calcium binding adaptor molecule 1, TLR: toll-like receptor, TNFα: tumor necrosis factor alpha, MCP-1: monocyte chemoattractant protein-1, MIP-1: macrophage inflammatory protein 1, GFAP: glial fibrillary acidic protein, CD88: cluster of differentiation 88, COX-2: cyclooxygenase-2, IFNγ: interferon gamma, gm-csf: granulocyte-macrophage colony-stimulating factor, C: complement cascade, BBB: blood brain barrier.

involving the immune and neuroendocrine systems (Sudo et al., 2004; Sampson et al., 2016; Singh et al., 2016) or through direct secretion by gut microbes of neurotransmitters (Yano et al., 2015) and metabolites (De Vadder et al., 2014; Sherwin et al., 2019). However, the importance of the gut in mediating brain function and behavior was ignited by the discovery that germ-free mice, which are devoid of microorganisms, have heightened stress responses (Sudo et al., 2004). In more recent work, the microbiota has been shown to influence complex behaviors such as social behavior, depression, and anxiety which are directly relevant to SCZ and other neuropsychiatric disorders (Desbonnet et al., 2014; Sherwin et al., 2019). Additionally, 19% of people with SCZ are comorbid for irritable bowel syndrome, which has a known inflammatory etiology (Gupta et al., 1997) compared to an occurrence rate of only 2.5% in the general population. Studying the role of the microbiota in disease states is challenging since it is highly sensitive to environmental changes. Therefore, most of the environmental risk factors for SCZ also impact the microbiota (Franklin and Ericsson, 2017), making it difficult to determine causation. However, recent work suggests a causative role for the microbiota in neuropsychiatric disorders and highlights the role of the immune system in linking the brain and gut in pathological conditions (Castro-Nallar et al., 2015; Yolken et al., 2015; Schwarz et al., 2018; Zheng et al., 2019; Zhu et al., 2019).

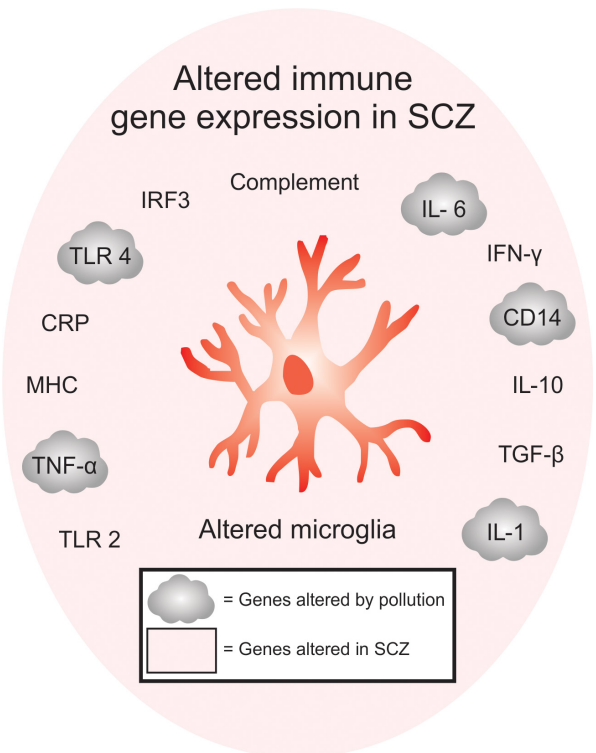

FIGURE 1 | Overlap between immune-signaling genes that are associated with pollution and SCZ. Of the immune genes that are associated with SCZ, many are also found to be altered either in humans or animals exposed to pollutants, offering a genetic point of convergence between changes in pollution-mediated inflammatory signaling and SCZ. Inflammatory gene expression, including TLR2, TNF-α, MHC, CRP, TLR4, IRF3, complement pathway, IL-6, IFN-γ, CD14, IL-10, TGF-β are altered in SCZ, while TNF-α, TLR4, IL-6, CD14 and IL-1 are altered in SCZ and after exposure to air pollution. These genes are specifically enriched in microglia.

The microbiota not only plays a key role in regulating host metabolism but also modulates inflammatory responses and neural function. Germ-free mice have multiple deficits in nervous system function including heightened hypothalamic-pituitary-adrenal (HPA) axis responses (Sudo et al., 2004), altered anxiety-like behaviors (Neufeld et al., 2011), increased motor activity (Diaz Heijtz et al., 2011), and impaired memory (Gareau et al., 2011), and social behaviors (Desbonnet et al., 2014). In healthy individuals, increased HPA axis function is generally associated with a suppression of subclinical inflammation due to the anti-inflammatory properties of glucocorticoids (Barnes, 1998). However, the ability of cortisol to suppress inflammation might be altered in SCZ, instead correlating with increased inflammation evident by an increase in IL-6 (Chiappelli et al., 2016).

The microbiota of people with SCZ has been found to contain more of the bacterial species *Lactobacillus* compared to healthy controls, and levels of this bacterium correlate with psychosis severity (Castro-Nallar et al., 2015; Yolken et al., 2015; Schwarz et al., 2018). In a recent study, gut microbiota from SCZ

patients was transferred into germ-free mice to test whether SCZ-relevant behavioral phenotypes were transmissible via their gut microbiome. Germ-free mice receiving fecal transplants from these patients had lower levels of glutamate and higher levels of glutamine and GABA in the hippocampus, and these mice exhibited locomotor hyperactivity and decreased anxiety-like and depressive-like behaviors, as well as increased startle responses relative to control mice that received fecal transplants from healthy subjects (Zheng et al., 2019). However, SCZ patients in this study were receiving antipsychotic treatment, which has been shown to alter the gut-microbiome (Bretler et al., 2019) so this could be a confounding effect. Transplantation of the gut microbiome from drug-free individuals with SCZ into antibiotic-treated mice caused SCZ-related phenotypes such as impaired learning and memory as well as increased psychomotor behaviors, while also leading to increased PFC dopamine and hippocampal serotonin levels compared to mice receiving microbiota transplants from healthy controls (Zhu et al., 2019), suggesting drug-independent effects of the gut-microbiome in SCZ.

Microbes are able to produce or aid in the production of multiple neurotransmitters, including serotonin, dopamine and GABA, but it is still unclear how the gut production of these neurotransmitters affects CNS function (Yano et al., 2015; Strandwitz, 2018). Additionally, gut microbiome transplantation or treatment with probiotics has been shown to, at least partially, reverse MIA-associated phenotypes in rodents, including deficits in anxiety-like, stereotypic and sensorimotor behaviors (Hsiao et al., 2013). The reversal of these phenotypes seems to be mediated through the normalization of gut permeability and microbe dysbiosis (Hsiao et al., 2013), suggesting that the gut microbiota can directly modulate immune responses even between a dam and its embryo. This is not surprising given that the microbiota has a well-studied role in inducing and maintaining the function of the host immune system.

The gut microbiome can affect the integrity of the blood-brain barrier (BBB), which facilitates increased neuroinflammation. The presence of gut microbes is necessary for the proper formation of the BBB during early development. Mice from germ-free dams have disrupted BBB maturation, which is evident by decreased tight junction expression both prenatally and postnatally. The hyperpermeability of the BBB in germ-free mice persists into adulthood, but can be rescued by microbiota transplantation from controls or through the administration of bacteria that produce short chain fatty acids (SCFAs) (Braniste et al., 2014), which are known to have anti-inflammatory effects and promote BBB integrity (Hoyles et al., 2018). As mentioned previously, the gut plays an important role in the differentiation of Th17 cells. Interestingly, the gut also promotes the infiltration of Th17 cells into the brain through the meninges where these cells secrete IL-17, which further promotes immune cell infiltration [reviewed in Cipollini et al. (2019)]. BBB endothelial cells express TLRs and therefore are able to respond to gut microbe components such as LPS, which can alter tight junction expression and promote immune cell infiltration into the CNS (Tang et al., 2017). The BBB and microbiome are both disrupted in SCZ; this works thus highlights the potential for crosstalk between these systems that might act synergistically to further contribute to neuroinflammation in SCZ.

Gut microbes produce metabolites that can cross the BBB and inhibit the function of mitochondria in the CNS (Hulme et al., 2020). A decrease in mitochondria density and altered structure has been observed in post-mortem SCZ brain tissue across multiple regions including the anterior cingulate cortex (Flippo and Strack, 2017; Roberts, 2017). This finding raises the intriguing possibility that gut microbe metabolites can contribute to SCZ pathology. While the identity of the CNS cell(s) affected by gut metabolites remains unclear, the dysfunction of mitochondria in microglia has been shown to alter cytokine production and inflammatory responses in the brain [reviewed in Culmsee et al. (2018)]. MIA, which increases the risk for SCZ, has been shown in mice to alter the structure of mitochondria in a disease-associated microglial subtype known as dark microglia, among the hippocampus (Hui et al., 2018). Taken together, these studies suggest that there is extensive interplay between risk factors for SCZ, such that signaling from the gut-brain axis and exposure to an early immune insult can alter the function of microglia and CNS mitochondria. Future studies could aim to target the gut microbiome to dually control BBB integrity and reduce neuroinflammation.

Gut microbiota dysbiosis can alter the maturation and function of microglia in the CNS, thus contributing to neuroinflammation (Erny et al., 2015; Thion et al., 2018). Germ-free mice have microglia with an immature morphology and gene expression profile in adulthood (Erny et al., 2015), suggesting that the microbiota impacts the maturation of microglia. The absence of microbes was found to not only affect microglial function but also impair innate immune responses, which were partially recovered by colonization with a more complex microbiome or by supplementation with SCFAs, which are a by-product of certain gut microbes (Erny et al., 2015). SCFAs might affect CNS function through their interactions with BBB endothelial cells (Braniste et al., 2014) or directly with the CNS considering that they do not require receptors to bypass the BBB (Frost et al., 2014). The lack of SCFAs could additionally lead to increased peripheral and central inflammation considering their well-known anti-inflammatory functions (Vinolo et al., 2011; Li M. et al., 2018).

Microglia also show sex-dependent differences in response to gut microbe sterility. Microglia from germ-free male mice displayed altered expression of immune genes and a more immature phenotype at juvenile stages whereas microglia from female mice were more affected in adulthood (Thion et al., 2018). These findings suggest that the maternal microbiome can regulate microglial function in the offspring brain (Thion et al., 2018), notably in the context of MIA exposure (Kim et al., 2017; Shin Yim et al., 2017), in a sexually dimorphic manner. Sex differences in microglial response to microbiome challenges are intriguing as they could partially explain the earlier onset of SCZ in males compared to females (Ochoa et al., 2012). MIA models also display sexual dimorphism in microglial properties and behavioral outcomes (Hui et al., 2018). However, much work is needed to understand whether microglia-induced sex differences are present in SCZ.

Without a doubt, the gut microbiome influences the development and maintenance of the immune and nervous systems, with significant crosstalk. In the context of SCZ, the metabolites and diversity of gut microbes may impact multiple disease symptoms. The microbiome links multiple risk factors for SCZ, including stress responses, by promoting immune activation and BBB disruption. Innate immunity of the brain, including microglial function, is sensitive to gut dysbiosis, making the gut microbiota an interesting target in SCZ. Probiotics and microbiome transplants should be further explored to improve symptom severity in people with SCZ. Additionally, precautionary steps could be taken in pregnant mothers to improve diversity of gut microflora, considering its profound impact on brain development. Future work should further explore the role of SCFA-producing microbes, considering that they exert anti-inflammatory effects and improve brain function and behavior. Taken together, gut microbes are positioned to alter immune responses to environmental challenges by regulating neuronal function, behavior, and microglial responses, all of which are altered in SCZ.

MIA ENHANCES RISK FOR SCZ BY ALTERING MICROGLIAL FUNCTION

It has become increasingly clear that immune challenges occurring during pregnancy increases offspring risk for varied neurodevelopmental and neuropsychiatric disorders, including SCZ. Specifically, maternal exposure during pregnancy to bacterial (Sørensen et al., 2009) or viral infections such as influenza, rubella or herpes (Pearce, 2001; Brown and Derkits, 2010) leads to lasting changes in offspring brain function and behavior (Estes and McAllister, 2016). Maternal infection has been extensively studied using animal models of MIA, which have provided a substantial amount of causative evidence for how early immune insults disrupt brain development and function (Knuesel et al., 2014; Estes and McAllister, 2016). MIA can be induced by exposing pregnant dams to immunogens that mimic an infection. The most common immunogens used to model MIA include polyinosinic:polycytidylic acid [poly(I:C)] and LPS which mimic viral or bacterial infection, respectively. These agents elicit immune responses that enable cytokines to pass through the placental barrier, activating placental and embryo macrophages, and leading to increased inflammation in the developing offspring (Wu et al., 2017). Although work is needed to normalize MIA protocols, particularly on the temporal level, and to understand the variability in reported results (Kentner et al., 2019), this animal model has provided insight into how maternal infection enhances the risk for various disorders. Here, we focus on progress that has been made in understanding prenatal immune challenges in mice and humans.

MIA impacts brain function in a circuit-specific manner and interacts with other risk factors for SCZ. These early immune insults can elicit a vast array of phenotypes in mice that are relevant to SCZ and ASD, including abnormalities in ultrasonic vocalization and sociability, increased repetitive behaviors, motor dysfunction, and deficits in sensorimotor gating and cognitive abilities such as working memory (Knuesel et al., 2014; Fernández de Cossío et al., 2017; Pendyala et al., 2017; Shin Yim et al., 2017). Some of these behavioral effects are sex-dependent (Haida et al., 2019). MIA-induced behaviors were accompanied by changes in specific brain areas such as altered hippocampal volume and cortical thickness, and changes in synaptic density and proteins (Estes and McAllister, 2016; Fernández de Cossío et al., 2017), which are also observed in SCZ (Glantz and Lewis, 2000; Hui et al., 2018; Onwordi et al., 2020). Alterations in amygdala-cortical circuitry have been implicated in SCZ (Benes, 2010) and a recent study showed that MIA enhances glutamatergic neurotransmission between these circuits by increasing synaptic strength in the exposed offspring (Li Y. et al., 2018). An exciting development in this field showed that MIA-induced deficits in neurodevelopment depend on inflammatory signaling through the maternal microbiome (Kim et al., 2017). MIA via LPS also disrupts BBB function by increasing its permeability, thus promoting neuroinflammation (Estes and McAllister, 2014; Simões et al., 2018). However, there is also evidence for no change in BBB permeability after MIA induced via poly(I:C) in mice (Garay et al., 2013), suggesting immunogen-dependent effects. These differences also emphasize the variability of MIA animal models and the need for experimental standardization.

Given that microglia are the primary innate immune cells of the brain, they provide rapid responses to immune insults and are greatly affected by systemic inflammation. MIA exerts its effects on neurodevelopment largely by disrupting microglial function and by priming them for altered responses later in life. Changes in the density of microglia are found in early postnatal MIA offspring in multiple cortical and subcortical regions including the anterior cingulate cortex, striatum and hippocampus (Zhang et al., 2018). Microglial involvement in MIA effects is evident through an increase in cytokine and chemokine signaling, in mouse hippocampus and basal forebrain, during late fetal development in response to either LPS (Schaafsma et al., 2017) or poly(I:C) (Pratt et al., 2013). A recent study showed that an MIA mouse model induced at embryonic day 9.5 with poly(I:C) led to an increased density of a pathological microglial subtype, called dark microglia, in the hippocampus of male versus female offspring (Hui et al., 2018). Dark microglia are almost exclusively observed in disease states or in aged animals, and exhibit greater levels of oxidative stress and hyper-ramified processes in closer proximity to synapses than typical microglia (Bisht et al., 2016). These studies highlight the ability of MIA to alter microglial state and function.

Moreover, MIA in mice alters the transcriptome and phagocytic activity of microglia in offspring (Mattei et al., 2017). Specifically, hippocampal microglia from male poly(I:C) mice displayed a downregulation of genes that encode cell surface receptors associated with phagocytosis (*P2ry6, Sirpa, Siglece, Cx3cr1, Fcgr1, Itgav)* (Mattei et al., 2017). These receptors are important components of the microglial 'sensome', which contribute to the regulation of microglia-neuron interactions and are important for the engulfment of neuronal material (Mattei et al., 2017; Hickman and El Khoury, 2019; **Figure 2**). Inflammatory abnormalities, such as increased levels of

SERPINA3, TNFα, IL-1β, IL-6, and IL-6ST, have been observed in the ventral midbrain in post-mortem SCZ tissue, and these results were also replicated in an MIA mouse model (Purves-Tyson et al., 2019). Importantly, these differences in immune markers from SCZ tissue could be accounted for by a subset of cases, including about 45% of high inflammatory cases. The ventral midbrain houses the majority of dopamine-releasing neurons in the brain, therefore MIA might contribute to SCZ pathology by disrupting immune-mediated wiring of dopaminergic circuits (Purves-Tyson et al., 2019). These findings are important because they link SCZ-associated neuroinflammation to dopaminergic abnormalities, which are a hallmark of this disorder.

Multiple studies targeting microglial signaling pathways were able to reverse MIA-associated neuropathology, suggesting that microglia are the main culprit in inducing neurological dysfunction in response to immune challenges. For example, a study that targeted colony stimulating factor 1 receptor (CSF-1R), which plays a role in microglial proliferation, was successful in reversing some MIA-induced phenotypes (Ikezu et al., 2020). Depleting and repopulating microglia by inhibiting CSF-1R was protective in mice exposed to poly(I:C) prenatally (Ikezu et al., 2020). Specifically, once the microglial population was renewed, not only were the deficits in repetitive and social behaviors reversed, but normal neuronal connectivity and microglia-neuron interactions were also restored (Ikezu et al., 2020). Another successful approach to restore typical microglial function targeted the peroxisome proliferator-activated receptor gamma (PPARγ) signaling pathway. PPARγ signaling is activated by fatty acids and reduces myeloid cell-induced inflammation via suppressing their production and/or secretion of inflammatory molecules (Bernardo and Minghetti, 2006). Agonists of PPARγ have been found to be protective in the context of MIA by inhibiting microglial expression of pro-inflammatory cytokines and surface antigens (Bernardo and Minghetti, 2006), suggesting that targeting microglial PPARγ signaling could be beneficial in offspring exposed to MIA (Zhao et al., 2019). In support of this, a recent study showed lower serum levels of PPARγ in patients with SCZ while levels of this biomarker decreased further with disease progression (Yüksel et al., 2019). Treatment with minocycline, a broad-spectrum anti-inflammatory and antibiotic drug that generally restores microglial functions, also reversed changes in microglial transcriptome and phagocytic activity in mouse offspring exposed to MIA (Mattei et al., 2017). Lastly, there is evidence that deep brain stimulation in rats can prevent some of the behavioral deficits associated with MIA specifically by reducing microglial pro-inflammatory responses (Hadar et al., 2017). Taken together, these data suggest that microglia play a critical role in MIA-induced brain dysfunction and that targeting microglia is a potential therapeutic approach to reverse MIA-induced phenotypes.

MIA is a risk factor for SCZ that depends on maternal immune signaling relayed to the fetal brain through the placenta. Maternal gut microorganisms have been found to play an important role in MIA-mediated deficits. A ground-breaking study showed that MIA phenotypes in exposed offspring are dependent on the presence of segmented filamentous bacteria in the maternal gut which promote Th17 cell differentiation, leading to increased IL-17a production (Kim et al., 2017). MIA phenotypes, including deficits in cortical development and behavioral abnormalities, were dependent on gut microbiome-mediated increases in IL-17a (Kim et al., 2017; Shin Yim et al., 2017). These data show that maternal microbe-induced immune signaling impacts fetal brain development with long-term consequences and that prenatal inflammatory insults can prime the gut-immune-brain axis, thus leading to altered CNS responses to immune challenges later in life.

MIA is an important model that has increased our understanding of how immune insults occurring during embryonic development can alter brain development. Although there is variability in data obtained using mouse models of MIA, notably due to differences in immunogen manufacture (molecular weight, endotoxin contamination, etc.), timing of immunogen administration, dosage, route of administration, housing conditions, timing of cage cages and mouse strain used (Careaga et al., 2018; Kentner et al., 2019; Kowash et al., 2019), understanding what causes these differences could aid in understanding the mechanisms underlying vulnerability versus resiliency to MIA (Meyer, 2019). In humans, only a subset of pregnant mothers who are exposed to a viral or bacterial infection have offspring who later develop SCZ (Estes et al., 2019). This is to be expected since immune activation is only one of the many risk factors for SCZ. Therefore, the variability in mouse models of MIA might be exploited to elucidate why certain sub-populations of individuals are at greater risk for SCZ. Since some mouse strains are resilient to MIA, the genetic differences between mouse strains could be used to identify protective versus susceptibility genes (Schwartzer et al., 2013). Overall, future work aimed at understanding such variability will likely be valuable in discovering only a subset of subjects are vulnerable to MIA.

It is interesting that MIA is a risk factor for both SCZ and ASD, since some of the neurological deficits observed in these disorders appear to be opposing. For example, SCZ is characterized by a significant loss of gray matter resulting in hypoconnectivity between the anterior hippocampus and PFC (Vita et al., 2012; Blessing et al., 2020), on which the neonatal ventral hippocampal lesion rodent model of SCZ is based (Joseph et al., 2018), whereas ASD is associated with hyperconnectivity (Supekar et al., 2013). How could the same risk factor play a role in such opposing phenotypes? We propose that the underlying genetic background and the time of exposure are important factors that determine the effects that MIA exerts on brain development. For example, SCZ is associated with genetic variation in the *C4* gene that led to enhanced *C4* expression (Sekar et al., 2016) whereas *C4*, *C3,* and *C1q* were found to be downregulated in ASD (Fagan et al., 2017). Differences in certain genes, such as complement genes, which have an established role in synaptic pruning (Stevens et al., 2007; Schafer et al., 2012; Sekar et al., 2016; Comer et al., 2020), could explain how MIA differentially contribute to disease phenotypes. Alternatively, the expression of *TLR3* and *TLR4*, which directly respond to poly(I:C) and LPS (Lu et al., 2008; Zhou et al., 2013), respectively, could differ between mouse strains with varying susceptibility to MIA and in humans predisposed to different NDDs. Lastly, it is not clear how recently emerging

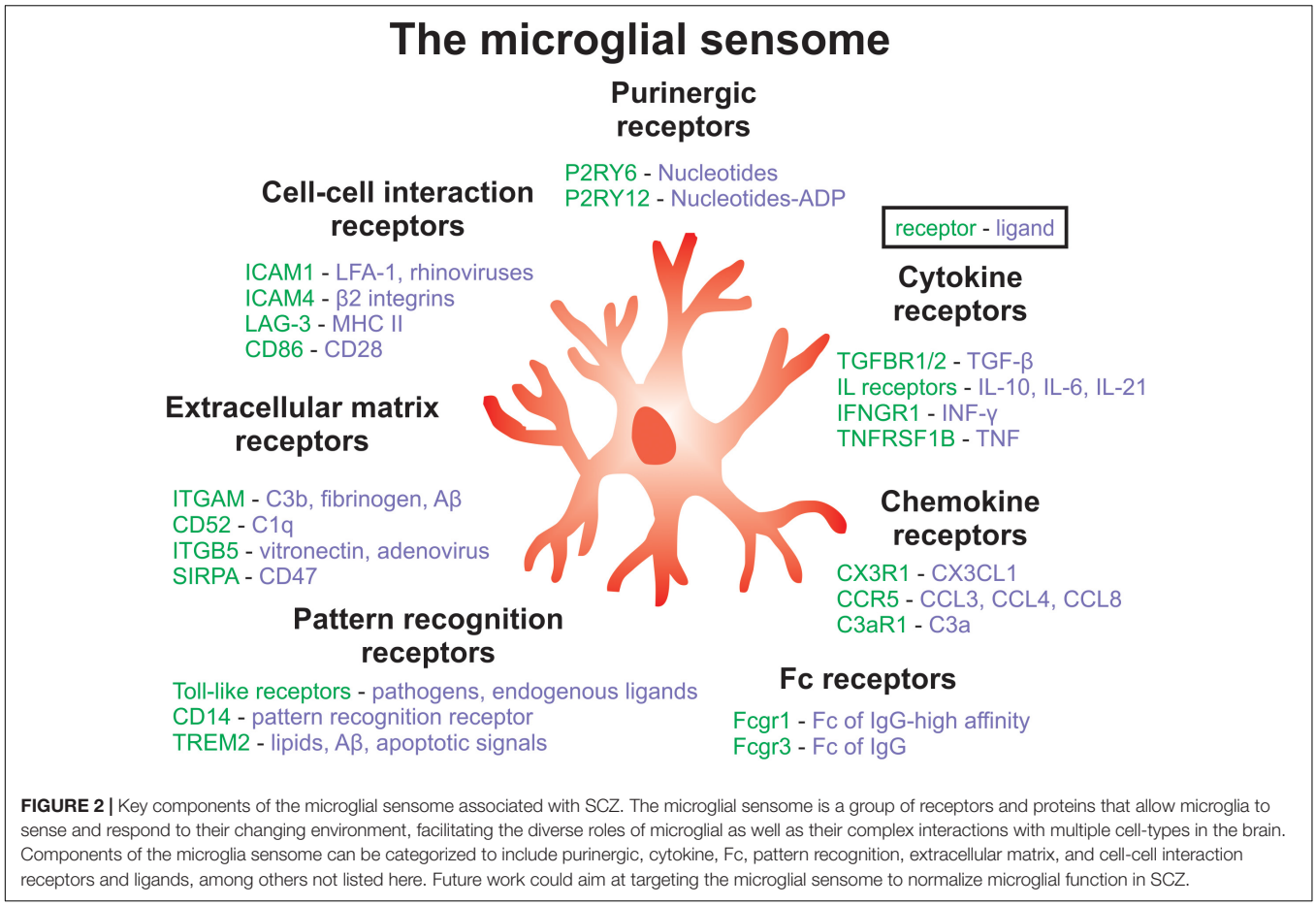

FIGURE 2 | Key components of the microglial sensome associated with SCZ. The microglial sensome is a group of receptors and proteins that allow microglia to sense and respond to their changing environment, facilitating the diverse roles of microglial as well as their complex interactions with multiple cell-types in the brain. Components of the microglia sensome can be categorized to include purinergic, cytokine, Fc, pattern recognition, extracellular matrix, and cell-cell interaction receptors and ligands, among others not listed here. Future work could aim at targeting the microglial sensome to normalize microglial function in SCZ.

viruses, such as SARS and MERS coronaviruses, might contribute to NDDs (Gretebeck and Subbarao, 2015; Fauci et al., 2020). It is also unknown whether the severe acute respiratory syndrome coronavirus 2, which caused the COVID-19 pandemic, leads to lasting consequences on brain development and behavior while preliminary data suggest that passive transfer of antibodies from mother to embryo is possible (Zeng et al., 2020).

STRESS-INDUCED INFLAMMATION AND MICROGLIAL DYSFUNCTION

Exposure to psychological stress or traumatic life events prenatally and during childhood or adolescence results in an increased risk for SCZ (Weinstock, 2008; Read et al., 2009; Kessler et al., 2010; Holtzman et al., 2013). Specifically, during critical periods of development, certain stressors, such as physical or mental abuse, socioeconomic disadvantage, living in an urban environment and neglect, all confer greater risk for SCZ (McGrath et al., 2004; Quidé et al., 2017; Popovic et al., 2019). Additionally, people with SCZ have altered physiological responses and increased vulnerability to stressful stimuli (Schifani et al., 2018). Thus, increased exposure and vulnerability to psychosocial stress, especially during critical periods of brain development, represents a significant challenge. However, cellular and molecular mechanisms that link early life stress (ELS) with increased risk for SCZ are still unclear. Nevertheless, evidence suggest that psychosocial stressors contribute to SCZ pathology by in part increasing neuroinflammation (**Figure 3**). A unified review was recently published focusing on the relationship between childhood trauma and psychosis, integrating results of epidemiological, clinical, neuropsychological and biological studies (Misiak et al., 2017).

Individuals with SCZ have altered physiological stress responses (van Venrooij et al., 2012; Schifani et al., 2018; van Leeuwen et al., 2018). Exposure to stress stimulates the sympathetic nervous system causing the secretion of epinephrine and norepinephrine, resulting in increased HPA axis function which leads to the release of stress hormones, such as cortisol, into the blood [reviewed by Chrousos (2009)]. These stress hormones alter an organism's physiology to promote activities that combat the stressor, such as increased cardiac function and glucose availability, while decreasing less urgent processes including digestion, reproduction, and immune function (Chrousos, 2009). In healthy individuals, cortisol led to the suppression of adaptive immunity and an increase in innate immunity due to the effects of glucocorticoids on inflammation (Barnes, 1998). Although cortisol has some anti-inflammatory effects, its ability to regulate inflammatory responses is altered

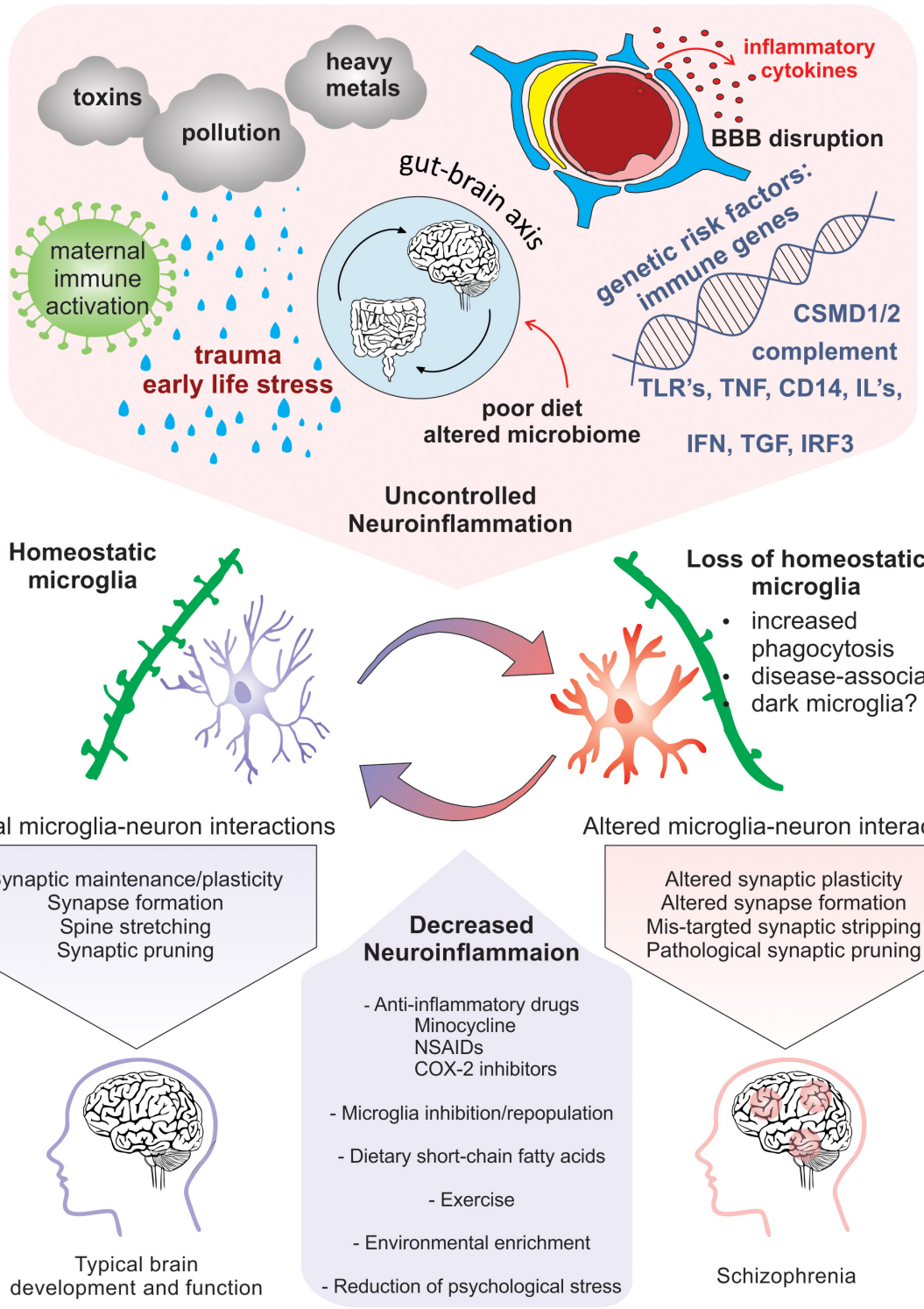

FIGURE 3 | Neuroinflammation-induced changes in microglia that are implicated in SCZ pathogenicity. Risk factors for SCZ that alter microglial function and enhance neuroinflammation include pollution, stress, nutrition induced gut-brain axis dysbiosis, viral infection, maternal immune activation, genetic predisposition, and cytokine secretion. Homeostatic microglia perform their immune sentinel role by interacting with neurons to guide circuit wiring during development. In an increased inflammatory milieu, loss of microglial homeostasis perturbs microglia-neuron interactions that may cause altered plasticity due to pathogenic synaptic formation, synaptic stripping, and pruning. Therapeutic approaches that promote homeostatic microglia through the reduction of neuroinflammation via anti-inflammatory drugs, microglial inhibition and repopulation, improved nutrition, environmental enrichment, and prevention of psychological stress could be potentially exploited to limit exacerbation of SCZ.

in SCZ. In healthy individuals, an acute stressor led to increased salivary levels of cortisol and a decrease in IL-6; however, in individuals with SCZ, an increase in cortisol was shown to be accompanied by an increase in IL-6 (Chiappelli et al., 2016). Additionally, chronic and ELS, which are risk factors for SCZ, are linked to increased immune activation (Chiappelli et al., 2016), as well as abnormal sensitivity and levels of glucocorticoids and their receptors (Webster et al., 2002; Sinclair et al., 2012; do Prado et al., 2017), disrupting the ability of cortisol to regulate inflammation (Miller and Chen, 2010). In this sense, stress-induced release of cortisol might increase inflammatory responses in people with SCZ instead of having anti-inflammatory effects such as seen in healthy individuals.

Although multiple studies have found an increase in HPA axis function in people with SCZ (Walder et al., 2000; Mondelli et al., 2010a; Chiappelli et al., 2016), others have reported a decrease in cortisol levels compared to controls in response to a stressor (Ciufolini et al., 2014; Lange et al., 2017; Glassman et al., 2018). The inconsistencies between these findings could be due variation including differences in stressor intensity, duration, time point of exposure (Lange et al., 2017), or administration of antipsychotics, which have been shown to alter cortisol stress responses (Houtepen et al., 2015). Despite these discrepancies, HPA axis dysfunction has been observed in first-episode psychosis prior to antipsychotic treatment (Ryan et al., 2004; Mondelli et al., 2010b; Mondelli et al., 2010a). Additionally, recent work has shown that regardless of differences in cortisol responses to acute stressors among people with SCZ, those with decreased cortisol responses to social stress had lower measures of social functioning (Tas et al., 2018). Therefore, understanding differences in cortisol responses and its relationship to immune function in SCZ could provide insight into the role of psychosocial stress on disease progression.

Prenatal psychological stress is associated with an increased risk of SCZ (Kofman, 2002; Weinstock, 2008; Pugliese et al., 2019). In mice, prenatal stress increased placental expression of several pro-inflammatory genes including *IL-6*, *IL-1B*, and *TNF*α specifically in males, and these changes were partially rescued by maternal administration of a non-steroidal anti-inflammatory drug (Bronson and Bale, 2014). Additionally, studies in mice have shown that male offspring exposed to prenatal stress displayed behavioral deficits including anhedonia and changes in stress responses that coincided with altered placental gene expression in males but not females, affecting *PPAR*α, the growth factor *IGFBP-1*, hypoxia-inducible factor 3a (*HIF3*α), and glucose transporter *GLUT4*, all of which have been implicated in immune system function (Mueller and Bale, 2008). Importantly, the placenta is a regulator of maternal-fetal immune initiation in offspring [reviewed in Hsiao and Patterson (2012)] and this interaction appears to be crucial given that prenatal dysregulation of the immune system can lead to altered immune responses postnatally (Bilbo and Schwarz, 2009; Pedersen et al., 2018). Maternal restraint stress resulted in offspring with altered microglial morphology and density in the cortical plate at embryonic stages and in neocortex at adulthood, and these prenatal-induced changes were reversed by blocking IL-6 (Gumusoglu et al., 2017), confirming that increased maternal expression of IL-6 can cause neuroinflammation in embryos by crossing the placenta (Dahlgren et al., 2006). Nevertheless, the role of maternal stress-induced inflammation and the specific involvement of the placenta in mediating its consequences are not fully understood.

ELS, such as childhood abuse or neglect, is a major risk factor for SCZ, however the mechanisms by which ELS induces changes in neuronal circuitry is not clear. Mounting evidence suggests that dysfunction of the immune system and microglia, especially, can contribute to brain miswiring and behavioral deficits after ELS (Na et al., 2014; Johnson and Kaffman, 2018). In humans and mice, ELS increases multiple blood pro-inflammatory markers including CRP, IL-1β, IL-6, IL-8, TNF-α (Hepgul et al., 2012; Marsland et al., 2017; Réus et al., 2017) while suppressing the anti-inflammatory cytokine IL-10, leading to depressive-like behaviors in mice (Réus et al., 2017). In line with these findings, ELS resulted in altered microglial gene expression, density, morphology and phagocytic activity during maturation in particular brain regions including the mPFC, striatum, anterior cingulate cortex and hippocampus (Cohen et al., 2016; Delpech et al., 2016; Bollinger et al., 2017; Wang et al., 2017; Banqueri et al., 2019; Réus et al., 2019). Chronic stress also altered microglial function by activating the P2X7 receptor, which induced the NLRP3 inflammasome thus increasing levels of mature IL-1β within the brain (Pan et al., 2014; Yue et al., 2017).

Since microglia play vital roles in brain development and homeostasis including neurogenesis, synaptic formation and elimination (Salter and Beggs, 2014; Hong et al., 2016; Tay et al., 2017b), their dysfunction could explain some of the neurological deficits observed after exposure to stress. Studies using RT-PCR from isolated microglia show that steroid hormone receptors, such as the glucocorticoid receptor, are abundant in microglia (Sierra et al., 2008), suggesting the possibility that stress could directly impact microglial function through glucocorticoid signaling. Indeed, a line of evidence suggests that stress can impact microglial proliferation, while blocking corticosterone synthesis or glucocorticoid receptor activity restored normal microglia density in mice (Nair and Bonneau, 2006; Duque and Munhoz, 2016). There is evidence that stress later in life can also induce changes in microglia, especially when these cells are primed by an environmental insult either prenatally or during early postnatal development (Catale et al., 2020). For instance, mice that were susceptible to repeated social defeat had microglial transcriptomes that were enriched for markers of phagocytosis, pro-inflammatory responses and reactive oxygen species compared to mice that were either resistant or not exposed to stress (Lehmann et al., 2018). Additionally, mice that were sensitive to repeated social defeat showed an increase in markers for extracellular matrix remodeling and BBB leakage, which coincided with an enhanced permeability of the BBB to a fluorescent tracer, and correlated with increased microglial phagocytosis of neuronal material (Stankiewicz et al., 2015; Lehmann et al., 2018). Additionally, microglial depletion by the CSF1R antagonist PLX5622 in a repeated social defeat mouse model protected against the behavioral abnormalities and prevented an increase in reactive oxygen species in the mPFC, nucleus accumbens and paraventricular nucleus (Lehmann et al., 2019). Together, these

data support that microglia play a vital role in stress-induced neuropathology by becoming more phagocytic, inducing the inflammasome and engulfing neuronal material.

Psychosocial stress might be more preventable than the other risk factors for SCZ. Reducing psychosocial stress in expecting mothers and young children or combating stress with exercise, nature exposure, yoga, or therapy could be used in individuals at risk for or diagnosed with SCZ (Entringer et al., 2009; Vancampfort et al., 2011; Brannigan et al., 2019). Some lines of evidence show that environmental enrichment can protect against or reverse many effects of stress, including ELS, by rescuing behavioral phenotypes, inflammatory responses, microglial function, and oxidative stress, notably in the mPFC (do Prado et al., 2016; McCreary and Metz, 2016; Dandi et al., 2018; González-Pardo et al., 2019), a region implicated in SCZ (Glantz and Lewis, 2000; Barch et al., 2001). However there is conflicting evidence concerning the ability of environmental enrichment to rescue these phenotypes in severe cases of ELS (Mackes et al., 2020). Alternatively, future studies could determine if treatment with anti-inflammatory medications can protect against stress-induced neuroinflammation since microglial depletion has been shown to be protective (Lehmann et al., 2019).

HOW THE PERIPHERAL IMMUNE SYSTEM GAINS ACCESS TO THE CNS IN SCZ

The link between BBB dysfunction and SCZ was first established when epidemiological studies revealed that about two-thirds of SCZ cases are diagnosed with comorbid conditions associated with deficits in endothelial cell function, such as metabolic syndrome and cardiovascular disease (Israel et al., 2011; Burghardt et al., 2014). Capillary wall endothelial cells form tight junctions with one another and are an integral component of the BBB along with pericytes, astrocytic endfeet, microglia, and the extracellular matrix that forms the basement membrane (Lassmann et al., 1991; Abbott et al., 2010; Bisht et al., 2016; Joost et al., 2019). The BBB restricts the passage of molecules between the blood and the brain to protect sensitive neural tissue from pathogens and immune molecules while allowing the passage of vital molecules such as glucose (Abbott et al., 2010). This allows the BBB to isolate the brain from peripheral immune responses; however, it has become increasingly clear that in pathological states the ability of the BBB to isolate the CNS from harmful immunological responses is disrupted (Bechter et al., 2010; Najjar et al., 2017).

Claudin-5, expressed in brain endothelial cells, forms a major component of the BBB barrier-forming tight junctions (Morita et al., 1999; Greene et al., 2019). Claudin-5 maps to a region on chromosome 22 where small deletions cause the 22q11 deletion syndrome, which is found in 30% of SCZ cases (Murphy, 2002; Motahari et al., 2019). People with this syndrome are haploinsufficient for claudin-5 and have increased odds of developing SCZ (Fiksinski et al., 2018; Greene et al., 2018). A recent study showed that during acute versus chronic inflammation, levels of claudin-5 are differentially expressed (Haruwaka et al., 2019). It is still unknown if microglial phagocytosis of tight junctions is also involved in SCZ, although this finding suggests that BBB dysfunction could be mediated through a decrease of molecules involved in tight junctions or BBB permeability.

Indeed, post-mortem mPFC tissue from SCZ individuals show changes in the endothelial cell gene expression of molecules involved in tight junctions and BBB permeability. People with SCZ can be divided into subgroups based on their extent of brain and serum inflammatory markers (Fillman et al., 2016). Cases of SCZ that have higher serum pro-inflammatory markers, which include about 40% of affected people (Fillman et al., 2016), also have greater gray matter loss in the mPFC, which is thought to underlie multiple symptoms of SCZ (Zhang et al., 2016). Compared to healthy controls, SCZ cases, especially high-inflammatory cases, have increased expression of the intercellular adhesion molecules ICAM-1 and VCAM-1 in endothelial cells from the PFC (Kavzoglu and Hariri, 2013; Cai et al., 2018; Nguyen et al., 2018). ICAM-1 and VCAM-1 interact with receptors on leucocytes to allow monocyte infiltration into the brain (Hermand et al., 2000). In endothelial cell cultures, ICAM-1 expression can be induced in a dose-dependent manner by the pro-inflammatory cytokine IL-1β (Cai et al., 2018). ICAM-1 expression has also been found to correlate with the expression of the macrophage marker CD163, and CD163-positive macrophages were found in close association with neurons in the frontal cortex of high-inflammatory SCZ cases (Cai et al., 2018). In this study, proteins that form endothelial cell tight junctions, including cadherin-5 (CDH5) and occluding (OCLN), were also upregulated in the frontal cortex (Cai et al., 2018), which highlights a compensatory mechanism to regain BBB integrity. Conversely, multiple studies have a found a decreased expression of CDH5 in the PFC of SCZ individuals, while genetic knockdown of CDH5 in mouse PFC led to BBB disruption and changes in behavior including deficits in learning, memory, sensorimotor gating, and anxiety-like behavior (Nishiura et al., 2017; Greene et al., 2018). The expression of tight junction genes could differ depending on the time point during SCZ progression, such that compensatory mechanisms could be elicited in later disease stages. In addition, the conflicting evidence for a leaky BBB in SCZ suggest that the BBB is compromised in only a subset of SCZ cases. The finding of subgroups of people with SCZ showing variable levels of systemic inflammation support this hypothesis. Together, these findings reveal the importance of studying subgroups of SCZ patients, based on systemic inflammation, to gain a more comprehensive understanding of the disease pathogenesis.

In addition to endothelial cells, pericytes and astrocytes have also been implicated in BBB dysfunction during systemic inflammation (Fabry et al., 1993; Nishioku et al., 2009; Chen et al., 2017; Banks et al., 2018). There is some evidence that pericytes can exit the perivascular space in response to LPS-induced inflammation in mice, while the extent of pericyte detachment correlated with microglial reactivity (Nishioku et al., 2009). Pericytes secrete cytokines, including IL-1 and IL-6, which are capable of disrupting endothelial cell tight junctions (Fabry et al., 1993). Disruption of the BBB in several mouse models of neuropsychiatric or inflammatory diseases

has been shown to affect microglial function, while dynamic neuroimmune interactions were described at the BBB in both health and diseased sates (Merlini et al., 2012; Borjini et al., 2019; Haruwaka et al., 2019). Although causal evidence is needed, multiple studies have found that microglial reactivity worsens BBB integrity in pathological states and that administration of the anti-inflammatory drug minocycline can improve BBB function (Yenari et al., 2006; da Fonseca et al., 2014; Shigemoto-Mogami et al., 2018). More work is still needed to understand whether or how the interplay between BBB dysfunction and microglia abnormalities contribute to the pathogenesis of SCZ. Complex cytokine signaling between the pericytes, endothelial cells, astrocytes and microglia is crucial for the development and maintenance of BBB integrity (Chen et al., 2017; Banks et al., 2018). Lastly, it was suggested that PFC hypoconnectivity in SCZ might result from altered blood flow regulated by pericytes, together with abnormalities in the structures of capillaries and astrocytic end feet (Uranova et al., 2010). As such, understanding the complex interactions between cell-types of the neurovascular unit and how they might be altered in response to inflammation in SCZ will likely be important.

Abnormal activity in multiple brain networks and regions are observed in SCZ (Uhlhaas, 2013). There is clear evidence that excitatory circuits are altered in SCZ (Glantz and Lewis, 1997, 2000; Uhlhaas, 2013). Blockade of *N*-methyl-D-aspartate receptors (NMDARs) in healthy subjects leads to psychotic symptoms and cognitive deficits that resemble those observed in SCZ (Balu, 2016). Additionally, both mRNA and protein levels of the NMDA subunits NR1 and NR2C are decreased in post-mortem SCZ brain tissue (Weickert et al., 2013). Recent evidence suggests that NMDAR function might be inhibited in SCZ by autoantibodies, which are produced against an organism's own tissue and are implicated in autoimmune disorders such as lupus (Becker et al., 2019). Circulating autoantibodies against glutamate and NMDARs were found to be present in approximately 20% of psychotic SCZ patients (Jézéquel et al., 2017). An increased BBB permeability might alter neuronal function by allowing the entry of autoantibodies against NMDARs into the brain, which have been shown in mouse models and neuronal culture experiments to suppress glutamatergic activity by altering the organization of NMDARs and their anchoring molecule ephrin-B2 (Kayser and Dalmau, 2016; Jézéquel et al., 2017; Kannan et al., 2017). Studies that interrogate specific cell-type and neural circuit responses will allow greater understanding of the impact of BBB permeability on brain function and open new opportunities to therapeutically modulate these pathways.

Beyond the BBB, peripheral inflammatory responses can gain access to the CNS via the meninges, the multi-layered protective tissue that surrounds the brain and spinal cord [reviewed in Rustenhoven and Kipnis (2019)]. Cytokines can accumulate in the dural CSF and cross into the brain, passing between endothelial cells that lack tight junctions (Louveau et al., 2015). Additionally, cytokine signaling specifically within the meninges has been shown to alter neuronal function by binding directly with receptors on neurons in frontal cortical regions and altering cognitive and social behaviors in mice (Derecki et al., 2010; Filiano et al., 2016). Meningeal T-cell production of multiple inflammatory molecules, including IL-17, IL-4, and INF-γ, have been shown to alter both excitatory and inhibitory circuitry and modulate cognitive function and social behavior (Derecki et al., 2010; Filiano et al., 2016; Ribeiro et al., 2019). Lastly, the CNS meningeal lymphatic system also offers a route for peripheral-central immune crosstalk. Since the brain does not contain a resident lymphatic system, waste removal is facilitated by cerebrospinal fluid draining through the meninges into the deep cervical lymph nodes, where interactions between CNS immune molecules and peripheral immune cells can occur (Louveau et al., 2015, 2018). In this manner, the peripheral immune system can gauge central immune status. In the aging brain, dysfunction of the meningeal lymphatic vessels leads to accumulation of harmful amyloid beta-protein toxicity and increase Alzheimer's pathology (Da Mesquita et al., 2018). Longitudinal imaging studies have shown that progressive brain matter loss is consistent with accelerated aging in patients with SCZ (Schnack et al., 2016). It remains to be determined whether therapeutic agents that boost lymphatic function by either increasing the diameter of the lymphatic vessels or cerebral spinal fluid drainage (Da Mesquita et al., 2018) could improve the cognitive and social deficits observed in SCZ.

DISCUSSION

Is SCZ an Inflammatory Disease?

There is growing evidence from both human and animal studies that many of the risk factors for SCZ converge on their ability to promote neuroinflammation, and that these effects are mediated in part by microglia. However, is there a pro-inflammatory phenotype in SCZ? Post-mortem and clinical studies show an increase in pro-inflammatory markers in people with SCZ compared to controls (Fillman et al., 2016; Sekar et al., 2016; Boerrigter et al., 2017; Lesh et al., 2018; Goldsmith and Rapaport, 2020; Pedraz-Petrozzi et al., 2020). Moreover, there is evidence for elevated levels of cytokines in blood samples from people with SCZ, whether they are medication-naive or receiving antipsychotic treatment, during episodes of psychosis (McKernan et al., 2011; De Picker et al., 2019; Mondelli et al., 2020; Steiner et al., 2020). Thus, such studies suggest that inflammation might contribute to the development of SCZ and also drive its progression and cyclic nature.

Schizophrenia cases can be sub-divided using either serum or post-mortem brain tissue levels of pro-inflammatory cytokines, which reveal that about 40% of SCZ cases have a high inflammatory expression signature (Fillman et al., 2016; Boerrigter et al., 2017; Cai et al., 2018). Although these studies suggest there are subtypes of SCZ patients, they do not provide information on their inflammatory states earlier in the disease development nor do they assay inflammation in the brain, which could differ from blood or CSF biomarkers of inflammation. There has been some success in longitudinal PET imaging studies that measure expression of translocator protein (TSPO), a non-specific marker of pro-inflammatory microglia, in the brain (Selvaraj et al., 2018). These studies show that SCZ is characterized by increased TSPO expression, which correlated

with greater gray matter loss (Selvaraj et al., 2018). However, there have been mixed results concerning PET measurements of TSPO with some studies showing increased TSPO binding in SCZ (Doorduin et al., 2009; Bloomfield et al., 2016) and others showing no correlation (Di Biase et al., 2017; Notter et al., 2018). Additionally, recent work revealed that neuronal activity can also drive the expression of TSPO (Notter et al., 2020). It is thus not clear if TSPO is a reliable marker for neuroinflammation (Sneeboer et al., 2020). The identification of more specific *in vivo* markers for neuroinflammation would be useful. Ideally, additional work should be done to specifically interrogate the extent of neuroinflammation in SCZ, in addition to peripheral inflammation, to determine if increased inflammation correlates with all or only a percentage of SCZ cases.

Given that SCZ is a highly heterogeneous disease, it is not surprising that there are different disease subtypes. Studies that have divided individuals with SCZ based on inflammatory markers have found more severe symptomology in those with higher levels of pro-inflammatory markers. Specifically, there is evidence for greater gray matter loss and poorer performance in language tasks (Fillman et al., 2016) and increased depressive symptoms (Bossù et al., 2015) in SCZ cases characterized by high inflammatory state. Consistent with this, therapeutics that reduce inflammation provide the greatest symptom improvement in neuropsychiatric cases associated with high inflammation. For example, inhibition of TNF was shown to improve symptoms in people with major depression, but only in those with heightened immune biomarkers (Raison et al., 2013; Weinberger et al., 2015). Additionally, various anti-inflammatory agents including aspirin, estrogen, N-acetylcysteine, COX-2 inhibitors, minocycline and fatty acids (Sommer et al., 2014) have been shown to improve symptom severity in SCZ, but there are some mixed findings of the efficacy of these therapeutics. Minocycline has been shown to reduce microglia and complement-dependent synapse removal in an *in vitro* model from patient-derived neuronal cultures while decreasing the risk for SCZ when administered to young adults (Sellgren et al., 2019), suggesting that targeting synaptic pruning via neuroinflammation would be therapeutic for SCZ and might directly target the disease process. Nevertheless, it is possible that there are discrepancies concerning the ability of some of these drugs to improve symptoms in SCZ because they might only be effective in high-inflammatory cases. Future work aiming to elucidate the differences between subtypes of SCZ could potentially allow for the development of more effective and targeted therapeutics. Although people with SCZ can be divided based on extent of inflammation, there is no denying the role of the immune system in this complex disease.

In line with this, microglia are significantly altered in SCZ and contribute to neural dysfunction by responding and contributing to neuroinflammatory signaling (**Figure 3**). In SCZ post-mortem tissue, microglia have been noted to have altered morphologies and densities in brain regions known to contribute to the symptomology of SCZ. Microglia engulfment of synaptic material is essential for the normal wiring of the brain and can contribute to pathological states when mis-regulated (Wake et al., 2009; Tremblay et al., 2010; Paolicelli et al., 2011; Schafer et al., 2012; Dejanovic et al., 2018; Filipello et al., 2018; Vainchtein et al., 2018; Weinhard et al., 2018; Comer et al., 2020). There is also evidence that microglia contribute to synapse formation during development, adolescence and into adulthood (Parkhurst et al., 2013; Miyamoto et al., 2016; Akiyoshi et al., 2018; Weinhard et al., 2018). Additionally, a two-photon *in vivo* imaging study in awake mice has shown that microglial contacts with synapses increase synaptic activity thus enhancing neuronal network synchronization (Akiyoshi et al., 2018). In this study, when MIA was induced with poly(I:C), microglia became reactive while neuronal synchronization decreased (Akiyoshi et al., 2018), suggesting that microglia contribute to network function and that their role in this process can be easily disrupted by immune responses.

Although there is evidence that microglia contribute to excessive synaptic pruning in SCZ, it is not clear if microglia-dependent synapse formation is also altered. Since much of the data collected from individuals with SCZ is from post-mortem samples, it is difficult to discern what is occurring on the synaptic level earlier in development. Recent studies suggest that more immature spine types can be differentially targeted in SCZ (MacDonald et al., 2017; Comer et al., 2020), therefore, it is possible that synapse formation mediated by microglia is also altered in SCZ. In a prenatal ventral hippocampus lesion model for SCZ, microglia displayed altered density, morphology and ultrastructure, together with increased expression of multiple complement genes including *C1q* and *C3* (Hui et al., 2019). This increase in microglial expression of complement proteins coincided with an increase of synaptic pruning in the PFC and behavioral deficits in rats, but was reversed by administration of minocycline (Hui et al., 2019). These studies highlight the necessary role exerted by microglia in normal brain development but also show their ability to drive neuroinflammation and contribute to pathology in disease states.

Indeed, there are multiple disease-associated microglial subtypes such as those seen in neurodegenerative disorders (Deczkowska et al., 2018) and dark microglia which were recently observed in SCZ post-mortem brain samples (Uranova et al., 2018). More work is needed to fully understand microglial subtypes that are more prevalent in disease states and how they contribute together to pathology, however data suggest they partially contribute to disease by enhancing synaptic pruning (Stratoulias et al., 2019). Future studies should also aim to develop more translational animal models so that *in vivo* studies can be performed to gain greater understanding into how microglia functionally impact synaptic development and circuit function in pathological states.

Does Inflammation Affect Specific Circuits and Neuromodulatory Systems?

Although there is no doubt that the immune system plays a critical role in shaping brain development and contributes to disease states when dysregulated, there is a need to understand which specific circuits and neuromodulatory systems in particular are most impacted by abnormal immune signaling. It is clear that complement proteins facilitate the removal of

synapses (Stevens et al., 2007) and that the upregulation of complement proteins contributes to circuit miswiring (Comer et al., 2020). However, SCZ is also characterized by alterations in inhibitory circuits (Dienel and Lewis, 2019), neuromodulatory systems such as dopamine (Howes et al., 2017) and glutamate (Uno and Coyle, 2019), and changes in the connectivity between brain regions such as the hippocampus and PFC (Sigurdsson and Duvarci, 2015). Do inflammatory responses alter specific neurotransmitter systems and networks differentially?

There is evidence that inflammatory responses target specific neuromodulatory systems and brain circuits. For example, changes to the gut microbiome driven by inflammation can alter the production of serotonin (Rogers et al., 2016), which is known to be disrupted in SCZ. Interestingly, MIA in rats has been found to increase the levels of dopamine in both the nucleus accumbens and mPFC (Luchicchi et al., 2016) of offspring, which is well-known to play a role in the positive symptoms of SCZ (Kesby et al., 2018). Additionally, MIA initially triggers hyperinhibition and neuronal miswiring, before leading to a reduced inhibitory drive (Thion et al., 2019). ELS in mice was shown to alter HPA circuity development, in addition to hippocampal and PFC function (Brenhouse et al., 2019). In addition, ELS is known to have an impact on inhibitory connectivity (Goodwill et al., 2018; Ohta et al., 2020), and previous work suggests that oxidative stress and/or neuroinflammation might underlie the changes in parvalbumin interneurons in response to ELS (Holland et al., 2014; Brenhouse et al., 2019). There is evidence for parvalbumin interneuron dysfunction in SCZ, as they have altered density in the frontal cortex of individuals with SCZ (Kaar et al., 2019). Interestingly, the meninges modulate cortical interneuron migration during development (Borrell and Marín, 2006); future work could interrogate whether changes in meningeal signaling, such as immune molecule signaling, could contribute to alterations in interneuron migration in SCZ.

The glutamate hypothesis of SCZ evolved from observations that NMDA receptor antagonists, such as ketamine, produce behavioral states similar to SCZ negative and positive symptoms in healthy human subjects (Krystal et al., 1994; Adler et al., 1998; Hu et al., 2015). As discussed throughout this review, studies have also reported spine dysgenesis and alterations in mRNA and protein levels of glutamate receptors in human SCZ post mortem tissue. The presence of circulating autoantibodies against glutamate and NMDARs in a subpopulation of psychotic SCZ patients further support this hypothesis (Ehrenreich, 2018). It has recently been shown that increased expression of the SCZ-associated gene *C4* led to a decrease in excitatory connectivity with no loss of inhibitory transmission, suggesting that excitatory synapses might be more vulnerable to elimination (Comer et al., 2020). In support of this, neuronal pentraxins, regulators of AMPA receptor trafficking, are interacting partners of C1q (Ma and Garred, 2018), providing a link between the complement pathway and excitatory synapse elimination. Another link between alterations in glutamatergic transmission and SCZ comes from studies in astrocytes, which have increased reactivity in SCZ (post mortem tissue) and are positioned to alter glutamatergic transmission through the regulation of glutamate biosynthesis, release, uptake and metabolism [reviewed in Mei et al. (2018)]. Lastly, the mobile genetic element, human endogenous retrovirus is associated with neuropsychiatric conditions and produces a protein that alters glutamate synapse structure and plasticity dependent on the presence of glial cells and neuroinflammatory signaling, contributing to altered behavior when expressed in mice (Johansson et al., 2020). Together, these studies suggest that inflammation might contribute to altered glutamatergic transmission through multiple mechanisms (**Figure 4**).

Although these studies suggest that risk factors for SCZ can exert specific effects on different CNS circuits, more work is needed to fully understand the mechanisms by which inflammation alters specific neuromodulatory systems and circuits. Studies that combine mouse models of SCZ and inflammation with whole-brain or mesoscopic imaging could shed light into how specific neuronal networks are impaired in SCZ (Sofroniew et al., 2016; Boido et al., 2019; Grandjean et al., 2020).

Impact of the Immune System on Synapse Development in SCZ

It is well-established that spine dysfunction is present in SCZ and is at least partially mediated by reductions in excitatory synaptic connectivity and plasticity (Glantz and Lewis, 2000; Glausier and Lewis, 2013; Berdenis van Berlekom et al., 2020). However, most of the work in this field has relied on human post-mortem tissue, so there is limited knowledge of what could be occurring earlier in development to drive these synaptic alterations. Recent work has highlighted the fact that the immune system works closely with the CNS to establish and refine neural circuits in healthy states as a part of normal development (Paolicelli et al., 2011; Tay et al., 2017b; Hammond et al., 2018). However, when this process is dysregulated, it can lead to pathology and the miswiring of the brain through synaptic loss (Schafer et al., 2012; Comer et al., 2020), which occurs in SCZ.

How are certain synapses selectively removed while others are protected? In the developing brain, there is a period of enhanced synaptogenesis followed by critical developmental periods characterized by experience-dependent refinement of synapses (Trachtenberg et al., 2002; Holtmaat and Svoboda, 2009). Synaptic elimination driven by sensory experience refines brain circuitry by optimizing connections between neurons. In SCZ, this process is thought to be dysregulated, thus leading to a loss of both excessive and necessary synapses, causing aberrant brain connectivity. Recent data suggest that more immature spine types, such as filopodia and thin spines (Cruz-Martín et al., 2012), are lost while larger, more established spines remain intact in SCZ (MacDonald et al., 2017). A similar phenotype was seen in mice overexpressing the mouse homologue of the SCZ-associated gene *C4*. In this *in vivo* model, synaptic loss observed in the PFC was specifically due to a loss of smaller spine-types while mushrooms spines were unaffected (Comer et al., 2020). This evidence is in line with previous work showing that

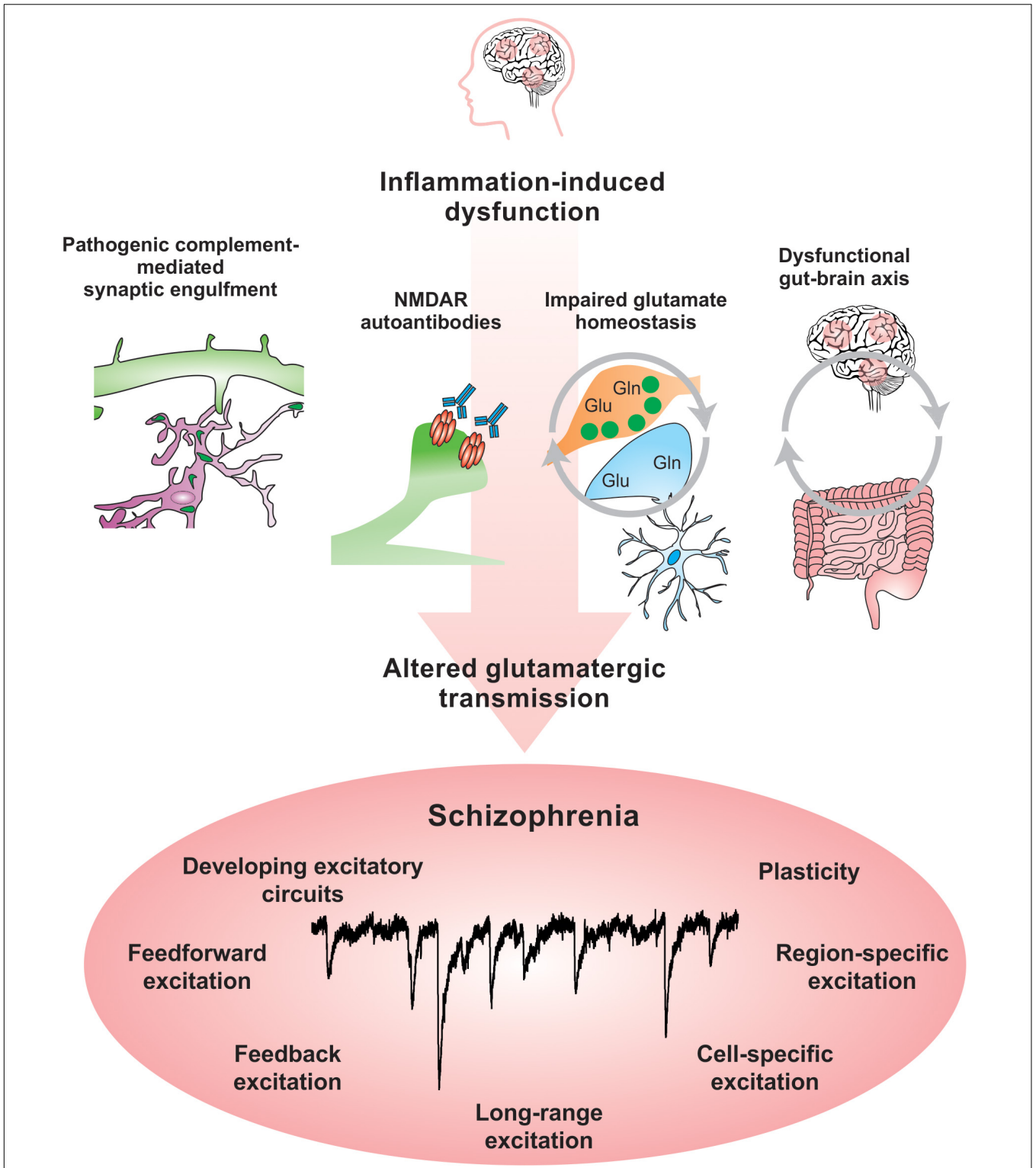

FIGURE 4 | Neuroinflammation-induced dysfunctions that alter glutamatergic transmission in SCZ. Changes in glutamatergic transmission are known to occur in SCZ. Neuroinflammation impacts excitatory circuitry in SCZ through complement-mediated engulfment of excitatory synapses, the production of autoantibodies against NMDARs, changes in glutamate homeostasis potentially mediated by alterations in astrocytes and changes in the gut-brain axis, a known regulator of glutamate synthesis that is able to impact CNS functions such as stress responses. These changes in excitatory transmission can alter brain circuitry, for example, by altering synaptic plasticity and long-range excitation.

complement-dependent synapse removal is activity-dependent and that connections with less activity are more likely to be eliminated (Schafer et al., 2012). Small spines were also shown to be preferentially contacted and eliminated upon microglial contact *in vivo* (Tremblay et al., 2010). Additionally, it has been suggested that immune signaling is able to protect more mature spines. There is increased expression of the "don't eat me" signal CD47 at synaptic inputs that are more active (Lehrman et al., 2018). In this way, the immune system would guide synaptic wiring by tagging synapses for removal while protecting other connections that are essential to the function of a circuit. However, more work is needed to support this idea and understand the mechanisms by which the immune system contributes to synapse-specific elimination versus stabilization.

It is also possible that in SCZ, spine loss is driven by the inability of circuits to produce "appropriate" connections. Therefore, the subsequent excessive pruning that occurs in SCZ could be due to the fact that neurons fail to produce adequate connections in the first place. This is relevant in the context of microglia since they regulate synaptogenesis (Miyamoto et al., 2016). Since most of the data obtained from people with SCZ is limited to post-mortem tissue, our information about what is happening on the circuit and synaptic level during development is limited. In this scenario, using mouse models to understand the role of microglia and immune signaling in synapse formation during the first weeks of postnatal development, when most synaptogenesis occurs (Cruz-Martín et al., 2010), is key. Future advances in the resolution and capabilities of *in vivo* human imaging studies notably through specific markers could help answer this question. It is encouraging that previous studies show a similar phenotype in mice that is seen in humans in terms of weaker synapses preferentially being eliminated (MacDonald et al., 2017; Comer et al., 2020). This could allow for studies in mice that more readily translate to humans. Understanding the mechanisms of complement-mediated circuit wiring is a worthwhile area of future study given it is both a mechanism of normal brain development and is implicated in multiple neurodevelopmental and neurodegenerative diseases. Lastly, enthusiasm has grown over the last decade to study marmosets in neuroscience research and an increase in the feasibility of genetic manipulations could provide an additional model to study how abnormal neuroimmune signaling contributes to SCZ (Okano et al., 2016; Servick, 2018).

Risk Factors Likely Interact Synergistically to Increase Odds for Developing SCZ

Here, we have highlighted the diverse risk factors for SCZ and how they impact the CNS by altering immune signaling. Likely, these risk factors act additively on certain signaling pathways to push vulnerable individuals past a certain threshold into a disease state. This field would benefit from future studies that aim to elucidate how the immune system regulates specific circuits and neuromodulatory systems to drive the diverse phenotypes observed in SCZ. Additionally, an in-depth understanding of the specific signaling networks compromised in SCZ may enable the restoration of typical immune-driven neurodevelopment after exposure to the various genetic and environmental risk factors described in this review.

AUTHOR CONTRIBUTIONS

AC, MC, M-ÈT, and AC-M wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Reelin Supplementation Into the Hippocampus Rescues Abnormal Behavior in a Mouse Model of Neurodevelopmental Disorders

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In the majority of schizophrenia patients, chronic atypical antipsychotic administration produces a significant reduction in or even complete remission of psychotic symptoms such as hallucinations and delusions. However, these drugs are not effective in improving cognitive and emotional deficits in patients with schizophrenia. Atypical antipsychotic drugs have a high affinity for the dopamine D_2 receptor, and a modest affinity for the serotonin 5-HT_{2A} receptor. The cognitive and emotional deficits in schizophrenia are thought to involve neural networks beyond the classical dopaminergic mesolimbic pathway, however, including serotonergic systems. For example, mutations in the *RELN* gene, which encodes Reelin, an extracellular matrix protein involved in neural development and synaptic plasticity, are associated with neurodevelopmental disorders such as schizophrenia and autism spectrum disorder. Furthermore, hippocampal Reelin levels are down-regulated in the brains of both schizophrenic patients and in rodent models of schizophrenia. In the present study, we investigated the effect of Reelin microinjection into the mouse hippocampus on behavioral phenotypes to evaluate the role of Reelin in neurodevelopmental disorders and to test a therapeutic approach that extends beyond classical monoamine targets. To model the cognitive and emotional deficits, as well as histological decreases in Reelin-positive cell numbers and hippocampal synaptoporin distribution, a synaptic vesicle protein, offspring that were prenatally exposed to maternal immune activation were used. Microinjections of recombinant Reelin protein into the hippocampus rescued impairments in object memory and anxiety-like behavior and recruited synaptoporin in the hippocampus in offspring exposed to antenatal inflammation. These results suggest that Reelin supplementation has the potential to treat cognitive and emotional impairments, as well as synaptic disturbances, in patients with neurodevelopmental disorders such as schizophrenia.

Keywords: maternal immune activation, Reelin, schizophrenia, autism spectrum disorder, neurodevelopmental disorders

INTRODUCTION

Schizophrenia affects up to 1% of the population and can cause life-long disability (Jaaro-Peled et al., 2010). Monoaminergic neurotransmitters are heavily implicated in the pathophysiology of schizophrenia and other psychotic disorders. Atypical antipsychotic drugs have a high affinity for the dopamine D_2 receptor and serotonin 5-HT_{2A} receptor (Miyamoto et al., 2005) and chronic administration of these drugs can significantly reduce or even completely remit positive psychotic symptoms, such as hallucinations and delusions (Miyamoto et al., 2005; Meltzer, 2013).

Despite some benefits of antipsychotic drugs, they do not often effectively treat the cognitive and emotional symptoms that the majority of schizophrenia patients also have (Nielsen et al., 2015; Howells et al., 2017; Ibi et al., 2017). Cognitive impairment in schizophrenia patients is selective and often includes dysfunction in attention, executive function, and working memory (Rodriguez-Blanco et al., 2017). Emotional symptoms, such as anxiety, are associated with more severe clinical features and worse outcomes (Temmingh and Stein, 2015). Deficits in cognitive processes and difficulties with emotional adjustment account for a significant proportion of psychosocial disabilities in patients with schizophrenia (Millan et al., 2012). Novel therapeutic drugs with strategic targets beyond the classical monoaminergic pathway are required to improve both cognitive deficits and emotional symptoms.

Reelin (*RELN* gene) is a large, secreted extracellular matrix glycoprotein that is a critical player in the modulation of neuronal development, synaptic plasticity, and spine formation/remodeling across the lifespan. During prenatal development, Reelin is expressed and secreted from Cajal-Retzius neurons in the outer layers of the developing cortex. Here, Reelin guides newly born neurons to their correct positions in an inside-out fashion (Frotscher, 2010). During postnatal development, when the Cajal-Retzius cells begin to die out in the cortex and hippocampus (Del Rio et al., 1996) inhibitory GABAergic interneurons begin to express and secrete Reelin (Pesold et al., 1998). This postnatally secreted Reelin acts to modulate axonal and dendritic outgrowth by regulating cytoskeleton stability via multiple independent and interconnected pathways (Wasser and Herz, 2017). In clinical studies, genetic linkage analyses have implicated *RELN* polymorphisms in the pathophysiology of neurodevelopmental diseases such as schizophrenia and autism spectrum disorder (ASD) (Ishii et al., 2016). Postmortem studies have also revealed decreased Reelin expression in the brains and the cerebrospinal fluid of patients with schizophrenia and ASD (Knuesel, 2010; Ishii et al., 2016). These studies suggest that down-regulation of Reelin signaling contributes to the pathophysiology of neurodevelopmental disorders and also raises the possibility that treating Reelin deficiency may help to improve schizophrenia and ASD symptoms (Rogers et al., 2013). Reelin injection into the brain to activate Reelin signaling pathways may thus be a novel therapeutic strategy for the treatment of neurodevelopmental disorders, though this remains untested.

Early disruptions of neurodevelopment contribute to both future psychiatric risk and to the underlying pathophysiology of neurodevelopmental disorders (Kushima et al., 2018; Gumusoglu and Stevens, 2019). There is evidence from multiple clinical studies that demonstrates an association between maternal inflammation during pregnancy and the development of neurodevelopmental disorders in offspring, including schizophrenia (Moreno et al., 2011; Brown, 2012; Kannan and Pletnikov, 2012) and ASD (Patterson, 2011).

Preclinical models of maternal immune activation (MIA) have been developed to examine the physiological mechanisms responsible for this association (Shi et al., 2003; Moreno et al., 2011). One common method of MIA is maternal administration of polyinosinic: polycytidylic acid (poly I:C), which is an agonist of toll-like receptor 3. Poly I:C activates the innate immune system in a manner similar to viral double-stranded RNA, which is produced in viral infection during the genetic replication of single-stranded RNA or as a secondary transcript by DNA viruses (Takeuchi and Akira, 2007).

Behavioral and anatomical differences in the offspring of pregnant mice or rats treated with poly I:C during gestational days (GDs) 9–17 (Shi et al., 2003; Meyer et al., 2005, 2006; Zuckerman and Weiner, 2005; Smith et al., 2007; Holloway et al., 2013), which roughly corresponds to late in the first and early in the second trimester in humans, have been analyzed. This method of MIA increases risk for both schizophrenia and ASD (Ibi and Yamada, 2015). Accumulating evidence demonstrates that the offspring of pregnant mice or rats treated with poly I:C exhibit augmented psychostimulant-induced hyperactivity and impairments in social interaction, prepulse inhibition (PPI), and memory (Meyer et al., 2005; Zuckerman and Weiner, 2005; Smith et al., 2007; Meyer, 2013). These behavioral phenotypes are thought to correspond to cognitive dysfunction and some domains of positive and negative symptoms in patients with schizophrenia (Arguello and Gogos, 2006).

Some characteristic neuropathological features of schizophrenia are also seen in the offspring of mothers treated with polyI:C during pregnancy (Gonzalez-Maeso et al., 2008; Jaaro-Peled et al., 2010). These features include a decrease in the number of Reelin-positive cells in the frontal cortex and hippocampus, reduced dendritic spine density, decreased dopamine D_1 and metabotropic glutamate 2 receptors, increased 5-HT_{2A} receptors in the prefrontal cortex, loss of parvalbumin (PV) in the hippocampal interneurons, and enhanced tyrosine hydroxylase in the striatum (Holloway et al., 2013; Meyer, 2013; Ibi and Yamada, 2015).

To evaluate the effects of Reelin protein signaling activation in the brain, we injected recombinant Reelin protein into the hippocampi of adult offspring of pregnant C57BL/6J mice that were intraperitoneally treated with poly I:C on GD 9. To the best of our knowledge, this is the first experimental study to investigate the effects of Reelin signaling activation as a therapeutic strategy for cognitive and emotional deficits and synaptic disturbances in an MIA-induced preclinical model of neurodevelopmental disorders.

MATERIALS AND METHODS

Animals

Pregnant C57BL/6J mice at GD 6–7 were obtained from Japan SLC Inc. (Hamamatsu, Japan). Dams were housed individually and habituated to the testing facility for a few days before experimental use. Mice were kept in a regulated environment (24 ± 1°C, 55 ± 5% humidity) under a 12-h light/dark cycle (lights on 7:45 a.m.) and provided food and tap water *ad libitum*. All experimental protocols including the use of laboratory animals were approved by the Animal Ethics Board of Meijo University and followed the guidelines of the Japanese Pharmacological Society (Folia Pharmacol. Japan, 1992, 99: 35A); the Interministerial Decree of May 25th, 1987 (Ministry of Education, Japan); and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Maternal Immune Activation (MIA) Model

At GD 9, pregnant mice received intraperitoneal injections of poly I:C (20 mg/kg; Sigma-Aldrich, St. Louis, MO) (i.p.), as previously (Esslinger et al., 2016; Gonzalez-Liencres et al., 2016). Control mice received intraperitoneal injections of sterile saline (0.9%). After treatment, pregnant mice were singly housed until parturition. Offspring were weaned and sexed on postnatal day (PD) 28. Males and females were caged separately, and littermates of the same sex were housed in groups of 3–6 animals per cage. Both experimental groups (vehicle and poly I:C) were composed of multiple independent litters to prevent litter effects. All litters included offspring of both sexes.

Purification of Full-Length Reelin

Recombinant Reelin protein was prepared using the Expi293 Expression System (Thermo Fisher Scientific, MA). Expi293F cells were transfected with a full-length mouse Reelin protein, which was fused at the N-terminal with a PA tag (Fujii et al., 2014). After 4 days, the conditioned medium containing the recombinant Reelin was harvested. To purify recombinant Reelin, the conditioned medium was incubated with anti-PA tag antibody (Wako, Osaka, Japan) coated-beads at 4°C overnight. Purified Reelin was then concentrated approximately 10-fold by an Amicon Ultra centrifuge filter (100,000 molecular weight cut-off, Merck, Darmstadt, Germany). To estimate the concentration, Reelin was detected by Coomassie blue staining and compared to bovine serum albumin (Ishii et al., 2015).

Stereotactic Hippocampal Reelin Injections

Offspring were anesthetized with a mixture (i.p.) of medetomidine hydrochloride (0.3 mg/kg; Wako), midazolam (4 mg/kg; Wako), and butorphanol tartrate (5 mg/kg; Wako), and then positioned between the ear bars of a stereotaxic frame (SR-6N; Narishige, Tokyo, Japan). Full-length Reelin (0.2 pmol/0.5 μL) protein or PBS (control) was delivered bilaterally with a Hamilton syringe at a rate of 0.1 μL/min for a total volume of 0.5 μL on each side. The needle was left in place for 5 min. Injection volume and concentration of recombinant Reelin protein was determined using previous studies (Rogers et al., 2011, 2013; Ishii et al., 2015). The following coordinates were used: -1.75 mm rostrocaudal, -2.0 mm dorsoventral, ±1.0 mm mediolateral from bregma (relative to dura). Immediately after removal of the needle, the skin was closed with tissue adhesive (Vetbond, 3M, St. Paul, MN). The injection point was confirmed by methylene blue microinjection (**Supplementary Figure S1**).

Behavioral Analyses

Behavioral analyses in the offspring of both saline- and poly I:C-treated dams were conducted at 8–20 weeks of age. To test for a rescue of the cognitive and emotional deficits seen in the MIA model of schizophrenia by recombinant Reelin injection into the hippocampus, we used a novel object recognition test on Day 4 post Reelin microinjection (Day 0). We then used the open-field test on Day 10 post microinjection.

Novel Object Recognition Test

Novel object recognition, a spontaneous form of memory, is derived from curiosity about novel objects (Leger et al., 2013). A novel object recognition test was performed, as described previously (Ibi et al., 2017). Male and female mice were individually habituated to an open box (30 × 30 × 35 cm high) for 3 days. During training sessions, two novel objects were placed in the open field and animals were allowed to explore the objects for 10 min under moderate light (20 lux).

During retention sessions, animals were placed into the same box 24 hr after the training session. One of the familiar objects used during the training sessions was replaced by a novel object, and the mice were allowed to explore the box freely for 5 min. A preference index for the retention session (the ratio of time spent exploring the novel object over the total time spent exploring both objects) was calculated to measure novel object recognition. During the training session, a preference index was also calculated as the ratio of time spent exploring the object that would be replaced by a novel object in the retention session to the total exploration time. The time spent exploring each object in both sessions was also recorded on video for subsequent blind scoring.

Open-Field Test

The open-field test poses a conflict between the endogenous mouse exploratory drive and their aversion to exposure in open, illuminated open areas. This test was used to examine both anxiety-like and locomotor behavior (Ibi et al., 2009). Mice were placed in the center of a square open arena (50 × 50 cm, wall height: 35 cm) and allowed to explore it for 60 min under bright illumination conditions (80 lux). Mouse activity was measured automatically using the EthoVision automated tracking program (Noldus Information Technology, Sterling, VA) (Ibi et al., 2009; Udagawa et al., 2015). The open-field was further divided into an inner square (40 × 40 cm) and an outer area (50 × 50 cm), which surrounded the inner square. Mouse movement was measured via a camera mounted directly above the open-field.

Measurements included distance and time spent in the inner and outer sections.

Spontaneous Locomotor Activity

Each mouse was placed in a standard transparent rectangular rodent cage (25 × 30 × 18 cm) under moderate illumination conditions (15 lux). Locomotor activity was measured for 120 min using an automated system of digital counters with infrared sensors (Scanet SV-10; Melquest Co., Ltd., Japan).

Immunohistochemistry

Mice were deeply anesthetized with ethyl carbamate (1.5 g/kg i.p., Katayama Chemical, Osaka, Japan) and perfused transcardially with saline, followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). Mouse brains were removed, post-fixed in the same fixative, and then cryoprotected. Twenty micrometer-thick coronal free-floating brain sections were made using a cryostat. Free-floating sections were transferred to 24-well dishes containing PBS. After blocking with 10% goat serum/PBS for 60 min, mouse anti-synaptophysin (Sigma-Aldrich SAB4200544, 1:500), rabbit anti-synaptoporin (Synaptic Systems, Göttingen, Germany 102002, 1:500), mouse anti-Reelin (Merck-Millipore MAB5364, 1:1000), mouse anti-PV (Sigma-Aldrich P3088, 1:2000), and rat anti-somatostatin (SST) antibodies (Merck-Millipore MAB354, 1:350) diluted in 10% goat serum/PBS were applied to the sections, which were then incubated overnight at 4°C. After washing in PBS, goat anti-mouse Alexa Fluor 568 and anti-rabbit Alexa Fluor 488 antibodies (1:3000; Invitrogen, Eugene, OR) were added to the sections for 2 hr at room temperature. Samples were imaged using an all-in-one Fluorescence Microscope (BZ-700, Keyence, Osaka, Japan) and a confocal-laser scanning microscope (LSM 800; Zeiss, Jene, Germany).

For the quantification of synaptophysin immunoreactivity, mean immunoreactivity signal intensity (Bregma -1.70 to -2.18 mm) in the hippocampus was measured using NIH image 1.62 software (Guan et al., 2009). For the quantification of synaptoporin-immunostained area, the area of immunoreactive region in the hippocampal DG (Bregma -1.70 to -2.18 mm) was measured using Keyence BZ-X Analyzer software (Keyence) according to previous reports (Latchney et al., 2014; Zuko et al., 2016). For quantification of the number of Reelin-, PV- and SST-positive cells, the number of hippocampal immunoreactive cells (Bregma -1.70 to -2.18 mm) in both hemispheres was counted. These analyses were performed by a blinded experimenter.

Statistical Analyses

Statistical analyses were performed and figures were produced using Prism software version 6 (GraphPad Software, Inc., San Diego, CA). As it was not possible to assume that behavioral data had a Gaussian distribution, these data are expressed as medians and interquartile ranges. Group-wise differences were evaluated using the Mann-Whitney *U*-test for comparisons between two groups.

Quantitative values obtained from immunohistochemistry are expressed as means ± standard errors (SEs). Unpaired *t*-tests were used to compare two groups.

Two-way ANOVAs followed by Tukey-Kramer *post hoc* test was used for multiple comparisons where relevant (e.g., Reelin injection and maternal poly I:C treatment). The percentage of aborted pregnancies was evaluated using a chi-square (x^2) test. The criterion for statistical significance was $p < 0.05$.

RESULTS

General Appearance of MIA Offspring and Conditions of MIA Pregnancy

After acute administration of 20 mg/kg polyI:C (i.p.) at GD 9, pregnant C57BL6J mice had a significantly increased percentage of aborted pregnancies [vehicle 25% (2/8), poly I:C 86% (43/50); $x^2 = 14.76$, $p < 0.001$], including either fetal resorptions (abdominal collapse without abortion of the dead fetuses) or frank abortions (premature discharge of dead fetuses). This result was consistent with a previous study (Ehninger et al., 2012). MIA did not affect adult offspring body weight at 9–12 weeks of age (mean ± SE: vehicle 23.53 ± 0.68 g, poly I:C 23.65 ± 0.34 g, $p = 0.88$). Moreover, offspring with MIA were viable and did not display any gross histological abnormalities in the hippocampus (data not shown). These results suggest that MIA negatively affected birth rate, but not offspring growth or the gross structure of offspring hippocampal layers.

Hippocampal Reelin Expression in MIA Offspring

Given a previous study demonstrating that MIA decreases the number of Reelin-positive cells in the hippocampus (Meyer et al., 2006), we examined Reelin expression in the hippocampi of adult offspring here. MIA decreased the number of Reelin-positive cells in the DG of the adult hippocampus but not in the CA1 or CA3 regions (**Figures 1A,B**). There was also no difference in the number of Reelin-positive cells in the medial prefrontal cortex between control and MIA offspring (mean ± SE: vehicle 8.41 ± 1.06, poly I:C 7.63 ± 1.40, $p = 0.65$) despite the fact that Reelin is a key modulator of cortical development and lamination (Herz and Chen, 2006).

Dysregulation of inhibitory GABAergic interneuron populations has been implicated in psychiatric disorders, such as schizophrenia, anxiety disorders, and ASD (Tremblay et al., 2016). GABAergic interneurons are classified as PV-, SST-, and serotonin 5-HT_{3A} receptor-positive interneurons. SST-positive neuronal population consist exclusively of Reelin-positive cells (Tremblay et al., 2016). We thus investigated the co-localization of Reelin and SST in the hippocampal DG of MIA offspring. We found that the percentage of Reelin-positive cells among SST-positive interneurons in MIA offspring was smaller than the percentage in controls (**Figures 1C,D**). Furthermore, the number of Reelin/SST double-positive cells (mean ± SE: vehicle 8.18 ± 0.88, poly I:C 4.26 ± 0.67, $p < 0.001$) and SST-positive cells (means ± SE: vehicle 16.36 ± 1.78, poly I:C 11.13 ± 0.80,

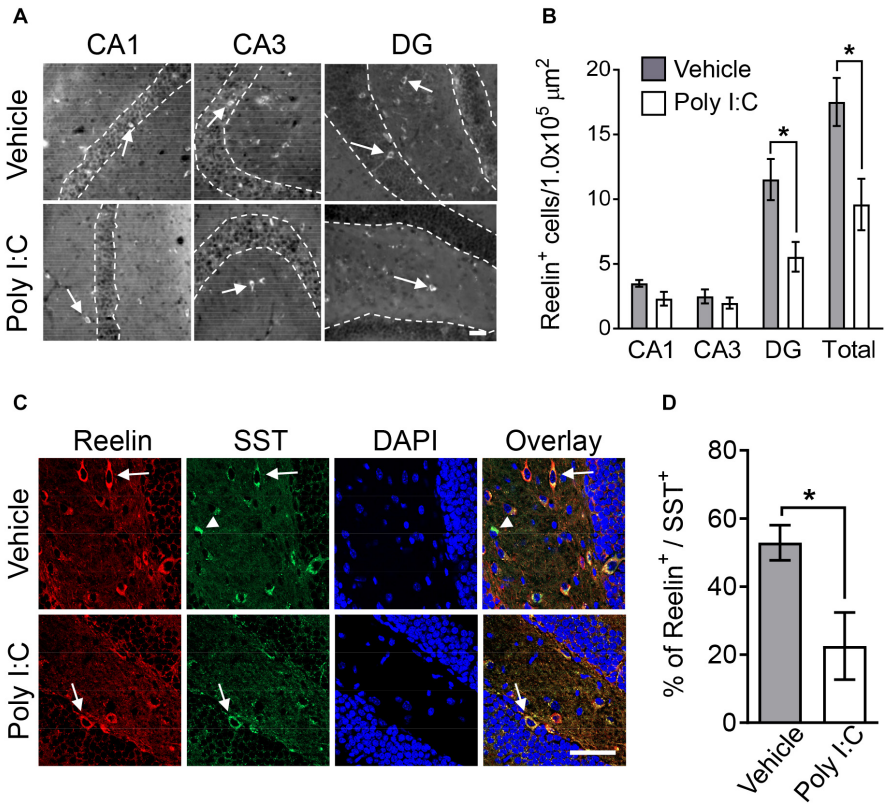

FIGURE 1 | Hippocampal Reelin expression in MIA offspring. Representative photographs showing Reelin-positive cells (arrows) in hippocampal subregions **(A)**. Quantification of Reelin-positive neuron numbers in hippocampal subregions **(B)**. Representative photographs of double-immunostained Reelin- and SST-positive cells (arrows: Reelin-positive/SST-positive, arrowhead: Reelin-negative/SST-positive) in offspring hippocampi **(C)**. Percentage of Reelin-positive cells among SST-positive cells in offspring hippocampi **(D)**. Values are means ± SE [Reelin staining n = 6–9 (vehicle: 5 males and a female, poly I:C: 7 males and 2 females), Reelin and SOM double-staining n = 3 (each group: 2 males and a female); from three independent dams]. Two-tailed t-test comparing MIA offspring vs. vehicle-treated control group offspring **(B,D)**. *$p < 0.05$. Scale bar: 50 μm.

$p < 0.01$) in the hippocampal DG was also significantly decreased by MIA.

Previous research has implicated deficits in PV-positive GABAergic interneurons in the pathogenesis of schizophrenia (Jaaro-Peled et al., 2010). Thus, we investigated the number of hippocampal PV-positive interneurons in MIA offspring here. Offspring hippocampal PV-positive interneuron numbers were not changed by MIA (mean ± SE; CA1 region: vehicle 4.06 ± 0.34, poly I:C 3.40 ± 0.72, $p = 0.35$; CA3 region: vehicle 3.86 ± 0.53, poly I:C 2.45 ± 0.62, $p = 0.11$; DG: vehicle 1.85 ± 0.29, poly I:C 2.55 ± 0.35, $p = 0.14$). Together, these results demonstrate that MIA reduced both Reelin- and SST-positive interneurons, but did not change the number of PV-positive neurons.

Hippocampal Synaptic Proteins in MIA Offspring

Multiple lines of evidence implicate synaptic dysfunction in the hippocampus in schizophrenia symptomatology (Tamminga et al., 2012; Osimo et al., 2018). For example, decreased hippocampal synaptic protein and mRNA levels in patients with schizophrenia have been previously reported (Osimo et al., 2018). Thus, we examined levels of hippocampal synaptic markers in offspring with MIA in the present study.

Immunoreactivity to synaptophysin (also known as synaptophysin 1), a presynaptic terminal marker of active synapses (Guan et al., 2009), was not changed in any hippocampal subregion by MIA (**Figures 2A,B**). However, immunoreactive area size for synaptoporin, a marker enriched in mossy fiber tracts (also known as synaptophysin 2) (Romer et al., 2011), was significantly decreased in MIA offspring compared to control mice (**Figures 2C,D**). These findings agree with previous reports of decreased mossy fiber synapse numbers in the hippocampus in schizophrenics (Kolomeets et al., 2007).

Effect of Reelin Injection on Behavioral Abnormalities in MIA Offspring

Given that hippocampal Reelin plays a crucial role in adult neurogenesis, synaptic plasticity and granule cell malformation (Frotscher, 2010; Jakob et al., 2017), a decrease in the number of Reelin-positive cells (**Figure 1**) may affect hippocampal function. Therefore, we examined the effects of increased exogenous

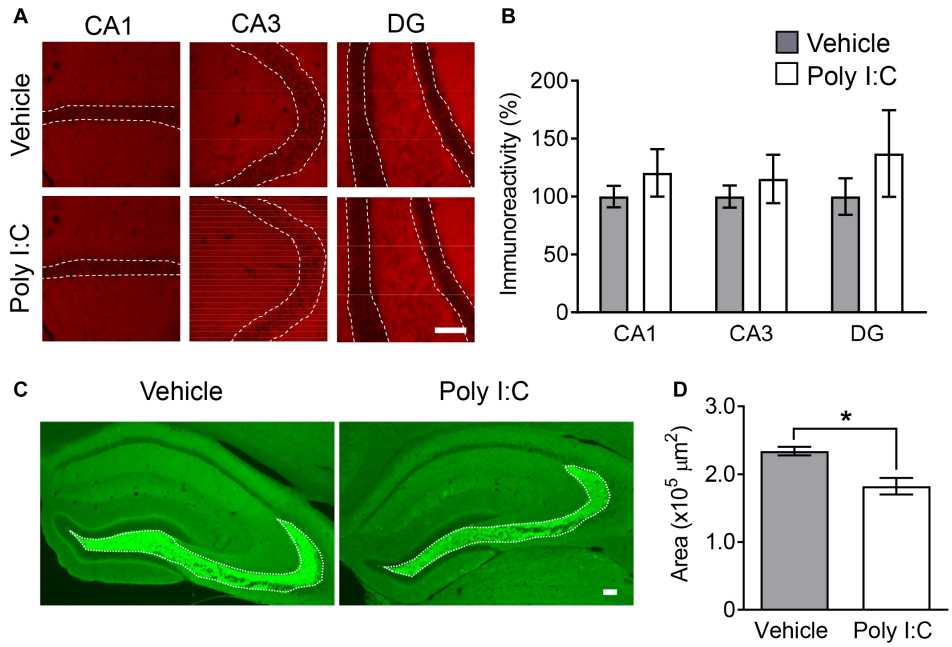

FIGURE 2 | Hippocampal synaptic markers in MIA offspring. Representative photographs showing synaptophysin **(A)** and synaptoporin **(C)** immunoreactivity in offspring hippocampi. Quantification of offspring hippocampal synaptophysin **(B)** and synaptoporin **(D)** immunoreactivity. Values are means ± SE [synaptophysin staining $n = 4$ (each group: 4 males), synaptoporin staining (each group: 2 males and a female); from three independent dams)]. Two-tailed *t*-test comparing MIA offspring vs. vehicle-treated control group offspring **(B,D)**. $^*p < 0.05$. Scale bar: 100 μm.

intra-hippocampal Reelin on behavior and hippocampal mossy fiber synapse numbers in MIA offspring. The novel object recognition test was carried out 4 days after Reelin injections and followed by the open-field test on Day 10 post-injection (**Figure 3A**). This schedule is based on previous studies in which behavioral assays were performed 5–10 days after Reelin microinjection into the lateral ventricle (Rogers et al., 2011, 2013; Ishii et al., 2015).

During novel object recognition task training sessions, all groups exhibited nearly equal exploratory preference for each object (**Figure 3B**: $F_{Poly\,I:C(1,34)} = 0.704$, $p = 0.41$; $F_{Reelin(1,34)} = 3.32$, $p = 0.078$; $F_{Interaction(1,34)} = 0.0028$, $p = 0.96$), indicating that neither MIA nor Reelin injection itself biased object preferences. In the test session, which was carried out 24 hr after the training session, two-way ANOVA showed a significant interaction effect between maternal poly I:C treatment and recombinant Reelin injection ($F_{Poly\,I:C(1,34)} = 1.576$, $p = 0.22$; $F_{Reelin(1,34)} = 0.30$, $p = 0.59$; $F_{Interaction(1,34)} = 11.45$, $p = 0.0018$) and *post hoc* analysis revealed that MIA-exposed offspring exhibited significantly lower exploratory preference for the novel object than did control mice (**Figure 3B**). This suggests that MIA impairs object recognition in offspring.

Deficits in object recognition were rescued with hippocampal Reelin injection, as these mice had significantly higher exploratory preference to the novel object than those of MIA-exposed offspring without Reelin supplementation. Finally, there was no difference in total object exploration time between the training and test sessions among all groups (**Figure 3C**: Training session $F_{Poly\,I:C(1,34)} = 0.11$, $p = 0.74$; $F_{Reelin(1,34)} = 1.042$, $p = 0.31$; $F_{Interaction(1,34)} = 1.27$, $p = 0.27$, Test session $F_{Poly\,I:C(1,34)} = 0.56$, $p = 0.46$; $F_{Reelin(1,34)} = 0.16$, $p = 0.69$; $F_{Interaction(1,34)} = 0.33$, $p = 0.57$), suggesting that neither MIA nor Reelin injection affected exploratory behaviors and/or motor function. Taken together, these results demonstrate that MIA impaired object recognition in offspring, which was rescued by hippocampal Reelin injection.

In the open-field test, mice were allowed to explore the open field freely for 60 min, as previously (Audero et al., 2013). Since hyperactivity was observed in MIA offspring during the first 30 min in a novel environment, their exploratory behavior was analyzed only in the final 30 min (**Supplementary Figure S2**: $F_{Poly\,I:C(1,126)} = 15.42$, $p = 0.0001$; $F_{Time(5,126)} = 5.30$, $p = 0.0002$; $F_{Interaction(5,126)} = 0.72$, $p = 0.61$). Two-way ANOVA showed a significant interaction effect between maternal poly I:C treatment and recombinant Reelin injection ($F_{Poly\,I:C(1,36)} = 2.71$, $p = 0.11$; $F_{Reelin(1,36)} = 0.73$, $p = 0.40$; $F_{Interaction(1,36)} = 12.45$, $p = 0.0012$) and *post hoc* analysis revealed that MIA offspring exhibited a significantly lower ratio of time spent in the inner area divided by that in the outer area (inner/outer ratio) than vehicle-treated control offspring. However, hippocampal Reelin treatment significantly increased ratio of time spent in the inner to outer zones of the open field among MIA-exposed offspring (**Figure 3D**). There was no difference in the total distance traveled among all groups (**Figure 3E**: $F_{Poly\,I:C(1,36)} = 2.12$, $p = 0.15$; $F_{Reelin(1,36)} = 0.29$, $p = 0.59$; $F_{Interaction(1,36)} = 1.77$, $p = 0.19$), suggesting that neither MIA nor hippocampal Reelin treatment affected exploratory behaviors or motor function in the open-field test. These results demonstrate that MIA increased

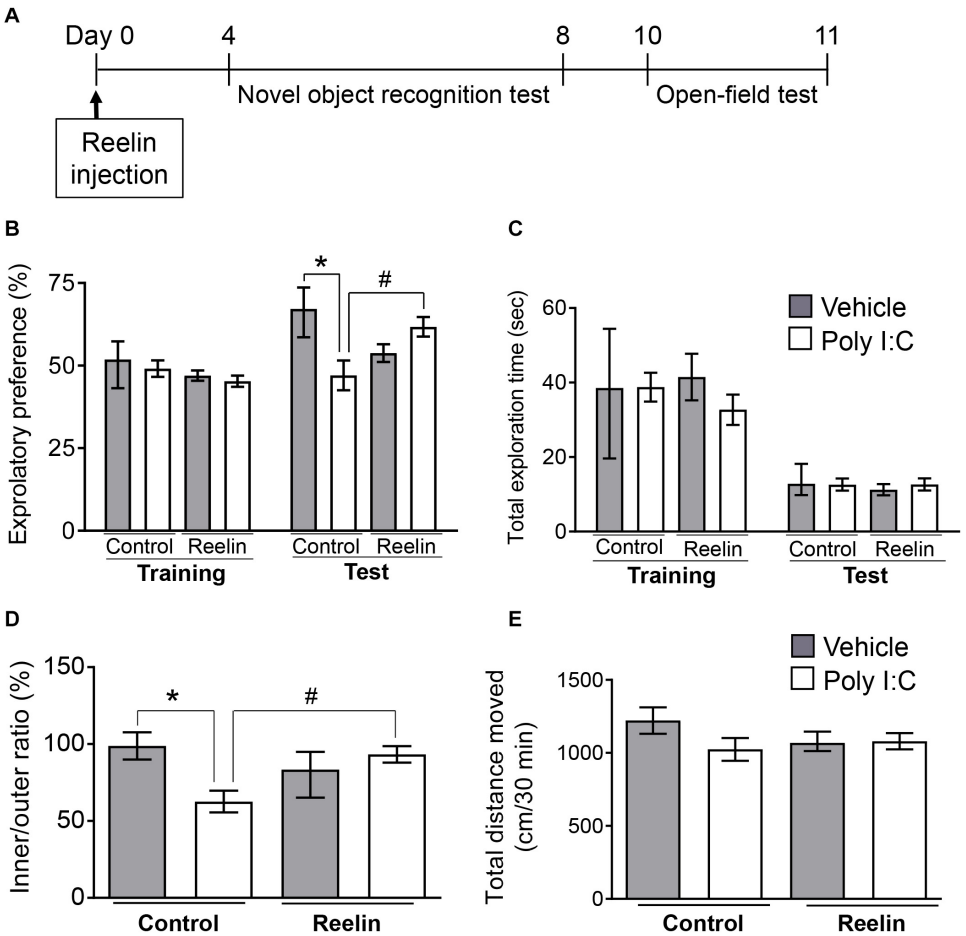

FIGURE 3 | Effect of Reelin injection into the hippocampus on the abnormal behavior of MIA offspring. Experimental schedule for Reelin injection into the hippocampus and subsequent assays **(A)**. Exploratory preference **(B)** and total exploration time **(C)** on the novel object recognition test. The test session was carried out 24 h after the training session. The time spent in the inside/outside (inner/outer ratio) of the field **(D)** and total distance traveled **(E)** in the open-field test, in which mice were allowed to explore the open field freely for 60 min. Exploratory behaviors were analyzed only in the final 30 min of the task due to consistent hyperactivity during the first 30 min. Values represent medians and interquartile ranges [novel object recognition test n = 8–12 (control-vehicle: 4 males and 4 females, control-poly I:C: 5 males and 5 females, Reelin-vehicle: 4 males and 4 females, reelin-poly I:C: 6 males and 6 females), open-field test n = 8–12 (control-vehicle: 4 males and 4 females, control-poly I:C: 6 males and 6 females, reelin-vehicle: 5 males and 5 females, reelin-poly I:C: 6 males and 6 females); from three or more independent dams]. Tukey-Kramer test $^{*}p < 0.05$ in the comparison between MIA offspring with hippocampal Reelin injection vs. vehicle-treated control group, $^{\#}p < 0.05$ for MIA offspring with hippocampal Reelin vs. PBS treatment.

anxiety-like behaviors in offspring, which were suppressed by hippocampal Reelin injection.

Effect of Reelin Injection on Synaptoporin Levels in MIA Offspring

Thirteen days post-surgery, we tested whether Reelin injection into the hippocampus reversed the down-regulation of synaptoporin-immunostained area in MIA offspring (**Figure 4A**). Two-way ANOVA showed a significant interaction effect between maternal poly I:C treatment and recombinant Reelin injection (**Figure 4C**: $F_{\text{Poly I:C}(1,14)} = 0.42$, $p = 0.53$; $F_{\text{Reelin}(1,14)} = 1.75$, p = 0.21; $F_{\text{Inteaction}(1,14)} = 11.19$, $p < 0.01$) and *post hoc* analysis revealed that the hippocampal synaptoporin-immunopositive area tended to decrease in offspring with MIA compare to controls (**Figure 4B**), which is consistent with **Figures 2C,D**. Meanwhile, the decrease of immunoreactive area to synaptoporin in offspring with MIA was significantly reversed by Reelin injection into the hippocampus (**Figure 4C**), demonstrating that Reelin supplementation may reverse MIA-induced mossy fiber deficits.

DISCUSSION

In the 65 years since the first antipsychotic was discovered, inhibition of neurotransmission via the dopamine receptor has proven to be an important strategy for the treatment of schizophrenia (Miyamoto et al., 2005; Stroup et al., 2006). However, these therapies are only effective in two-thirds of all patients, and their efficacy is limited by the lack of an effect on negative and cognitive symptoms (Nielsen et al., 2015;

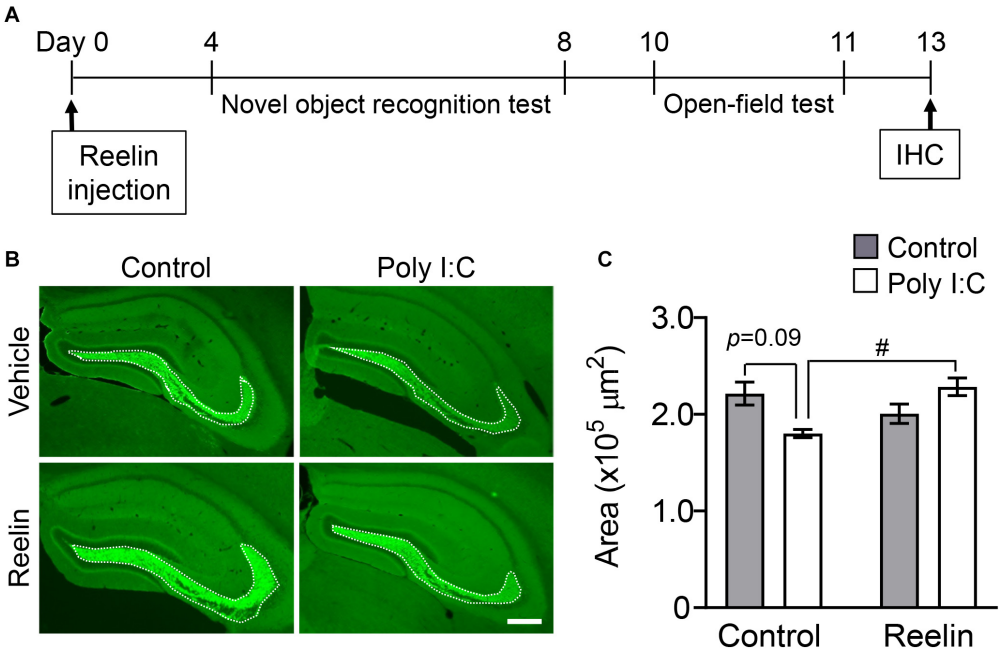

FIGURE 4 | Effect of Reelin injection into the hippocampus on synaptoporin levels in MIA offspring. Experimental schedule of Reelin injection into the hippocampus and subsequent assays **(A)**. Representative photographs showing synaptoporin immunoreactivity in the hippocampi of MIA offspring **(B)**. Quantification of hippocampal synaptoporin immunoreactivity in offspring **(C)**. Values indicate means ± SE [n = 3–6 (control-vehicle: 3 males and a female, control-poly I:C: 3 males, reelin-vehicle: 3 males and 2 females, reelin-poly I:C: 3 males and 3 females); from three independent dams]. Tukey-Kramer test $^{\#}p < 0.05$ in the comparison between MIA offspring with hippocampal Reelin injection vs. PBS treatment. Scale bar: 300 μm. IHC: Immunohistochemistry.

Howells et al., 2017). Therefore, there is an urgent need to identify new molecular targets to address the various dimensions of schizophrenia symptoms. In the present study, Reelin injection into the hippocampus reversed impairments in object recognition memory and anxiety-like behavior, as well as the decreased in synaptoporin-immunoreactive area in the hippocampus, in an MIA animal model of schizophrenia. A previous study reported similar effects of Reelin, with intraventricular injections of Reelin rescuing synaptic dysfunction and hippocampus-dependent cognitive impairments in a mouse model of Angelman syndrome, a genetic disorder which impairs nervous system development (Hethorn et al., 2015). These results indicate that Reelin may be a new molecular target for the treatment of neurodevelopmental disorders.

It is critical that synaptic disturbances are assessed to better understand the pathophysiology of neurodevelopmental disorders such as ASD and schizophrenia (Pocklington et al., 2014). Disruptions in synaptic formation and remodeling have been observed in the brains of patients with neurodevelopmental disorders (Penzes et al., 2011). In patients with schizophrenia, decreased protein and/or mRNA levels of various synaptic markers (Osimo et al., 2018) have been reported. These changes in expression levels may serve as a marker of synaptic disturbance. In particular, synaptoporin, which is exclusively enriched in granule cell axons, is a synaptic vesicle marker for hippocampal mossy fibers (Romer et al., 2011). Patients with schizophrenia have both decreased synaptoporin levels (Guillozet-Bongaarts et al., 2014; Chang et al., 2017) and mossy fiber deficits (Kolomeets et al., 2007), indicating that synaptoporin expression may be a good marker for hippocampal synaptic disturbances in schizophrenia. In the presents study, we demonstrated synaptoporin deficit in MIA offspring, even in the absence of gross anatomical alterations in the brain or neuronal loss. Our findings support the hypothesis that neurodevelopmental disturbances, but not neurodegeneration, are central to the etiopathogenesis and disease progression of neurodevelopmental disorders.

MIA leads to hippocampal synapse disturbances, which may be rescued by the activation of the Reelin signaling pathway. In double knockout *Reeler* mice, which lack Reelin and the Reelin receptor [apolipoprotein E receptor 2 (ApoER2)/very low density lipoprotein receptor (VLDLR)], there are similar hippocampal histological abnormalities as those that occurred here in MIA offspring. Notably, these abnormalities include the aberrant migration of granular cells, as well as deregulated projections of mossy fibers (Drakew et al., 2002). Given that Reelin-positive interneurons are abundant near the dentate granular cells of the hippocampus, secreted Reelin may activate ApoER2/VLDLR on granular cells in the DG, which could lead to the formation of mossy fibers sprouts from granular cells. Thus, decreased numbers of Reelin-positive cells may play a causative role in the down-regulation of synaptoporin-immunostained area in MIA offspring. This possibility is supported by our present results, in which Reelin injection rescued synaptoporin deficit in the hippocampi of MIA offspring (**Figures 4B,C**).

The downstream Reelin signaling pathway should be considered when theorizing about the molecular mechanisms underlying its possible therapeutic effects. Reelin receptors (ApoER2 and VLDLR) can phosphorylate Disabled-1 (Dab1), which is a Reelin adaptor protein (Herz and Chen, 2006). This pathway downstream of Reelin activates phosphatidylinositol-3-kinase (PI3K) and subsequently stabilizes the cytoskeleton, contributing to cell migration. Further, Reelin activates the N-methyl-D-aspartate (NMDA) receptor, and in turn the cAMP response element binding protein (CREB), leading to enhanced synaptic plasticity and development (Herz and Chen, 2006).

Indeed, mice either with Reelin supplementation into the brain (Rogers et al., 2011) or with overexpression of Reelin (Pujadas et al., 2010) exhibit enhancement of cognition and long-term potentiation (LTP). In addition, single nucleotide polymorphisms (SNPs) in the *APOER2*, *VLDLR*, and *DAB1* genes are associated with cognitive impairments in patients with schizophrenia (Verbrugghe et al., 2012). We have further identified a novel exonic deletion of *RELN* in a patient with schizophrenia (Sobue et al., 2018), and mice with this *RELN* mutation exhibit schizophrenia-like behaviors and histological abnormalities (Sobue et al., 2018). While these results suggest that signaling pathways downstream of Reelin may contribute to its therapeutic effects (Herz and Chen, 2006; Rogers et al., 2011; Verbrugghe et al., 2012; Hethorn et al., 2015), the molecular mechanisms by which Reelin recovers brain dysfunction in MIA remain unknown. Further study in preclinical models of neurodevelopmental disorders is required to clarify the effects of Reelin downstream from its lipoprotein receptors.

Regarding the microinjection of recombinant Reelin, the present study has a limitation not to clarify how far injected recombinant Reelin spread. Instead of recombinant Reelin, we have investigated the diffusion of a dye (methylene blue) injected into the hippocampus (**Supplementary Figure S1**), in which it looks confined to the hippocampal DG, suggesting that recombinant Reelin may stay around the DG following the injection. In addition, we have already found that injection of recombinant Reelin into the hippocampus had no effect on the hippocampal Reelin protein levels in mice at the end of behavioral analyses, 2 weeks after the injection (data not shown), which is consistent with a previous report to indicate that recombinant Reelin injected into the brain is maintained only in a few hours, and subsequently degraded (Rogers et al., Learn Mem 2011). On the other hand, some studies have demonstrated that injection of recombinant Reelin into the brain increases the number of spine and enhances synaptic plasticity even 5–10 days after the injection, which is corelated with the amelioration in cognitive function (Rogers et al., 2011, 2013; Hethorn et al., 2015; Ishii et al., 2015). These previous findings raise a possibility that recombinant Reelin injection into the hippocampus activates the Reelin receptors (i.e., ApoER and VLDLR) around the hippocampal DG, leading to the enhancement of synapse formation/function as well as cognition. These effects may be supposedly maintained in a certain period of time even though injected recombinant Reelin had been degraded in a few hours after the injection. Further study is required to elucidate the mechanism underlying the long-lasting effect of injected Reelin on synapse and cognition.

Prenatal stressors including MIA and physical restraint decrease hippocampal Reelin expression in offspring (Meyer et al., 2006; Palacios-Garcia et al., 2015). DNA hypermethylation of the *RELN* promoter also contributes to Reelin down-regulation (Palacios-Garcia et al., 2015). Clinical evidence supports increased methyl donor S-adenosylmethionine in the brains of patients with schizophrenia (Guidotti et al., 2007), and an association between hypermethylation of the *RELN* promoter (Grayson et al., 2006) and down-regulation of the corresponding protein (Reelin) in the brains of these patients (Guidotti et al., 2016). This suggests that hypermethylation of the *RELN* promoter in both animal models and in patients with neurodevelopmental disorders epigenetically suppresses Reelin expression in the hippocampus, leading to cognitive and synaptic disturbances. The release of this suppression from hypermethylation of the *RELN* promoter may thus decrease down-regulation of Reelin protein expression in the brain, which could serve as an alternative therapeutic strategy to direct Reelin injection in the brain.

Another therapeutic approach to the treatment of Reelin dysregulation in schizophrenia may be the inhibition of the enzyme that degrades Reelin. A recent report demonstrated that A Disintegrin and Metalloproteinase with Thrombospondin motifs 3 (ADAMTS-3) is the major enzyme involved in Reelin cleavage and inactivation (Ogino et al., 2017). Previous reports have already demonstrated that mice with truncated inactive Reelin exhibit schizophrenia-like behaviors such as hyperactivity, social withdrawal, and memory deficits (Sakai et al., 2016; Sobue et al., 2018). Inhibition of ADAMTS-3 may therefore reduce the amount of inactive Reelin and help to improve the symptoms seen in neurodevelopmental disorders. Futures studies should examine these impacts preclinically for potential translation to humans.

In the present study, we have demonstrated that MIA enhanced the locomotor -activity (**Supplementary Figure S2**) and anxiety-like behavior (**Figure 3**) in offspring, supposedly associating with positive and negative symptoms, respectively. Further, offspring with MIA exhibited the impairment of recognition memory (**Figure 3**), reflecting with cognitive symptom in schizophrenia (McGuire et al., 2013). Meanwhile, MIA had no effect on the social behaviors and sensorimotor gating in social interaction and PPI tests, respectively [social interaction: $p = 0.26$ (Mann-Whitney U test); PPI: $F_{PolyI:C(1,27)} = 0.072$, $p = 0.79$ (two-way ANOVA)]. These results suggest that MIA model prepared under our experimental condition may show only a part of behavioral abnormalities as observed in schizophrenia, which partially achieve an adequate level of behavioral validity as previously reported (Jones et al., 2011). On the other hand, the most studied and reproducible cognitive impairments in schizophrenics is working memory deficit (Elvevåg and Goldberg, 2000). In rodents, working memory can be measured in various tasks such as the eight-arm radial maze test and delayed non-matching to sample position operant conditioning task (Dudchenko, 2004). For the further verification of validity as an animal model

of schizophrenia, memory function in the present MIA model should be investigated in future studies by using such behavioral cognitive tests.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics Board of Meijo University.

AUTHOR CONTRIBUTIONS

DI, TaN, ToN, KY, and MHi designed the experiments, analyzed the data, and wrote the manuscript. GN, NK, and RT performed the experiments. MS, TK, and MHa helped with purification of recombinant Reelin protein. All authors reviewed, edited, and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fncel.2020.00285/full#supplementary-material

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Self and the Brain. The Immune Metaphor

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One of the fundamental questions in neuroscience is how brain activity relates to conscious experience. Even though self-consciousness is considered an emergent property of the brain network, a quantum physics-based theory assigns a momentum of consciousness to the single neuron level. In this work, we present a brain *self* theory from an evolutionary biological perspective by analogy with the immune *self*. In this scheme, perinatal reactivity to self inputs would guide the selection of neocortical neurons within the subplate, similarly to T lymphocytes in the thymus. Such self-driven neuronal selection would enable effective discrimination of external inputs and avoid harmful "autoreactive" responses. Multiple experimental and clinical evidences for this model are provided. Based on this self tenet, we outline the postulates of the so-called autophrenic diseases, to then make the case for schizophrenia, an archetypic disease with rupture of the self. Implications of this model are discussed, along with potential experimental verification.

Keywords: neurologic self, immune self, neurogenesis, schizophrenia, autophrenic disease

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A JOURNEY FROM CONSCIOUSNESS TO CELL BIOLOGY

Since the philosopher David Chalmers raised "the hard problem of consciousness" (1), referring to the interrelationship between brain activity and the content of conscious subjective experience, there have been numerous attempts to explain consciousness from a scientific approach. Innovative approaches have arisen from neurophenomenology (2), functional neuroimaging tools (3) and from cognitive and evolutionary psychology (4). Through most of the Western science, consciousness has been considered as an emergent brain process: the result of the integration of either global neural synchronization (5, 6), or of many asynchronic microconsciousness (7), whereas a role for the single neuron unit is left aside. However, based on quantum physics, it has been hypothesized a cell-based theory that located the momentum of consciousness within the neuron microtubules (8, 9). Yet, it has been largely unexamined the question of a cell-based consciousness from a biological viewpoint underlying microcircuitries (10).

Here, we explore the gap between self-consciousness and neural activity from an evolutionary biological perspective (11). There is a fundamental ontological assumption that the central nervous system (CNS) evolved along the immune system (IS) by natural selection to better define individuals' identity and interactions (12–14). We formulated the hypothesis that the distinction of self/nonself (internal/external) inputs by the brain has a cell basis similar to the immune self. The immune self/nonself recognition enables immune effective function without jeopardizing tissue integrity (15). We

inferred this *self* brain hypothesis by analogy of basic principles between these two complex network systems, although through divergent processes and scales. Contemporary systems biology aims to understand the rules governing dynamic regulatory networks across different scales (genes, RNA, proteins, cells, systems, metasystems, organism). Self-organization underlies a generic property of many complex networks and of gene regulatory networks in particular, which control cell ontogeny (16, 17).

Comprehensive understanding of immune physiology has often been anticipated by clinical immunopathology. For instance, autoimmune diseases in vertebrates are induced by failure or imbalance in recognition of the tissues of the body itself. However, autoimmunity or low level self-reactivity is essential for normal immune response. Within the CNS, an inappropriate reactivity to self inner signals would risk of an "*horror autotoxicus*"[1] (18) severely interfering with any cortical sensory, motor, and cognitive processes and therefore with consciousness. Inappropriate reactivity to self may characterize what we have called autophrenic diseases [from Greek αμτο- (*auto*), "self" and φρήν *(phrēn*), "mind, brain"], including epilepsy or schizophrenia (SZ). According to this novel view, epilepsy may represent excessive reactivity to self[2], defined by sudden firing of a subset of neocortical neurons that causes unrealistic sensations (visual, olfactory, gustatory, auditory), involuntary jerking and altered self-consciousness (19). In the case of SZ, it would occur a dysfunction of subsets of neocortical neurons that discriminate *self* body signals as nonself, resulting in the rupture of the psychoneurological self (20, 21). Indeed, false fragmented perceptions are lived as a real self/nonself conflict and severely affect the patient's awareness. These pathologies might suggest a role for the self at the brain subscale microcircuitries or even at the cellular level.

Immune self/nonself discrimination is instructed mainly during embryogenesis and early in life, through selection processes resulting in massive cell death that ensures efficient and highly specific response and eliminate potential highly autoreactive lymphocytes. The principles guiding brain cortex functional organization during embryonic neurogenesis have not been fully investigated. If, following our argument, that were the case that a *self* principle governs brain function, such instruction in *self* should be developed during neurogenesis. Based on theoretical grounds and biological observations, we hypothesized that self-driven selection processes based on neuron connectivity occur within the brain cortex (11). In this article, to translate our hypothesis into a model, we firstly recapitulate well-established immune principles to hypothesize similar brain solutions at the system level in a unified evolutionary account; and secondly, we present a body of arguments to prove the plausibility of the hypothesis based on clinical and experimental observations. We then expand the analogy to outline the postulates for autophrenic diseases by extrapolating the postulates that define autoimmune diseases.

Evolutionary Immune-Based Model for Brain Memory

The IS comprises at least two inextricably intertwined orders of recognition and memory that were developed through evolution: innate and adaptive immunity, the latter initially acquired in jawed vertebrates. Innate immunity displays a broad and pseudospecific quick and transitory response to stimuli (trained memory), essential for the survival of the organism as it is the only immunity present in invertebrates and plants. Adaptive immunity serves a highly specific and controlled response to a given antigen, and accounts for a long-term memory that, upon an ulterior *rendezvous* with this antigen is translated in a more accelerated, focused, and heightened response (22). Adaptive specificity stems from a bewildering diversity of unique T and B cell receptors repertoire, representing thus a huge qualitative jump in fine specificity to a changing environment by increasing complexity and efficiency of the response. Interestingly, innate immune cells (antigen presenting cells) drive antigen-specific memory activation and clonal selection of T lymphocytes' pools.

We built on the similarity of this functional organization with the vertebrate CNS looking side by side innate/implicit and adaptive/explicit orders of recognition and memory (23). Through these analogies, our model challenged the traditional view of the hippocampus only ascribed to the explicit/adaptive memory by an innate/implicit memory structure and essential mediator to explicit (declarative) memory. Although the hippocampus and adjacent structures of the medial temporal lobe (MTL) share function homology to the mushroom bodies of invertebrates (such as insects and crustacea) (24–26) and are evolutionarily ancient brain regions, the involvement of MTL in short-term associative memory is recent (27, 28). Hence, MTL bridges both implicit and explicit processes, which is not a new idea, but in line with kinetic models (29), functional neuroimaging studies (30, 31), and processing-based models (32). Explicit/adaptive memory is founded in an overwhelming diversity of neocortical neurons spatially and dynamically arranged to cope with extremely precise recognition that enables high cognitive abilities. Similar to the IS, "innate" brain regions (MTL) guide the formation of long-term and highly accurate explicit/adaptive memories through specific synapses between ensembles of neurons (engrams) within the neocortex (33). The proposed classification of brain memory based on a singular immune standpoint opens up a broader evolutionary insight on the role of the MTL in implicit and explicit memory strategies of encoding, storage, and retrieval (23). For instance, the widely studied patient H.M., with extensive bilateral hippocampal lesion, is an eloquent case supporting that highly specific (explicit/adaptive) memory resides within the neocortex, and corroborates the psychological distinction between short- and long-term memory (34).

Our epistemological approach explores recognition and memory brain systems by an immune-based analogy to better align the functional network architecture within an evolutionary

[1] Paul Ehrlich proposed the term "*horror autotoxicus*" to designate the unwillingness of the organism to the production of autoantibodies "amboceptors directed against its own tissues".

[2] Hereinafter "self" alludes to biologic self (at cell level) although otherwise specified.

context. This standpoint has not been previously undertaken but to applying IS principles into artificial intelligence computation (for instance, data encryption and storage, intruders' detection and recall algorithms, information rate efficiency, machine learning, etc.) (35, 36). Depicting similarities in basic biological principles between the IS and the CNS does not mean diminishing their gross differences or pretending to tackle the self, which lies beyond the boundaries of cell biology. In this essay, we intend to offer a new perspective on how brain emergent properties may be constrained by cell biology.

Instruction in Self: Thymopoiesis Meets Neurogenesis

The IS and the CNS constitute two complex network systems in open exchange that sense and respond to the environment while preserving the identity and integrity of the organism (homeostasis). This information processing function requires the discrimination between a dynamic self (internal signals and symbiotic interactions) and nonself (i.e. the outer world and the other), property shaped through evolution up to a degree of high specificity (37). In addition to higher specificity and specialization, evolution has led to an ever-increasing complexity at genetic, molecular and cellular regulatory interactions (complementarity, positive and negative feed-forward and feed-back loops) to determine overall response within each system. It can be asserted that the specificity of T lymphocytes and neocortical neurons as a whole delineate our identity (immunological and cognitive) as individuals, which is mostly acquired during embryogenesis and shortly after birth. Hence, each one of us is equipped with a unique repertoire of T lymphocytes and neocortical neurons (38, 39) to face the external world early in life. These cells will be thereafter selected by novel stimuli from the external world through connections with innate cells/structures, triggering activation of cells ensembles; and the repertoire of lymphocytes and neurons will be then shaped according to external experience (40). The whole process underpins the extraordinary plasticity of these systems and the concept of individual history. Dealing with the notion of time is indeed an intricate issue of the IS and the CNS, which has equally fascinated physicists, biologists, and philosophers approaching the brain in particular. To put this temporal relationship in plain terms, these paradoxical "anticipatory" specific cells (T lymphocytes, neocortical neurons) can be selected at any given moment by external cues to become "past" memory cells ensembles and then travel forward to "the present" -now- during recall, while being key to modifying the "future" behaviour of the organism (definition of cognition).

The anticipatory repertoire of T lymphocyte receptors (TCR) is generated by combinatorial gene rearrangement within the thymus (thymopoiesis). T-lymphocytes subsequently undergo a multi-step process of selection in response to self (41–43). Those T lymphocytes that weakly respond to self stimulus (antigen) happen to survive, while those that do not react with self die, (positive selection), and those that react too strongly with self are most of them eliminated (negative selection) or preserved as regulatory T lymphocytes (44, 45). Thus, the self principle governing thymopoiesis determines the future immune response. As Janeway's proverbial assertion dictates, "the immune system evolved to discriminate infectious non-self from noninfectious self" (43). Thus, autoimmunity (low level autoreactivity) is an inherent constituent of immune homeostasis, meaning that all peripheral T cells are self-reactive (46). The process described above is greatly simplified to focus on general mechanisms, and even if TCRs are highly specific, restriction of antigen presentation by the major histocompatibility complex (MHC) further increases the diversification of the individual response and is subjected to relative degeneracy.

How does the exquisite brain organization contribute to effective self/nonself recognition? And moreover, can we infer that there is a role for cognitive self at the neuron (engram) level? The underlying principles and mechanisms generating the functional specificity and diversity of neocortical neurons are far from being well known.

In a recent work, we suggested proceeding from an immune angle to address these questions and testing its validity or refutability. From this standpoint, we positioned the *self* as the axis of cortical neurogenesis (11), which would allow normal brain functioning and prevent costly autoreactivity. In parallel with the immune "logic", we sustained that the brain evolved to discriminate perceptible non-self from non-perceptible self. Neocortical neurons selection would be guided according to the degree of self recognition, by which too low and too high self-reactive neurons would undergo programmed cell death. Such self-driven neuronal selection would remove neurons exhibiting none or excessive reactivity to self signals. As a result of this selection process, neurons exhibiting low self-reactivity would discriminate any novel external stimulus, fundamental for effective neuronal response, and tolerance induction in the neocortex (11). This would also mean that all neocortical neurons are somehow self-reactive. Interestingly, neocortical neurons are not only "perceptive" of the outer world but highly interconnected *via* associational projections to fulfil the needs of the organism by using internal and external information.

Neurogenesis is written in chemical and electromagnetic language, whose code has been explored from multiple perspectives. We present below a body of arguments to test the presented hypothesis based on experimental and clinical observations, notwithstanding the obvious limitations derived from current gaps in knowledge and to methodological barriers[3].

i. Internal cues during neocortical neurogenesis primarily instruct neuronal selection:

-The most prevailing model for the development of neural circuits (Hebbian plasticity) states that synaptic connections are strengthened by correlated activity between pre- and post-synaptic neurons, while weakened by uncorrelated activity or lack of activity. Neural activity-dependent regulation is involved in cell type specification, dendritic branching, synaptic maturation and learning and memory through a complex program of gene regulation (47). However, this theory does not explain to date whether and how activity-dependent

[3]References to the original papers will be made when these provide essential contribution to the discussion.

mechanisms sort out neurons during neurogenesis. Two sequential waves of programmed cell death (PCD) regulated each by two distinct gene programs occur at the cortex subplate during embryogenesis and early postnatal life: a first wave at ventricular (VZ) and subventricular zones (SVZ) that purges up to 70% of progenitor cells showing spontaneous voltage-dependent activity evolving to synchronized small networks; and a second wave that further selects around ≈30% of mature neurons at postmitotic zones once coherent neural circuits with thalamocortical and cortico-cortical connections have been established [reviewed in (48)]. According to our model, to delete neurons by internal (self)-reactivity criteria, which goes beyond neuron-quality control, the whole process evokes striking similarity with the two independent PCD waves of positive and negative selection of lymphocytes during thymopoiesis in: developmental timing (embryonic and postnatal); stepwise functional segregation according to cell activity (primarily of inactive progenitor cells and of synaptically-driven maturing neurons afterwards); cell specification and migration-maturation gradient; balanced specific excitatory and inhibitory cell subsets (49–52). We suggest that PCD purges primarily those neurons that do not show a reaction to internal self-signals and afterwards eliminates those that overreact to these internal signals (11).

-*In vitro* and *in vivo* findings support that endogenous spontaneous firing rates at the neocortex may guide PCD waves during neurogenesis (53, 54). It remains unclear whether this neural activity functions in an instructive or in a permissive way, that is, if there are specific patterns of neural activity leading the neuronal fate; or in the contrary, it is that the mere presence of neural activity is sufficient for the neuronal survival. In fact, it has been described a rich repertoire of organized spontaneous activity patterns within the neocortex *intra utero* and perinatally, whereby depolarization of transmembrane voltage potentials above a certain threshold affects neurons survival and network organization (52, 55–57). By analogy to the presentation of the wide array of body self antigens (ectopically) to lymphocytes during thymopoiesis for *self* instruction, we hypothesize that the neurogenesis harbors the huge cast of *self* electrical signals that will instruct and select by PCD the repertoire of neocortical neurons. If this is so, the search of promiscuous gene expression within the cortex of those *self* electrical signals to promote self tolerance—the equivalent to the autoimmune regulator (AIRE) gene by thymus stroma—would be of great interest. We have not found experiments addressing specific-spike series or specific molecules within the SVZ/subplate in relation with differential neuronal sorting and circuit formation that may explain specific pattern-dependent regulation in neuronal segregation.

-Experimental models of cortical neurons xenotransplantation may shed light on the issue of a potential host *self* brain instruction. After single human pluripotent-stem cells (PSC)-derived cortical neurons xenotransplanted to the neonatal (P0/P1) mouse brain into the subplate, neurons integrated in the mouse cortex. Around 17% of transplanted single neurons matured and displayed responses to sensory stimuli that resembled those of the host neurons, stressing the specific nature of the circuitry. The authors suggested that the host brain provided not only permissive environment but also instructive cues regulating precise circuit formation (58). The host *self* instruction in human neurons seemed to be restricted during maturation at subplate by presynaptic partners (thalamus or cortex). The experiment was performed in neonatal mice, showing ulterior fine-tuning to external stimuli. By contrast, very limited synaptic integration results when the PSC-derived human cortical neurons were transplanted in bulk into the mouse cortex, which are less accesible to receive inputs from the host brain (59).

ii. The presence of specific excitatory (E) and inhibitory (I) neuron subsets within the neocortex assures homeostasis and functionality.

-A cortical organoids model from induced-PSC generates E and I neurons, i.e. glutamate and gamma-aminobutyric acid (GABA) neurons, respectively, which account for the generation and maintenance of oscillatory activity and synchronization of the network. Small-scale functional electrophysiological networks by these neurons subsets coordinate information flow resembling preterm neonatal brain activity (60). Inhibitory neurons act as a necessary "self-check" for excessive or prolonged responses by which the cortex precisely regulates functional effective connectivity. Impaired E/I balance is associated with several diseases, such as epilepsy and SZ.

-Stimulus-specific E and I assemblies have been described in the ferret primary visual cortex (61) and posterior parietal cortex of mice (62), pointing to selective inhibition by GABAergic neurons, similarly to antigen-specific regulatory T cells, controlling excessive responses and maintaining homeostasis and tolerance to self.

Recent work has shown that positive selection of I neurons (early postnatally) occurs and is coordinated by activity-dependent connections to E neurons (63, 64). The generation of the combinatorial code of unique neuron-tag molecules, such as protocadherins (65), seems to regulate a critical window of PCD of cortical interneurons (66). Adequate balanced networks of I and E neurons is adjusted by consecutive waves of PCD (48, 63)-. This phenomenon further supports the concept of neuron-specific selection by *self* E neurons, providing an evolutionary advantage for the rapid increase in pyramidal neurons in the primate lineage (63).

iii. Lesion studies can yield valuable information about the putative contributions of neural selection *in utero* to cortex functionality.

-Malformations of the cortical development (MCD) may be due to a broad array of disorders that disrupt the tightly spatiotemporally orchestrated process of neurogenesis (proliferation, migration, differentiation, synaptogenesis, apoptosis, synaptic pruning). MCD may affect the neuronal pool and connectivity of specific circuits, causing a wide spectrum of cognitive deficits, seizure disorders or neurospychiatric diseases, such as schizophrenia or autism (67). Depending on the time and degree of the neurodevelopmental

insult, clinical onset can be delayed (latency) thanks to compensation mechanisms through E-I interactions or other plasticity mechanisms, or to the time lapse to acquire a task that relies upon the specific neurobiologic substrate. A main feature is thus the fine regional and functional specificity of the affected neocortical neurons, which translates into hyperexcitability (anti-self) interfering their related circuits.

-The self brain hypothesis can be fully integrated into the programmed changes described for differentiation and maturation sequences of cortical neurons during neurogenesis and also provides a new dimension to the whole biological process. Defects or interferences in these developmental changes would result in excess of specific autoreactive E neurons, contributing to the hyperexcitability and ultimately in epilepsy. The view we present here might add conceptually important elements to the understanding of epileptogenesis. Accumulative or dysbalanced action potential firing of individual autoreactive neurons may disrupt the ensemble of specific circuits. The earlier the insult, the more severe or intractable the disorder, with persistent deleterious effects despite the high plasticity of the immature brain (68, 69). Subtle alterations on electrical activity during neurogenesis affect neuronal segregation and connectivity, and can cause many forms of epilepsy. These observations may suggest that specific features of neural activity (rather than just the presence of neural activity) are important for the neuronal selection during neurogenesis, pointing to the hypothesis that endogenous specific neural activity is instructive for neuronal selection within the neocortex. This fact may also reflect the purging function of non-reactive or highly self-reactive neurons. Our theory may also provide the basis for therapeutically significant avenues of development. Currently available pharmacological treatments of epilepsy are mainly symptomatic, none is curative or preventive. Moreover, anticonvulsivants show suboptimal effectiveness with long-term detrimental neurologic effects. New functional tools based on specific cellular resolution biomarkers to identify the hyper-reactive E neurons populations may favour new therapeutical interventions to selectively block these circuits. Also, these neuronal resolution biomarkers could propel the dissection of specific circuits and hopefully the development of new drugs based on pathophysiological mechanisms.

-Gene lesions associated to control of apoptosis during neurogenesis underlie several types of MCD, resulting in epileptogenesis when not to perinatal lethality (70). Given that genes and epigenetic modifications regulating the survival of specific populations of neurons are now beginning to be elucidated, advances within the field will foster progress in understanding cortical neuron segregation and neural circuits during neurogenesis (48, 71).

-Murine models of MCD, in which targeted chemical and physical insults during early development within the SVZ induce pronounced cortical hyperexcitability and reproduce the pathological and clinical findings of congenital forms of epilepsy [reviewed in (72)]. Timing and location (region and layer) of the induced lesion are key to the MCD clinical expression, suggesting that specific alteration of neuronal seggregation processes lead to hyperexcitability and altered connectivity.

iv. Our hypothesis challenges the currently accepted alternative hypothesis of "instruction from external inputs":

-In the auditory system, the selection and wiring of neocortical neurons within central sensory areas precedes the formation and priming of sensory receptors, circuits that will be refined later on by external inputs (73, 74). Before hearing onset, the precise temporal pattern of spontaneous pre-hearing activity is crucial for the formation of precise tonotopy in the central auditory pathway, supporting the role of self-instruction orchestrated development.

-Extreme examples or experiments of nature, such as complete unimodal sensory deprivation or anophthalmia (bilateral congenital absence of eyes) may give relevant insight into this issue. The connections patterns of organization in the cortex visual areas in the absence of retinal waves and visual experience of anophthalmic patients are not significantly different from normal sighted individuals (75–77). This finding may suggest that the visual retinotopic architecture of the neocortex does not primarily depend on external sensory instruction, but that *in utero* neural activity primarily shapes functional properties of cortical networks (75, 77).

Therefore, from many directions we find support for the working hypothesis that self/nonself discrimination is the result of a biological process primarily instructed from early neurogenesis by host self signals, to build an extensive repertoire of neocortical neurons. In both the IS and the CNS, each post-selection repertoire would thus represent, respectively, a mirror image of the immune and neurological reality that we are able to sense and with which we can constantly interact (**Figure 1**). This primary neuronal repertoire and neural circuits will be secondarily refined by external inputs during development, an activity-dependent process that is plastic. The entire process would endow the brain with a cell basis for consciousness and hence self-consciousness.

CONCEPTUAL, CLINICAL AND EXPERIMENTAL ARGUMENTS OF AUTOPHRENIC DISEASE. REVISITING SCHIZOPHRENIA

In order to formulate a general scheme and a case example, we will firstly take advantage of the well-established criteria defining autoimmune disease (AD) (78) to draw the principles of autophrenic disease in order to evaluate the self model in brain pathology (**Table 1**). Secondly, we will apply these principles to predict SZ pathophysiology. ADs are multifactorial conditions that result from the complex interplay of risk and protective factors, in which autoreactive T lymphocytes induce specific tissue damage or dysfunction. Intrinsic (genetic, epigenetic, endocrine, and psychoneurological), extrinsic (environmental), and stochastic factors induce cumulative effects that eventually

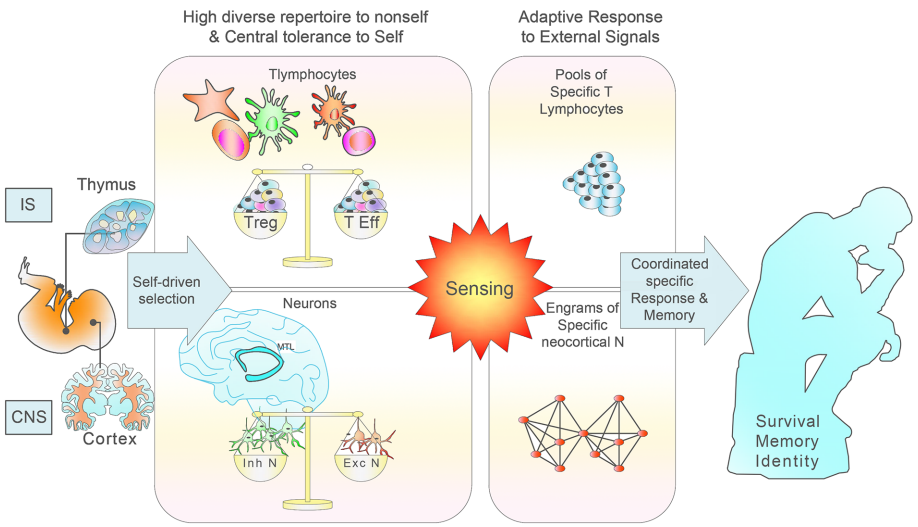

FIGURE 1 | Scheme of the normal IS and CNS development, maturation and functioning based on self education. During the generation of T lymphocytes in the thymus, it occurs a multi-step process of selection in which the great majority of highly self-reactive T cells die, leading to an immunocompetent and self-tolerant pool of naïve T lymphocytes. Clonal deletion is incomplete, as many self-reactive T cells find their way to the periphery where they constitute a constant risk for the development of autoimmune disease. During neurogenesis, the effective removal of most autoreactive neurons relies on a coordinated multi-step selection, where most low reactive neurons to self would be able to discriminate external signals. A number of selection mechanisms is of fundamental relevance for neocortical neuron development, responsiveness to external signals and tolerance induction to internal signals. Matching of electrical signals through sequential layers allows combinatorial signals with increasing complexity in a unique and extremely precise way.

lead from health to illness, with a waving course. A key pathophysiological role of innate immunity in AD has been unveiled (98). An interesting phenomenon is how disparate AD share immune mediators (autoimmune tautology) (99), while differ in the target tissue or organ, highlighting that specificity is given by the antigen-specific autoreactive T lymphocytes. A pro-inflammatory/anti-inflammatory cytokine imbalance favours differentiation and amplification of these target-specific autoreactive T lymphocytes.

On the basis of this proposed neurobiological self model, the nomenclature autophrenic disease designates complex multifactorial diseases in genetically susceptible individuals, modulated by endocrine and immunological factors, as well as psychological events in life (11). They define excessive or aberrant responses of autoreactive excitatory cortical neurons to specific endogenous neural inputs and/or defective inhibitory neurons, which disrupt certain cortical brain structures and functions (**Figure 2**). Similarly to autoimmune tautology, a cortical E-to-I neurotransmitter imbalance characterizes autophrenic diseases. By analogy with autoimmune pathogenesis, evolutionarily ancient brain structures (in particular the limbic system) would be expected to play a primary role. To complicate the scenario even further, autophrenic disease may be triggered by autoimmunity, as autoreactive T lymphocytes can target specific neurons' subsets (92, 93).

SZ is a prevalent mental disease characterized by a severe and disabling course in which the rupture of the psychic self is nuclear to the disease (100). Endocrine factors, such as male predominance, clinical onset in adolescence or early adulthood, and worsening at postpartum may play a part, coincidentally with cognitive maturation of the prefrontal (PFC) and parietal cortices (101, 102). Foetal and early childhood immune priming, such as maternal infection or active brain inflammation, are strongly associated with susceptibility to disease (103). Cumulative evidence across different experimental approaches (copy variant numbers, rare and *de novo* variants, genome wide association analysis, transcriptome and 3D genome structures) has restored its original conception as a neurodevelopmental disease, which stretches the way back to neurogenesis during embryonic development (88, 104, 105). SZ shows strong heritability estimated from twin studies of 79% (106, 107), while is highly polygenic with very low individual impact. Main mutations involve synaptic connectivity and chromatin remodelling (108). Transcriptome analyses of epigenetic regulated genome have revealed specific cell-type-dysregulation in the frontal lobe of SZ patients (109–111). In addition, a role of activated microglia during neurogenesis that affect neuronal segregation and connectivity has been hypothesized (112, 113). It is postulated that SZ is a heterogeneous large scale dysconnectivity syndrome (114). According to age of onset, SZ has been classified in a rare but severe childhood form with widespread cognitive impairment; and an early adult form with predominantly PFC-related verbal and executive abilities decline (115). Hallucinations and passivity phenomena (delusions of alien control), with disrupted discrimination between the external and internal inputs, are cardinal to SZ. In particular, auditory (audible thoughts, voices arguing and commenting about the patient in third person), visual and cenesthesic hallucinations are common (first rank symptoms). Overactivity in primary and secondary sensory areas seem to be

TABLE 1 | Model of autoimmune disease criteria proposed by Rose and Bona based on 1957 Witebsky's postulates (78).

	AUTOIMMMUNE DISEASE	AUTOPHRENIC DISEASE
Concept	- Excessive or inappropriate adaptive immune response against the antigens of the body itself (autoAg) - Loss of tolerance to autoAg - Tissue damage and/or dysfunction, chronic inflammation	- Excessive or inappropriate specific excitability against the neural signals of the body itself - Loss of tolerance (inhibition) to self-signals - Alteration of specific cortical region architecture and function (local and distant)
Direct evidence	- Activation of autoreactive T lymphocytes or autoAb targeting Ag-specific tissues or organs - Disease-specific autoAb inducing dysfunction (cell damage, binding to inhibitory or stimulatory receptor or enzyme or hormone) - Replication of disease by passive transfer of pathogenic autoAb/autoreactive T lymphocytes - Proliferation of T lymphocytes *in vitro* in response to autoAg - Induction of disease by xenotransplantation of human target tissue plus sensitized T lymphocyte to severe combined immunodeficient mice - *In vitro* cytotoxicity of T lymphocytes towards cells of the target organ	- Disease-specific highly autoreactive excitatory cortical neurons and engrams at specific brain regions (hyperactivity of sensory cortices during hallucinations) inducing disrupted connectivity and cortical dysfunction (79–83) - Cortico-subcortical hyperconnectivity within sensorymotor areas, while reduced PFC-thalamic connectivity (84) - Replication by disinhibitory action of NMDAR antagonists (i.e. ketamine) through blockade of E-to-I synapses - Hyperactivation of neurons in response to self-produced sensory stimuli (85)
	- Desensitization with low dose and repeated exposition to autoAg	- Beneficial effects of non-invasive brain stimulation, such as slow rTMS (86) and direct stimulation on auditory hallucinations and negative symptoms refractory to antipsychotics to reduce brain excitability (87)
Indirect evidence	- Genetically induced disease models - Experimental immunization or animal models of spontaneous autoimmunity	- Genetically induced disease models: abnormal patterns of oscillatory activity and firing in PFC (88), but not HP in DISC1 knock-down mice (89) - Experimental SZ by somatostatin interneuron dysfunction at PFC (90) - 3D neural tissue model organoids 15q11.2 microdeletions (91) - AutoAb targeting neuronal antigens that disrupt synapsis and cause functional dysconnectivity in a subgroup of SZ (92, 93).
	- AutoAb located at the site of lesion (as well as immune complexes) - Adoptive regulatory T cell therapy in autoimmune diseases (95)	- Aberrant anti-self-firing synapses at affected regions (disturbed gamma band synchrony) (94) - Grafting of GABA-ergic progenitors can reduce seizures and psychosis (96)
Circumstantial evidence	- Association with other autoimmune diseases. - Shared mediators (AD tautology)	- Association with other autophrenic diseases: epilepsy (9% to 52%) (97), depression, autism (30%), etc. - Shared mediators among different neuropsychiatric diseases.
	- High risk and protective HLA haplotypes, thymogenesis and other immune-related genes - Lymphocytic infiltration of the organ, especially if there is a restriction in V gene usage - Favorable response to immunomodulation and immunosuppression.	- Main neuron-specific genetic signatures (neuronal connectivity and synaptic plasticity). - Protective HLA haplotypes - Neuron-type-specific and cortical region-specific epigenetic linking genetic expression signature. - Favorable response to neurotransmitters' inhibitors, anti-epileptic medications, lithium, electrostimulation (rTMS and transcranial direct or alternating current stimulation) and electroconvulsive therapy.

Ab, antibody; Ag, antigen, DISC1, disrupted in schizophrenia; HLA, human leukocyte antigen; E-to-I, Excitatory-to-Inhibitory; HP, hippocampus; NMDA-R, N-Methyl-D-Aspartate receptors; PCF, prefrontal cortex; rTMS, repetitive transcranial magnetic stimulation.

Autoimmune diseases in strict sense must fulfil at least three criteria of direct evidence and most of those of indirect and circumstantial evidence. We show autophrenic disease postulates in parallel and specifics applied to the case of schizophrenia.

involved in aberrant gamma oscillations to endogenous inputs from pyramidal neurons (83) despite absence of actual external stimuli (81, 82, 85). Some authors have explained this aberrant neuronal firing to inner signals in terms of feed-back loops and distant connectivity involvement with excessive expectation, by which altered recognition of endogenous signals were misattributed as coming from an external source (79). Experimental evidence suggests that SZ patients show similar or even outperform healthy controls on visual discrimination skills. More difficult is to explain negative symptoms (e.g., alexithymia) in terms of *self*, which might be understood by local and long-scale brain connectivity disturbances (80, 114).

The E/I imbalance is considered key for SZ pathophysiology (116). However, the non-uniform distribution of the E/I imbalance—such as distinct hyper- o hypodopaminergic (117) and hyper- or hypogabaergic brain regions and cortex areas (118)—suggests their secondary role in SZ pathophysiology. This uneven distribution dampens the therapeutic efficacy of current drugs. A profound defect in inhibitory GABAergic interneurons has long been established as the most common finding (119). GABAergic defect is region-specific, with decreased expression of the neurotransmitter GABA, decreased ability to generate gamma oscillations, decreased GABA receptors and low inhibitory neuron markers at the basal ganglia, the visual cortex and in the cerebrospinal fluid (120). In contrast, GABA is increased at PFC in unmedicated patients (188, 121). Excessive dopamine and glutamate (due to hypofunctioning N-methyl-D-aspartate receptors) within the hippocampus and striatum (79, 122), account for overactivity of the primary and secondary sensory areas that induce misperceptions, while both

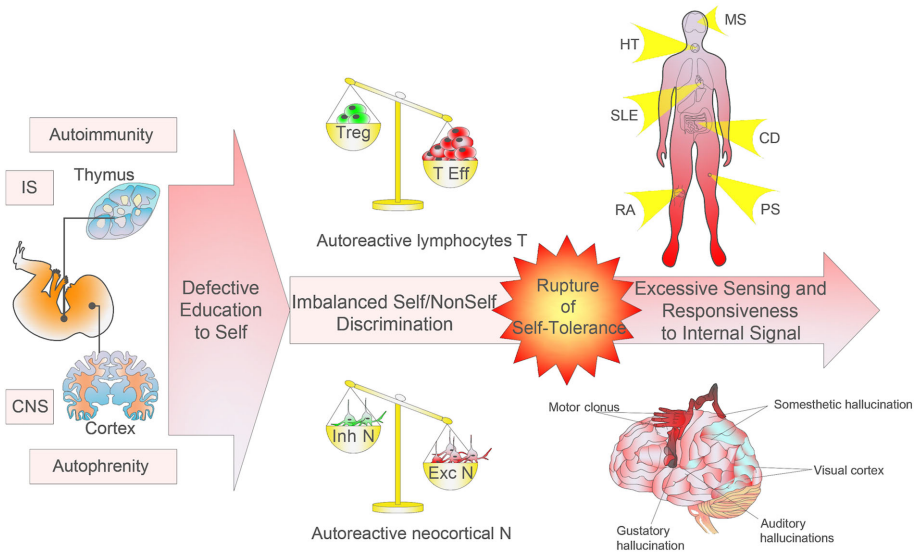

FIGURE 2 | Imbalanced self/non-self education and discrimination. The research on monogenic autoimmune syndromes has shown the relevance of mutations harbored by defined proteins of thymic selection processes and the development and activation of regulatory T cells. Environmental factors operating in a genetically susceptible host may trigger or exacerbate the pathological autoimmune process, induce mutations in genes coding for immunoregulatory factors, or modify immune tolerance or regulatory and immune effector pathways. Autoimmune diseases can affect any site and any organ system in the body. In parallel, monogenic autophrenic diseases are related to protein mutations involved in cortical neurogenesis, synaptic plasticity and connectivity, and chromatin remodelling in specific cortical areas or functions, which can affect any body's function. The neocortex shows an extremely fine topography and plays essential functions of sensory, motor, and cognitive processes. The autophrenic diseases so conceived may provide a better understanding of the complex neurobiology of neurodevelopmental disorders. Although the autoimmune/autophrenic diseases affect specifically adaptive/explicit recognition, the pathophysiology is induced by alteration in evolutionarily more ancient cells constraining adaptive response (i.e. antigen presenting cells and the limbic system, respectively).

neurotransmitters are decreased at brain cortex. Conventional therapies have been directed to modulate the E/I balance, with partial effectiveness and potentially severe side-effects.

We suggest that E/I imbalance arises from dysregulation between self-specific excitatory and inhibitory neurons (or synapses) at circumscribed cortical areas. Involvement of the cortical areas responsible for specific recognition and explicit memory (primary and secondary sensory areas) might underscore a perceptual basis of self-consciousness. Thus, the dysregulation between self/nonself-reactive neurons would be the key event in the autophrenic disease, with subsequent local E/I imbalance and altered connectivity processing and metacognition. In this context, our theory advocates for the identification through single-cell resolution biomarkers of specific hyper-reactive neurons and the ensemble of their circuits as a new means for better understanding these diseases and for the exploitation of more specific blocking strategies. Alternativelly, targeting specifically GABA inhibitory neurons at the former circuits might be effective to control hyperexcitability. Promising strategies targeting specific cell types are being advanced by calcium imaging (123). Selective optogenetic activation of individual cortical neurons can trigger relevant ensembles and modify behavioural responses in mice, supporting a causal link between the cortical neuron (*self*-reactive neuron in our view) and learned behavior (124, 125). In other order of strategy, a psychodynamic approach would be interpreted as a means to "desensibilize" autoreactive neurons by modifying the context of presentation of the input from MTL or basal ganglia.

DISCUSSION AND FUTURE PERSPECTIVES

The IS and the CNS are genuine self-referential systems. The analogies between the two systems for cell recognition and memory strategies may support that general principles are operating. In the IS, discrimination between self and nonself is based on certain criteria of reactivity to self (46). Autoimmunity is an implicit constituent of cell immune homeostasis. Its deregulation may lead to autoimmune disease. In this work, we present a cell brain *self* theory from an evolutionary biological perspective by analogy with the immune *self*. At the cell level, we postulate that the extreme precision of recognition and association that enables the brain to perceive, memorize, anticipate, and to act is primarily instructed by reactivity to self during early life, when main neocortical structures and circuitries are organized. The implicit assumption of the biologic brain *self* theory is that autophrenity is consubstantial to brain physiology and homeostasis. Early life individual environmental exposure is key to refine the functional structure by means of a trade-off between chance (contingency) and necessity (adaptation). As for autoimmunity, the concept of autophrenity extends to self-regulation within the network.

The actual role of the immune self/nonself principle to determine the outcome of the immune response is finely tuned to a spatio-temporal dimension. The response will depend not only in the antigen *per se* but in anatomic location (site-specific) and contextualization of the immune challenge (126). To illustrate the point made, if any antigen is delivered, how this antigen will be seen by the IS will be tuned by intrinsic (quantity, duration of exposition, location, etc.) and extrinsic factors that will differentially impact the response (effector against tolerant). Among the intrinsic factors, the valence, perceived at the system level as danger/reward or as discontinuity (surprise)/continuity (127), is key to the outcome and mainly driven by innate immunity. Likewise, the response of the CNS is not only determined by the stimulus *per se* but strongly conditioned by the context of presentation through innate structures (mainly the limbic system) as a danger/reward valence, by evolutionarily more sophisticated feelings (128) and by previous experience (129). This valence modulation has impressive therapeutic potential and is being exploited in strategies as, for instance, desensitization therapies in allergic diseases and by cognitive-behavioural psychotherapies (130), respectively.

The structure of cognition is metaphorical, built on pattern recognition at different scales (13). We endeavoured to explore the neurologic self as a metaphor of the immune self at the cellular level, which provides grounds for understanding complexity from another biological system logic. Through this immune analogy, our hypothesis provides a potential guiding principle, which may add both biologically and, likely, therapeutically significant avenues for development. The exploration by this approach of cortical neurogenesis might offer a bottom-up explanation of the system functioning as a whole, and a new insight into some neuropsychiatric diseases.

The present work addresses the autophrenic disease by analogy with autoimmune disease. Other approaches, such as computational phylogenetic analysis of homologue genes, which code for the receptor pathways of neocortical neurons across species, could add complementary experimental verification of the theory, which could trace the plausible evolutionary sequence. This theory constitutes the basis for current ongoing work.

SZ semiotics is already shifting to link mental phenomena with underlying neurobiological mechanisms (131, 132) given the overlap among psychiatric manifestations and diseases. As long as current theories about etiologically complex illnesses like SZ remain open, we hope our theory will help to change SZ understanding. Following this reasoning, our theory points at aberrant anti-self neuronal responses behind anti-self neuropsychiatric disorders in a more meaningful dimension from a biological point of view. The brain self theory opens a new conceptual reflection on the gap between biological and conscious self. In his incompleteness theorem, Kurt Gödel decried the incapability for any formal system to be proven or disproven from within the system. Given the inherent limitations to consistently approach the functioning of the brain from brain logic, our inferential perspective from an immune metaphor would argue for its consistency. The brain self theory is beheld by a number of biological, experimental, and clinical findings in SZ that deserve further investigation. Typical positive SZ symptoms like hallucinations may help to better understand how excessive self-reactive excitatory neuronal activity of the neocortex may compromise the discrimination between the external world and internal experience and so alter the structure and connectivity of affected areas and distant circuits.

We expect that an understanding of neurobiology in terms of *self* at a wide-system functioning will open new targeted therapeutic strategies in disparate anti-self brain diseases and hopefully inspire further investigation.

AUTHOR CONTRIBUTIONS

SS-R wrote the first draft. SS-R and FF have equally contributed to the conception, design, critical revision, and final approval of the manuscript. SS-R and FF agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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REVIEW
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From Maternal Diet to Neurodevelopmental Disorders: A Story of Neuroinflammation

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Providing the appropriate quantity and quality of food needed for both the mother's well-being and the healthy development of the offspring is crucial during pregnancy. However, the macro- and micronutrient intake also impacts the body's regulatory supersystems of the mother, such as the immune, endocrine, and nervous systems, which ultimately influence the overall development of the offspring. Of particular importance is the association between unhealthy maternal diet and neurodevelopmental disorders in the offspring. Epidemiological studies have linked neurodevelopmental disorders like autism spectrum disorders, attention-deficit-hyperactivity disorder, and schizophrenia, to maternal immune activation (MIA) during gestation. While the deleterious consequences of diet-induced MIA on offspring neurodevelopment are increasingly revealed, neuroinflammation is emerging as a key underlying mechanism. In this review, we compile the evidence available on how the mother and offspring are both impacted by maternal dietary imbalance. We specifically explore the various inflammatory and anti-inflammatory effects of dietary components and discuss how changes in inflammatory status can prime the offspring brain development toward neurodevelopmental disorders. Lastly, we discuss research evidence on the mechanisms that sustain the relationship between maternal dietary imbalance and offspring brain development, involving altered neuroinflammatory status in the offspring, as well as genetic to cellular programming notably of microglia, and the evidence that the gut microbiome may act as a key mediator.

Keywords: maternal diet, nutrient imbalance, inflammation, genetic programming, microglia, gut microbiome, neurodevelopmental disorders, schizophrenia

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INTRODUCTION

Nutrition is of course essential to the maintenance of life, but it is particularly fundamental at the onset of life during the antenatal and early life periods of growth and development of organs and systems. Although diet holds great importance for proper development, macronutrients (carbohydrates, proteins, fats) and micronutrients (vitamins, minerals) are often consumed

by pregnant and/or lactating mothers in inadequate proportions (Garcia−Casal et al., 2018). Across the world, ∼39 million pregnant women are estimated to be obese [body mass index (BMI) over 30] or overweight (BMI: between 25 and 30) due to poor nutrition (Chen et al., 2018), ∼32 million of pregnant women are anemic, ∼19 million of pregnant women suffer from vitamin A deficiency, while millions of pregnant women suffer from iron, folate, zinc, and/or iodine intake deficiency (Garcia−Casal et al., 2018). Malnutrition includes maternal undernutrition and nutrient deficiency but also excess of some key nutrients, like carbohydrates and fats, that often lead to maternal overweight and obesity (World Health Organization [WHO], 2020).

It is not surprising that maternal diet can profoundly impact fetal and early postnatal development of a mother's progeny (Garcia−Casal et al., 2018). Indeed, malnutrition during *in utero* and early life, notably due to inappropriate quantity and quality of nutrients consumed by the mothers, can affect the offspring's growth, metabolism, immune function, brain, and cognitive development (Ahmed et al., 2012; Godfrey et al., 2017; Chen et al., 2018). In this review, we address this worldwide issue associated with maternal diet by focusing on its potential long-term impact on neurodevelopment in the progeny. We strive to provide an up-to-date view of adequate nutrition during pregnancy and its effect on mothers and their offspring. Furthermore, we provide insights into diet-induced inflammatory status, microbiome as well as genetic/epigenetic changes, and their association to neurodevelopmental disorders as potential underlying mechanisms.

MATERNAL DIET IN PREGNANCY

Even prior to conception, diet plays an important role in enabling implantation of the embryo and placentation of the future mother. Women planning for pregnancy require an increased intake of folate equal to 400 μg/day, often ingested as dietary supplement (Parisi et al., 2014), prior to conception until the 12th week of pregnancy (Plecas et al., 2014). During pregnancy, several suggested essential nutritional requirements (i.e., carbohydrates, fats, proteins, vitamins—A, B, and C—and minerals—iodine, iron, magnesium, selenium, zinc) almost double (see **Table 1**) (Katamay et al., 2007; Ares Segura et al., 2016; Kominiarek and Rajan, 2016; Mousa et al., 2019). This increased need mainly occurs in the second and third trimesters of gestation, i.e., the main period of fetal growth (Nnam, 2015). Then, after pregnancy, the nutrient requirements for breastfeeding mothers also differ from those in non-pregnant state; increasing for some nutrients (i.e., vitamins—A, B2, B5, B6, B8, B12, C, and E—and minerals—selenium and zinc) while decreasing for others (i.e., proteins, vitamins—B3 and B9—and minerals—iodine, iron, and magnesium) (Katamay et al., 2007; Ares Segura et al., 2016; Kominiarek and Rajan, 2016; Mousa et al., 2019).

Increased intake of macronutrients (fats, carbohydrates, and proteins) and micronutrients (vitamins and minerals) is generally recommended in the nutritional guidelines for pregnant women. In addition, guidelines exist about excluding certain food sources that may contain teratogens (substances that are known to have damaging effects on the embryo), unsafe bacteria (e.g., certain dairy or fish products), as well as avoiding alcohol and caffeine (200 mg/day) consumption (Plecas et al., 2014; Martin et al., 2016) and lowering salt (or sodium chloride) intake (Katamay et al., 2007). The so-called dietary supplements are also recommended when dietary consumption alone does not fulfill nutrient requirements, such as in women following a vegetarian/vegan diet, living in cold climates or with malabsorption disorders (Kominiarek and Rajan, 2016).

As we will further detail, overall, maternal diet is critical for the progeny's proper development and maturation. Inadequate supply of macro- and micronutrients may cause a broad range of adverse outcomes for the fetus, ranging from premature birth and

TABLE 1 | Selected macro- and micronutrients recommended daily consumption for non-pregnant, pregnant, and breastfeeding women (Katamay et al., 2007; Ares Segura et al., 2016; Kominiarek and Rajan, 2016; Mousa et al., 2019).

Nutrients	Recommended intake		
	Non-pregnant	Pregnant	Breastfeeding
Macronutrients			
Carbohydrates (g/day)	130	**175**	N/A
Fats and fatty acids			
n-6 α-Linolenic acid (g/day)	12	**13**	N/A
n-3 Linoleic acid (g/day)	1.1	**1.4**	N/A
Proteins (g/day)	46–50	**60–71**	**62–65**
Micronutrients			
Vitamins			
Water-soluble			
B1 (thiamin) (mg/day)	1.1	**1.4**	**1.4**
B2 (riboflavin) (mg/day)	1.1	**1.4**	**1.6**
B3 (niacin) (mg/day)	14	**18**	**17**
B5 (pantothenate) (mg/day)	5	**6**	**7**
B6 (mg/day)	1.3	**1.9**	**2.0**
B8 (biotin) (μg/day)	30	30	**35**
B9 (folate) (μg/day)	400	**600**	**500**
B12 (cobalamin) (μg/day)	2.4	**2.6**	**2.8**
C (mg/day)	75	**85**	**120**
Fat-soluble			
A (μg/day)	700	**770**	**1300**
D (μg/day)	5	5–15	5–15
E (mg/day)	15	15	**19**
K (μg/day)	90	90	90
Minerals			
Calcium (mg/day)	1,000	1,000	1,000
Iodine (μg/day)	150	**220–250**	**190**
Iron (mg/day)	18	**27–60**	9–27
Magnesium (mg/day)	310-320	**350–360**	310–320
Phosphorous (mg/day)	700	700	700
Selenium (μg/day)	55	**60**	**70**
Sodium (mg/day)	<2,000	<2,000	<2,000
Zinc (mg/day)	8	**11**	**12**

N/A, not available. Increased recommended intake are highlighted in bold.

neurodevelopmental defects (neural tube, cognitive, and motor) to death (Nnam, 2015; Martin et al., 2016).

Maternal Supplements

To support the dietary intake of pregnant or nurturing women, supplements are often recommended in nutritional guidelines, especially when the necessary nutrients cannot be fully obtained from their diet (Kominiarek and Rajan, 2016). The extent to which these supplements confer beneficial effects varies with the specific nutrients. For instance, calcium supplementation studies show reduced preeclampsia and preterm delivery in higher risk group of pregnant women without improving outcomes for the newborn (Hofmeyr et al., 2018). Similarly, zinc supplements had a small positive effect on decreasing preterm births (Donangelo and King, 2012). In contrast, supplementation trials with *n*-3-long chain poly-unsaturated fatty acids (PUFAs) during pregnancy and lactation revealed improved general cognitive score of 2–5 years old children, without significant and specific improvement reported on cognition, language, or motor development (Gould et al., 2013). For vitamin B9 supplement, studies suggest a beneficial effect mainly in decreasing risk of birth defects (Maria De-Regil et al., 2010). Vitamin B12 supplementation helps normalize maternal cholesterol plasma levels, as well as lipid metabolism in the offspring (Khaire et al., 2015b). Still, additional unbiased studies with bigger sample sizes are needed to determine the real beneficial effects of supplementation for certain nutrients such as iodine (Harding et al., 2017). Supplementations of iron and vitamin C, which can assist with iron absorption, are routinely recommended during pregnancy due to a twofold increase in need and the common occurrence of anemia during pregnancy (Kominiarek and Rajan, 2016). However, in the case of vitamin C supplementation, no significant effect has been observed on pregnancy complications (i.e., intrauterine growth restriction, preeclampsia, preterm labor, stillbirth) (Rumbold et al., 2015). Cosupplementation has been proven beneficial in certain cases, notably the cosupplementation of magnesium, zinc, and vitamin D was shown to decrease inflammation and oxidative stress in women with gestational diabetes (Jamilian et al., 2019).

Although the intake of supplements seems to have beneficial effects for the most part, a majority of the studies investigating the effects of supplements have overlooked demographic and lifestyle factors (e.g., maternal age, ethnicity, comorbidities, physical activity) that may interact in producing the reported pregnancy outcomes. Therefore, the results of these studies should be considered and interpreted with caution, and future investigation should consider demographic and lifestyle factors, together with other potential factors influencing supplements absorption and outcomes (e.g., dose, food source, and method of absorption).

Maternal Adaptation in Pregnancy

Several strategies are in place within the future mother's organism to ensure an optimal nutrition and development of the progeny (see **Figure 1A**). As metabolism changes during pregnancy from an anabolic (building up) to a largely catabolic (breaking down) state (King, 2000), nutrient absorption by the mother's intestine is increased, while their excretion from the mother's kidneys and gastrointestinal tract is altered (Zhang et al., 2013; Plecas et al., 2014). In addition to these metabolic changes, nutrients are redirected to the placenta and mammary glands, as well as mostly transferred to the developing fetus (Nnam, 2015). To accommodate this increased requirement of blood flow for nutrient and oxygen delivery to the placenta (Plecas et al., 2014; Nnam, 2015), the mother's blood volume also increases by 35–40%, representing an expansion of 45–50% of the plasma volume and 15–20% of the erythrocyte population (Nnam, 2015).

The placenta itself is critical to nutrient transfer from the mother to the fetus, where most nutrients cross by diffusion, while other nutrients require facilitated diffusion or active transport through the placenta (McArdle et al., 2008; Desforges and Sibley, 2009; Plecas et al., 2014; Lewis et al., 2018). Nutrients, then, enable cellular growth, migration, differentiation, and fetal development, where amino acids, the building blocks of proteins, are particularly of utmost importance (Desforges and Sibley, 2009; Plecas et al., 2014) and where glucose provides about 75% of the fetus energy needs (Plecas et al., 2014).

Absorption and the Gut Microbiome

Another important adaptation that takes place in pregnancy pertains to the microorganisms that live in our intestines and help process our dietary intake (see **Figure 1A**). The gut microbiome is critical for both the harvest and storage of energy sources (reviewed in Krajmalnik-Brown et al., 2012). While energy demands mainly increase during the latter part of pregnancy, adjustments begin to already take place early in pregnancy (King, 2000).

Although pregnancy is a physiological state, many of the body's metabolic and immune adaptations resemble those of dysfunctional states in non-pregnant individuals with metabolic syndrome, such as increased energy harvest, adiposity, and decreased insulin and leptin sensitivity (Koren et al., 2012; Gohir et al., 2015b). These changes may prove necessary to provide for the high-energy demands of fetal growth during the third trimester and milk production during breastfeeding after birth. Moreover, there is some evidence that contributing to these adaptations are concomitant changes in the gut microbiome (Collado et al., 2008; Gohir et al., 2015b). Pregnancy-associated changes include a general increase in the amount of bacteria living in the gut, although phyla diversity is reduced (Collado et al., 2008). In addition, *Firmicutes* and *Bacteroidetes*, the two normally predominant phyla (90%) of the gut microbiome (reviewed in Ley et al., 2006; Rinninella et al., 2019) do not appear to undergo important alterations with pregnancy (Collado et al., 2008). However, the *Proteobacteria* phylum and *Actinobacteria* phylum (mainly *Bifidobacterium* spp.), which are respectively associated to increased inflammatory status, and immune stimulation and metabolic function, are increased from the first to the third trimesters (Collado et al., 2008; Rinninella et al., 2019).

Changes in the gut microbiome are important to consider because to meet the body's needs, 10–30% of energy intake is harvested in the large intestine where undigested carbohydrates and proteins are further processed (fermented). With the help of the resident microbes, this fermentation process results

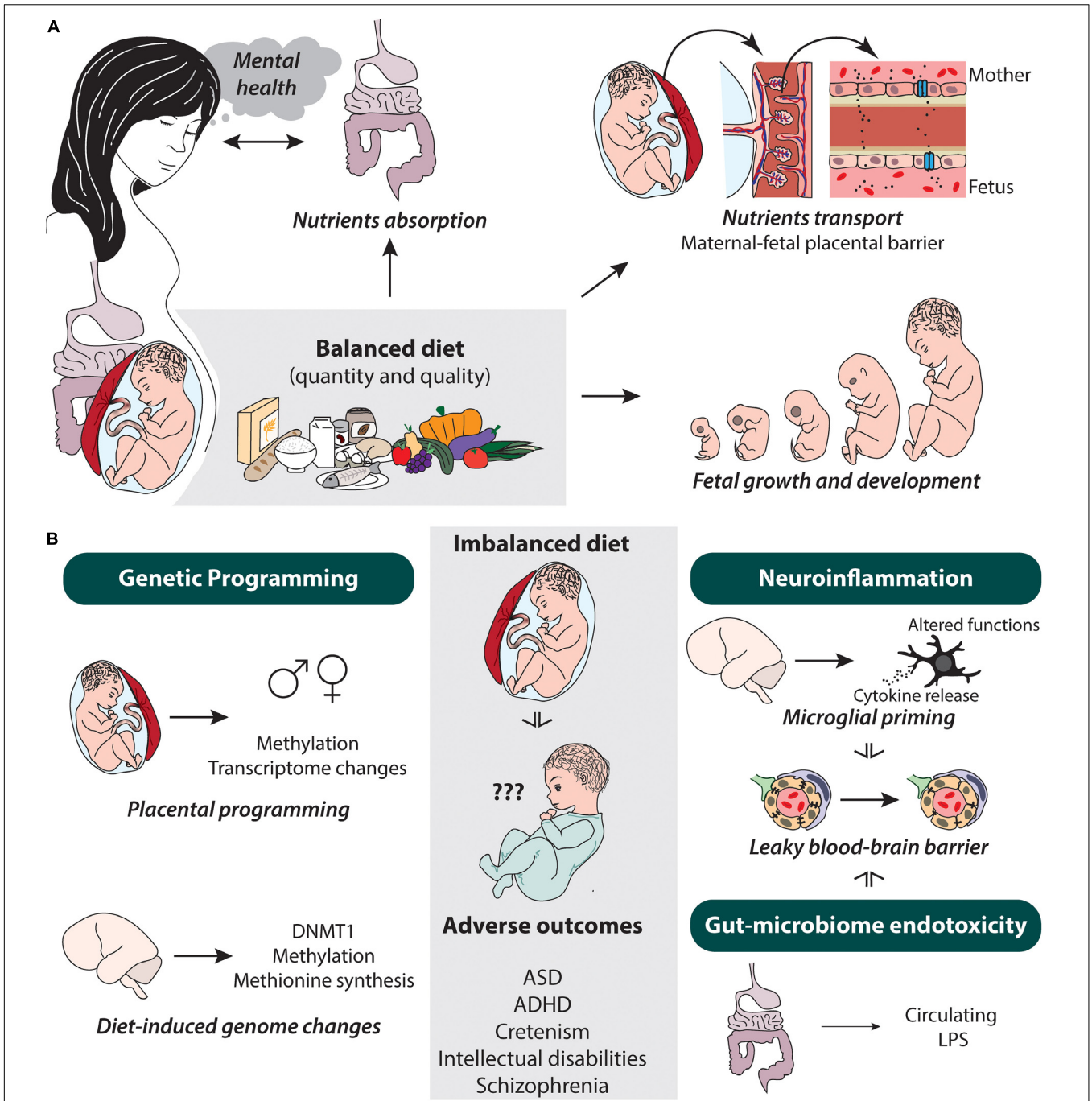

FIGURE 1 | Potential dietary-mediated factors altered by the pregnant mother's diet and putative changes and mechanism occurring in the progeny. **(A)** Balanced diet influences on nutrient absorption and transport, as well as fetal growth and development in pregnant women. Their gut microbiome can also influence mental health state of the pregnant women. **(B)** In the offspring, diet can influence genetic programming (i.e., placental and genome-wide), neuroinflammation (i.e., microglia, cytokines, and blood–brain barrier leakiness), and gut microbiome endotoxicity, which in turn causes adverse neurodevelopmental outcomes such as ASD, ADHD, cretinism, intellectual disabilities, and schizophrenia. ♂, male; ♀, female; ASD, autism spectrum disorder; ADHD, attention-deficit-hyperactivity disorder; DNMT1, DNA (cytosine-5)-methyltransferase 1; LPS, lipopolysaccharide.

in the production of monosaccharides and short-chain fatty acids (SCFA): primarily acetate, propionate, and butyrate in a 3:1:1 proportion (Macfarlane and Macfarlane, 2003). These metabolites have somewhat ambiguous functions, contributing to lipogenesis (monosaccharides) that can ultimately lead to the increase in adipose tissue and insulin resistance. This is in part due to an increase of circulating free-fatty acids and the proinflammatory cytokine production associated to some

types of adipose tissue expansion and SCFAs propionate and butyrate (Krajmalnik-Brown et al., 2012; Gohir et al., 2015a; Jiang et al., 2019).

However, SCFAs appear to also be an important source of fuel, because of the ease with which they are absorbed through the gut by non-ionic diffusion (Kamp and Hamilton, 2006), dependent on the pH, with slight acidity, presumed from bacterial metabolic activity (Schönfeld and Wojtczak, 2016). SCFAs then travel *via* the bloodstream to the liver where they are metabolized also with relative ease not requiring protein binding, transportation, and transmembrane translocation (reviewed in detail in Schönfeld and Wojtczak, 2016). Importantly, SCFAs are not stored as adipose tissue and appear to stimulate energy expenditure as they modulate the liver's metabolism of carbohydrates and lipids by inhibiting glucose production, glycolysis, thus contributing to prevent hyperglycemia and promoting fat oxidation (Wu et al., 2005; Gao et al., 2009). In addition, SCFAs also regulate gut satiety hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) and because SCFAs can cross the blood–brain barrier, they can further have direct effects in the brain and they are known to centrally stimulate another satiety hormone: leptin (Canfora et al., 2015). Lastly, SCFAs appear to have beneficial immune regulatory properties, increasing the anti-inflammatory functions of regulatory T cells in the colon (Smith et al., 2013).

In the context of pregnancy, while more research is required, all of these features of SCFAs are thought to be favorable, with high-fiber diets leading to high quantities of acetate in production and having a protective effect against asthma in the offspring (Thorburn et al., 2015). The gut microbiome is further involved in synthesis and absorption of micronutrients like vitamins and amino acids (Rodríguez-Concepción and Boronat, 2002; Bäckhed et al., 2005; Gill et al., 2006, latter reference for supplementary detailed tables). This finding proposes an additional mechanism by which altered microbiome could impact pregnancy (Gohir et al., 2015a), since there is already an increased urine excretion of water-soluble micronutrients (Ladipo, 2000) like vitamins B6, B12, folate (B9), and thiamin (B1), which are crucial for fetal development (Jans et al., 2015).

The gut microbiome increases macronutrient absorption and synthesis, which helps in building energy stores and regulates the immune system. Since pregnancy is for the most part anabolic, this is not inherently negative except when there is overconsumption of macronutrients, for instance of fats, which we will detail in section "Gut Microbiome-Mediated Endotoxicity," and which appears to alter microbial communities in a way that impacts general metabolism and micronutrient synthesis even when fatty diets are consumed prior to conception (Gohir et al., 2015a). Notably, some metabolites that are produced by the gut microbiome can play a beneficial role in the body's regulation of inflammation thus protecting the fetus.

Mental Health and the Gut Microbiome

Psychosocial adaptation is another big aspect of pregnancy and that can be linked to diet and the gut microbiome. An altered gut microbiome can also affect pregnancy in another important way: through its effect on maternal mental health (see **Figure 1A**). It is increasingly recognized that the gut commensal bacteria have both direct and indirect effects on cognition and behavior. In a landmark paper, germ-free mice, which are born to a sterile environment and are thus not colonized by bacteria, were shown to have an inadequate development of their stress response through the hypothalamus-pituitary-adrenal axis, hence altering anxiety-like behavior (Sudo, 2014). The alterations were reversed when the mice were colonized by specific strains of "good" gut bacteria early during development but not if the intervention was done in adulthood (Sudo et al., 2004). Some animal models of depression show that microbiome is altered and in healthy human volunteers, probiotics have been shown to alleviate distress (Cryan and Dinan, 2012), and others have shown that consumption of foods that are rich in fat and/or carbohydrate alleviate anxiety (Wurtman and Wurtman, 1995; Hurley et al., 2005; Teegarden and Bale, 2008). The reality is that both anxiety and depression during pregnancy and in postpartum stages are common and can lead to adverse outcomes often encompassing preterm birth and low birth weight (reviewed in Dunkel Schetter and Tanner, 2012) as well as behavioral alterations during childhood (reviewed in Rees et al., 2019). Less understood is the degree to which general socioeconomic situation (Stringhini et al., 2010) and chronic stress contribute to the suboptimal management of pregnancy and diet (reviewed in Monk et al., 2013), which in turn, could underlie immunological and endocrine alterations, as well as anxiety and depression affecting the fetus and the maternal behaviors postpartum (Dunkel Schetter and Tanner, 2012). Notwithstanding, we know that the gut microbiome is critically involved in modulating the stress and immune response, which are important features of both anxiety and depressive disorders (reviewed in Morris et al., 2017; Peirce and Alviña, 2019).

SPECIFIC NUTRIENT IMBALANCE AND INFLAMMATION

Macronutrients

Inappropriate availability of macronutrients—in deficiency or excess—can have long-term effects on the development of several body systems of the progeny (e.g., metabolic, circulatory, pulmonary). Protein is a macronutrient that when insufficiently consumed can lead to severe developmental consequences, including intrauterine growth restriction, impaired brain growth, and neurocognitive deficits (Monk et al., 2013). Meeting adequate protein requirements during pregnancy is most important during the second and third trimesters, when growth and development of both maternal and fetal tissues is accelerated (Kominiarek and Rajan, 2016). Studies on rodent adult offspring have demonstrated that low protein intake during pregnancy led to macrostructural changes of the brain such as decreased cerebrovascular density (see **Table 2**) (Bennis-Taleb et al., 1999). However, carbohydrate and fat consumption has become a pressing nutritional focus as worldwide, millions of pregnant women in developed countries suffer from obesity or overweight (Chen et al., 2018) mainly due to excessive carbohydrate and fat intake (Bleich et al., 2008). Critically, some of its negative consequences are tied to inflammation (Bolton and Bilbo, 2014;

TABLE 2 | Maternal and fetal outcomes of essential nutrient imbalance.

Nutrients	Imbalance	Outcomes		References
		Mother	Fetus/child	
Proteins	↓	N/A	Brain structural changes ↓ cerebrovasculature	Bennis-Taleb et al., 1999
Carbohydrates	↑	↓ hypertension ↓ adverse outcomes	↓ adverse outcomes	Gabbe and Quilligan, 1977; Kattah and Garovic, 2013; Sanjarimoghaddam et al., 2019
	↓ or ↑↑↑	Metabolic disruptions	Metabolic disruptions, death, stillbirth	Koski et al., 1986; Yamashita et al., 2000
Fatty acids (*n*-6/*n*-3)	↓ *n*-3	↑ postpartum depression	N/A	Horvath et al., 2007; Kim et al., 2017; Hoge et al., 2019
	↑ *n*-3	↓ preterm delivery in high-risk pregnancy	N/A	
	↑↓ *n*-6/*n*-3	N/A	Altered neurodevelopment	
Fats (saturated and unsaturated)	↑	Obesity, overweight, diabetes, restricted intrauterine growth, preeclampsia, C-section	Stillbirth, metabolic disorders, altered behaviors (anxiety, cognitive, social, motor)	Bilbo and Tsang, 2010; Sullivan et al., 2010, 2015; Tozuka et al., 2010; Peleg-Raibstein et al., 2012; Sasaki et al., 2013; Bolton and Bilbo, 2014; Castanon et al., 2015; Grissom et al., 2015; Tarrade et al., 2015; Graf et al., 2016; Ohta et al., 2017; Montalvo-Martínez et al., 2018; Thompson et al., 2018; Winther et al., 2018; Cordner et al., 2019; Smith et al., 2019
Ketone bodies	↑	Mortality, morbidities in the long term (cardiac, gastrointestinal, renal complications)	Growth alteration, altered behaviors (anxiety, ADHD, cognition, depression)	Rizzo et al., 1991; Sussman et al., 2013, 2015; von Geijer and Ekelund, 2015; Kanikarla-Marie and Jain, 2016; Nnodum et al., 2019
Vitamin A	↓	Night blindness, anemia, ↓ immune responses	Death, delayed growth, fetal malformations	Sommer and Davidson, 2002; Spiegler et al., 2012; Bastos Maia et al., 2019
	↑	Miscarriage	Fetal malformations	
Vitamin B9 (folate)	↓	Miscarriage, restricted intrauterine growth, preeclampsia	Fetal malformations	Fekete et al., 2012
Vitamin B12 (cobalamin)	↓	Preterm delivery	Anemia, fetal malformations, and altered behaviors (cognitive, motor)	Pepper and Black, 2011; Rogne et al., 2017; Sayar et al., 2020
Vitamin D and calcium	↓	Implantation failure, placental insufficiency, diabetes, miscarriage, preterm delivery, preeclampsia, C-section, poor immune response/tolerance	Fetal malformation, metabolic disorders (diabetes, hypertension, stroke, coronary artery disease), atopic disorders (asthma, eczema, hay fever), CNS disorders (ASD, multiple sclerosis, schizophrenia)	Merewood et al., 2009; Shin et al., 2010; Heyden and Wimalawansa, 2018
Sodium chloride (salt)	↑	Hypertension, restricted intrauterine growth, placental abruption, preeclampsia, comorbidities (cardiovascular, renal, hepatic CNS disorders)	Death, growth delay, cardiovascular, renal, CNS disorders (ASD, ADHD, schizophrenia)	Kattah and Garovic, 2013; Kleinewietfeld et al., 2013; Mao et al., 2013; Ha, 2014; Choe et al., 2015; Seravalli et al., 2016; Stocher et al., 2018; Afroz and Alviña, 2019; Faraco et al., 2019; Riise et al., 2019; Lahti-Pulkkinen et al., 2020
Iodine	↓	Hypothyroidism	Death, growth delay, cretinism, hypothyroidism, goiter	Skeaff, 2011; Kominiarek and Rajan, 2016; Pearce et al., 2016
	↑	Iodine-induced hypothyroidism, hyperthyroidism	N/A	Pearce et al., 2016
Iron	↓	Anemia, placenta hypertrophy, inflammation, poor milk quality	Death, anemia, growth delay, metabolic disorders (obesity, high blood pressure), altered neurodevelopment (neurotransmitter metabolism, neurotransmission, myelination), altered behaviors (cognitive, motor, emotive, psychology)	O'Connor et al., 1988; Huang et al., 2001; Gambling et al., 2002, 2004; Lozoff and Georgieff, 2006; Alwan and Hamamy, 2015; Means, 2020
Zinc	↓	Preterm delivery, prolonged labor, hypertension, increased risk of infection	Death, fetal malformations, growth delay, seizure, altered behaviors (anxiety, hypotonia, ADHD, social deficits)	Donangelo and King, 2012; Roohani et al., 2013; Grabrucker et al., 2014, 2016; Sauer and Grabrucker, 2019

↓, reduced; ↑, increased; ↑↓, imbalance; ↑↑↑, excessive; ASD, autism spectrum disorders; ADHD, attention-deficit-hyperactivity disorders; C-section, cesarean section; CNS, central nervous system; N/A, not available.

Buyken et al., 2014; Castanon et al., 2015; Ludwig et al., 2018; Montalvo-Martínez et al., 2018).

Carbohydrates and Fats

Carbohydrates are involved in several important bodily processes, including energy supply, glycemia control, insulin metabolism, cholesterol and triglyceride metabolism (Slavin and Carlson, 2014; Holesh et al., 2020), as well as maintenance of the gut microbiome's health and diversity (Turnbaugh and Gordon, 2009). They are mainly found in whole-grain foods (Plecas et al., 2014) and also in fruits, vegetables, and milk products (Slavin and Carlson, 2014; Holesh et al., 2020). Carbohydrates are classified into three main categories: sugars (i.e., simple sugars like mono- or disaccharides and complex sugars like oligo- and polysaccharides), starches (i.e., complex polysaccharides made of several glucose molecules produced by plants), and fibers (i.e., non-digestible complex carbohydrates) (Slavin and Carlson, 2014; Holesh et al., 2020). Carbohydrates act as the main energy source for maintaining pregnancy and lactating processes, the offspring's growth and development (Plecas et al., 2014; Martin et al., 2016), as well as milk production (Ares Segura et al., 2016; Martin et al., 2016) (see **Table 2**).

The type of carbohydrate has been shown to determine different inflammatory properties. In fact, meta-analysis looking at high-sensitivity C-reactive protein (CRP) and interleukin (IL)-6, which are inflammatory signals of the immune system, in human participant from countries across the world, have revealed a significant correlation between fiber intake and an anti-inflammatory effect, whereas whole grain was associated with an increase of inflammatory markers (Buyken et al., 2014).

Dietary recommendations for global fat intake, both saturated and unsaturated, are mainly addressed to non-pregnant women. While dietary recommendations have been made for certain essential fatty acids, α-linolenic acid (*n*-6 PUFA) and linoleic acid (*n*-3 PUFA) in particular, which are needed more in pregnancy (see **Table 1**) (Mousa et al., 2019), special considerations need to be taken in terms to quantity, as well as type of fats consumed during pregnancy.

Diets with high levels of fats (regardless of their saturation state) can induce maternal overweight or obesity and diabetes, notably by sustaining a proinflammatory state, as demonstrated in diverse animal models of maternal high-fat diet (mHFD) (Bolton and Bilbo, 2014; Castanon et al., 2015; Montalvo-Martínez et al., 2018). Beyond the increased fat mass, rodent models have also revealed an inflammatory state associated to mHFD (e.g., increase of cytokines IL-2, IL-4, IL-6) (Castanon et al., 2015; Graf et al., 2016; Bordeleau et al., 2020; Kretschmer et al., 2020). Indeed, *in vitro* experiments confirmed that IL-6 can promote apoptosis of endothelial cells, thus impairing placental vasculature and leading to intrauterine growth restriction *in vivo* in a mHFD mouse model (Kretschmer et al., 2020). Moreover, mHFD can also lead to behavioral changes in the mothers inducing increased anxiety-like behaviors (Thompson et al., 2018), which can negatively impact offspring's maternal care (Reck et al., 2018) (further detailed in **Table 2**). Prospective longitudinal study on pregnant women revealed that under stressful-perceived situations, women tend to consume more proinflammatory diet, such as a diet high in fats (Lindsay et al., 2018). Considering that more than three quarters of pregnant women experience low to moderate mood during gestation (Woods et al., 2010), high fat consumption during pregnancy may be more prevalent than we conceive.

One essential type of fat, especially required during pregnancy and breastfeeding, is the so-called PUFAs. In the maternal diet, omega 3 (*n*-3) PUFAs are found in fish oil or linseed oil, where fish source of *n*-3 PUFA is notably more efficient at providing necessary PUFAs to the progeny's brain (Fernandes et al., 2012). PUFAs are formed by an acyl chain of at least 18 carbons with one acid (–COOH) end and one methyl ($–CH_3$) end (Fernandes et al., 2012). Essential PUFAs include *n*-3 PUFAs linoleic acid and omega 6 (*n*-6) PUFAs α-linolenic acid. These two PUFAs can synthetize other PUFAs of the same unsaturation class (*n*-3 or *n*-6, indicating the position of the first double bound, C = C, on the chain). Since *n*-3 and *n*-6 compete for the enzymes desaturases and elongases, dietary intake of linoleic acid and α-linolenic acid influences their conversion rate (Taha et al., 2014; Bentsen, 2017). During the third trimester of pregnancy up to the first 2 years after birth in humans, arachidonic acid (*n*-6 PUFAs) and docosahexaenoic acid (*n*-3 PUFAs) are the most abundant PUFAs measured in the brain (Hadley et al., 2016). During this time, PUFAs have been shown to contribute to brain growth (Hadley et al., 2016), neurogenesis (Kawakita et al., 2006; Tokuda et al., 2014), synaptic plasticity, and neuronal wiring in animal and clinical human studies (Owens and Innis, 1999; Hamazaki et al., 2005; Darios and Davletov, 2006). Of note, *n*-6 PUFA is less reliant on dietary intake than *n*-3 PUFA (Taha et al., 2014).

n-3 PUFAs, like docosahexaenoic acid, can directly interact with transcription factors involved in inflammatory processes—nuclear factor-κB (NFκB) or peroxisome proliferator-activated receptor γ (PPARγ)—and thus, modulate the maternal and placental inflammatory status. Although the specific underlying mechanism remains under investigation, it was postulated that *n*-3 PUFAs might act on lipid mediators and help maintain placental functions during pregnancy through their anti-inflammatory properties (Akerele and Cheema, 2016; Lewis et al., 2018). Supplemental intake of docosahexaenoic acid in male mouse fed with a diet high in fats was also shown to soothe hypothalamic high-fat diet (HFD)-induced inflammation by decreasing suppressor of cytokine signaling 3 (SOCS3) signaling and promoting the Janus kinase (JAK)/protein kinase B (Akt) pathway. Other than its action on inflammation, *n*-3 PUFAs taken in this context helped to normalize the metabolic energy-balance (Cheng et al., 2020). However, imbalance of PUFAs such as excess of *n*-3 PUFAs, may inhibit the production of crucial proinflammatory cytokines during gestation (Akerele and Cheema, 2016). Cyclooxygenases and lipoxygenases can convert PUFAs into short-lived hormones—eicosanoids—that possess inflammatory properties (e.g., prostaglandins, thromboxanes, lipoxins, and leukotrienes). In animal model and human studies, *n*-6 PUFAs-derived eicosanoids have been commonly described as proinflammatory, however, they can also contribute to inflammatory resolution, while *n*-3-derived eicosanoids are anti-inflammatory (Phillis et al., 2006; Calder, 2009; Innes and Calder, 2018). High dietary intake of

n-6 PUFAs has been long believed to be linked to heightened inflammation but, enhanced inflammation was not consistently observed in different human studies (Innes and Calder, 2018). Excessive consumption of fats and/or sugar, a hypercaloric diet, can also promote a proinflammatory status in the pregnant woman or nurturing mother (Montalvo-Martínez et al., 2018).

Another important contribution of dietary fats during pregnancy is in the production of ketone bodies. Fatty acids can be broken down in the liver into ketone bodies—3-hydroxybutyrate and acetoacetate—(Paoli et al., 2013; Paoli, 2014), which are distributed throughout the body as a metabolic substrate, i.e., as fuel instead of glucose. With their metabolic changes during gestation and lactation, including reduced insulin sensitivity, women usually have an elevated level of circulating ketones (Paterson et al., 1967; Nnodum et al., 2019). Ketone bodies pass freely through the placenta or mother's milk, and they provide supplemental energy to the developing fetus. For the developing central nervous system (CNS), ketones not only can act as an energy source but also be used in lipogenesis as a lipid precursor. Moreover, ketones can modulate CNS functions, notably by partaking in adenosine triphosphate (ATP) synthesis and carbon pathway (Edmond et al., 1985; Williamson, 1985; Bronisz et al., 2018). While ketones possess important roles for fetal and neonate development, the consumption of a ketogenic diet and its implication during pregnancy is complex and it remains largely unclear whether it is beneficial or detrimental.

Ketogenic High-Fat Diet

One HFD that gained a lot of popularity in the past years is the ketogenic diet, initially used for its anticonvulsant and protective effects in neurodevelopmental disorders (Yudkoff et al., 2007). With ketone-induced metabolic changes, ketogenic diet have been suggested to alleviate symptoms or features when consumed by individuals with neurologic or neuropsychiatric disorders like autism spectrum disorder (ASD), epilepsy, or schizophrenia (Yudkoff et al., 2007; Kraft and Westman, 2009; Gano et al., 2014; Ruskin et al., 2017a,b). In addition, it is also known for its relatively rapid effects in reducing weight (e.g., obesity), inflammation, and metabolic alterations (Ludwig, 2020). Over the years, variations of ketogenic diets have been proposed (Shilpa and Mohan, 2018) but typically it involves 80–90% of the calories coming from lipids (high-fat/low-carbohydrate, moderate proteins). Upon 3–4 days of fasting or ketogenic diet intake, there is an increased production of ketone bodies and metabolism shifts from glycolysis to ketosis in several organs including the brain (Yudkoff et al., 2007). Mild ketosis is a physiological process that is known to be induced in fasting, lactation, shortly after exercising or muscular activity (Dashti et al., 2004). In fact, mild ketosis was a "normal" metabolic-state preagriculture, and it is still observed in some populations (e.g., Inuit in the Artic, First Nation groups in Canada) (Ludwig et al., 2018). Of note, ketosis is different from ketoacidosis, which can occur in pathological conditions such as uncontrolled type 1 diabetes, where a lack of insulin prevents most organs from using the available glucose, this leads to ketone bodies being produced in excess of 20 mmol/L while the body attempts to eliminate excess glucose *via* urine, causing a lowering of the pH of the blood and dehydration with potentially fatal consequences (Mullins et al., 2011; Paoli, 2014).

While ketogenic diet improves insulin sensitivity in non-pregnant individuals (Dashti et al., 2004), it was suggested that ketogenic diet might not be as beneficial for pregnant women and their progeny (see **Table 2**) (von Geijer and Ekelund, 2015; Kanikarla-Marie and Jain, 2016; Nnodum et al., 2019). Indeed, the outcome of a ketogenic diet on the offspring is complex; it may also differ according to the type of fats consumed, which change the gut microbiome: high proportions of saturated and monounsaturated fats appear to have a negative impact on its diversity while high polyunsaturated does not (Paoli et al., 2019; Wolters et al., 2019). This can then impact metabolic and inflammatory status and underlying health conditions (e.g., overweight, diabetes, neurological, neuropsychiatric) of both the mother and the developing fetus.

In contrast to a conventional HFD, ketogenic HFD has been proposed to induce more of an anti-inflammatory profile in non-pregnant individuals. In fact, ketogenic diets have reported a decrease in cellular stress by reducing reactive oxygen species production and enhancing antioxidant activities, as well as elevating circulating levels of PUFAs through the increased activity of fatty acids oxidation (Gano et al., 2014). In the context of maternal immune activation (MIA), a postnatal ketogenic diet in the offspring demonstrates a protective effect (Ruskin et al., 2017b); however, it remains to be investigated if this protective effect on the postnatal brain is due to anti-inflammatory and metabolic modulation by the ketones and/or acting *via* the gut microbiome (Ang et al., 2020).

Micronutrients

Other important considerations during pregnancy pertain to deficiency in micronutrients such as vitamin A (Garcia−Casal et al., 2018), vitamin B9, vitamin B12, vitamin D, calcium, iodine, iron, or zinc, among others (Blumfield et al., 2013; Visentin et al., 2016; Garcia−Casal et al., 2018). Maternal imbalance or inappropriate intake can lead to detrimental outcomes for both the mother's pregnancy (e.g., preeclampsia, intrauterine growth restriction) and the offspring's development (e.g., stillbirth, growth delay, risk of developing disorders detailed in **Table 2** for each nutrient). Among those essential micronutrients, several share inflammatory properties, which, in the context of pregnancy, could contribute to the detrimental outcomes on both pregnancy and progeny.

Vitamin A

Vitamin A is an essential nutrient found in food from animal sources like dairy products, liver, and fish oil, as well as in food from vegetal sources (e.g., fruits, leaves, tubers). Vitamin A from vegetal sources is poorly absorbed, however, compared with animal sources (Bastos Maia et al., 2019). Vitamin A is involved in several physiological functions through its active oxidized forms: retinaldehyde and retinoic acid. Retinaldehyde is involved in visual function, whereas retinoic acid can act as ligand for the nuclear retinoic acid receptor and regulate the transcription of genes involved in reproduction, development, growth, and

immunity. During pregnancy, vitamin A and its derived products are needed by the mother for placental maintenance and by the embryo for the formation and development of various organs (i.e., hearth, eye, kidney, lung, limbs, spinal cord, and brain). The placenta stores vitamin A that mobilizes to the fetus during prenatal development. This storing process ensures an adequate delivery of retinoids in cases of maternal insufficient intake to protect the developing fetus (reviewed in Spiegler et al., 2012).

Vitamin A through its metabolized active form, retinoic acid, can modulate immune homeostasis by binding to retinoic acid and retinoid receptors, which then acts as and interacts with transcription factors (Spiegler et al., 2012; Oliveira et al., 2018). As such, retinoic acid can modulate inflammatory processes—including infiltration of immune cells, production of cytokines [e.g., IL-1β, IL-4, IL-6, IL-10, IL-12, IL-18, interferon (IFN)-γ, transforming growth factor beta (TGF-β), tumor necrosis factor (TNF)-α], and other inflammatory mediators [NFκB, NOD-like receptor family pyrin domain-containing protein 3 (NLRP3)]—in a variety of tissues (Kang et al., 2000; Wang et al., 2007; Oliveira et al., 2018; Elshal et al., 2019; Alatshan et al., 2020). Thus, dietary intake of vitamin A can influence inflammatory response. For instance, in the context of dermatitis, low vitamin A exacerbates its severity partially through an increase of T cell release of immunoglobulins (i.e., IgG1, IgE) and cytokines (i.e., IL-4, IL-13) (Yang et al., 2020).

Other than immune mediators, vitamin A can modulate the integrity of the intestinal barrier by promoting expression of tight junction proteins (i.e., claudin-1, occludin, zonula occludens-1) (He et al., 2020). By doing so, it may modulate trafficking of metabolites coming from the diet or produced by the gut microbiome. In pregnancy, this could imply that vitamin A can influence maternal and fetal outcomes directly on the immune system or indirectly through the gut-immune axis.

Surprisingly, in a recent study on ulcerative colitis, it was demonstrated that increased levels of retinoic acid are associated with higher levels of proinflammatory cytokines (i.e., IL-17, INF-γ) and lower levels of anti-inflammatory cytokines (i.e., IL-10) in the intestinal mucosa of patients. It was postulated by the authors that in the presence of inflammation, retinoic acid maintains inflammation by upregulating proinflammatory molecules (Rampal et al., 2020). Therefore, it seems that the role of vitamin A during inflammatory processes is complex and may be modulated by the diet and interacts with the inflammatory state of the person, whether it is a chronic inflammatory disorder or an immune-privileged state like pregnancy.

Essential Vitamin B

Vitamin B9 or folate is a water-soluble B vitamin found in green-colored vegetables and citrus fruits. Its synthetic form—folic acid—is most stable when used in supplements (Kominiarek and Rajan, 2016). Folate itself is involved in the synthesis of DNA, RNA, and some amino acids (Fekete et al., 2012; Stamm and Houghton, 2013; Kominiarek and Rajan, 2016) as well as methylation reactions (Stamm and Houghton, 2013). Therefore, folate is important during periods of placentation, implantation of the embryo, embryogenesis, and fetal growth (Maria De-Regil et al., 2010; Parisi et al., 2014; Kominiarek and Rajan, 2016). During embryogenesis and fetal growth, the need for folate is highly increased reaching 600 mg/day (from 400 mg/day in non-pregnant women) (Kominiarek and Rajan, 2016).

Vitamin B12 or cobalamin acts as a cofactor with folate in DNA methylation (Pepper and Black, 2011; Khaire et al., 2015a,b). Cobalamin is also involved in lipid metabolism (Khaire et al., 2015a,b). B12 deficiency during pregnancy can arise in cases of women with vegetarian or vegan diets, as well as women with intestinal diseases that result in a malabsorption condition (Rogne et al., 2017).

Vitamin B insufficiency has been associated with higher levels of neuroinflammation and oxidative stress, while supplementation of vitamin B reduces oxidative stress and inflammation by increasing oxidative metabolism that may promote energy storage and developmental processes (Ford et al., 2018).

Vitamin D and Calcium

Vitamin D and calcium are closely related in terms of their metabolism. Obtained either through dietary consumption or mostly synthetized by the skin in contact with sunlight, the active form of vitamin D promotes calcium absorption (Curtis et al., 2014; Kominiarek and Rajan, 2016). Dietary sources of vitamin D include eggs and fish or commonly supplemented juices and milks (Kominiarek and Rajan, 2016). Proper absorption of both vitamin D and calcium are critical to bone growth and calcification (Curtis et al., 2014; Kominiarek and Rajan, 2016), immune and inflammatory functions, as well as cellular differentiation (Kominiarek and Rajan, 2016). In the embryo, the vitamin D and calcium needs increase during the main periods of skeleton formation and calcification, which start at the beginning of the embryonic stage (formation of a cartilaginous skeleton) and end during the last trimester of pregnancy (ossification of the skeleton) (Curtis et al., 2014). Pregnant women with vegan or vegetarian dietary habits as well as woman living in cold climate (Kominiarek and Rajan, 2016; Zhou et al., 2017) or with darker skin have a higher risk of vitamin D and calcium deficiency (Kominiarek and Rajan, 2016).

Vitamin D possesses a key role in the suppression of inflammation. Indeed, *ex vivo* placental experiments demonstrated that treatment with different forms of vitamin D, 25OHD3, or $1,25(OH)_2D_3$, attenuates lipopolysaccharide (LPS)-induced inflammation (Liu et al., 2011). Vitamin D can also modulate proliferation, differentiation, survival, maturation, and cytokine release of several immune cells including dendritic cells, macrophages, T cells, and B cells (Guillot et al., 2010).

On the contrary, depletion of vitamin D receptor or hydroxylase Cyp27b1 exacerbates inflammatory mediator levels (Liu et al., 2011). Low intake of vitamin D additionally promotes a proinflammatory status, due to the reduced vitamin D-induced inhibitory action on the adaptive immune response and inflammation (Shin et al., 2010).

Salt

Salt or sodium chloride is easily obtained in western diet with processed food often enriched in salt (Ha, 2014). However, excessive consumption of sodium chloride can cause renal

(Ha, 2014), cardiac diseases, including hypertension (Ha, 2014; Choe et al., 2015), CNS disorders (Kleinewietfeld et al., 2013; Faraco et al., 2019), and inflammation (Kleinewietfeld et al., 2013). *In vitro* studies helped to clarify the inflammatory properties of salt. For instance, sodium chloride-hypertonic stress can act as a chemoattractant to immune cells like macrophages, thus modulating their migration and mobility (Müller et al., 2013). Human and mouse macrophages treated *ex vivo* with high concentration of salt possessed a proinflammatory signature, both at the gene and protein levels, which was exacerbated following immune challenges induced by LPS (Zhang et al., 2015).

In *in vivo* studies in rodent models and humans, elevated consumption of salt was shown to promote immune activities of macrophages (Zhang et al., 2015; Guo et al., 2018), T cells (Guo et al., 2018) and dendritic cells (Xiao et al., 2020), which in turn exacerbated the onset of immune diseases (e.g., colitis, *lupus erythematosus*, lung injury) (Zhang et al., 2015; Guo et al., 2018; Xiao et al., 2020).

In maternal adipose tissue, high-salt diet increases the expression level of inflammatory molecules [e.g., IL-1β, TNF-α, *cluster differentiation* (*Cd*) *68*] in mice (Reynolds et al., 2014). In another independent study in mice, elevation of inflammatory gene expression was also reported in macrophages from the lungs [i.e., *C-X-C chemokine ligand 1* (*Cxcl1*), *Il6*, inducible nitric oxide synthase (*iNOS*)] and kidneys (i.e., *iNOS*), but not from the brain or adipose tissue (Zhang et al., 2015). Controversially, immunosuppression properties of high-salt diet—such as inhibition of IFN-γ/JAK/signal transducer and activator of transcription (STAT) pathway—was recently reported in mouse kidney cells (Arai et al., 2017). It thus seems that salt inflammatory properties may vary depending on the cell type studied. Current knowledge into the maternal-fetal effect of high-salt diet during pregnancy and lactation limits our capacity to assess the extent of salt-induced inflammatory changes in the context of maternal diet as well as neurodevelopmental disorders.

Minerals: Iodine, Iron, and Zinc

Together with iron and zinc, iodine is one of the minerals most commonly found deficient in the diet of pregnant women (Garcia−Casal et al., 2018). Iodine is a critical element of thyroid hormone synthesis found in seafood products as well as fortified iodized salt (Kominiarek and Rajan, 2016; Pearce et al., 2016). Thyroid hormones are important for fetal and newborn neurodevelopment by modulating cellular migration and differentiation, synaptogenesis, as well as myelination (Bernal, 2007). Maternal iodine consumption is thus critical for the fetus until its own thyroid begins producing thyroid hormones, around the second trimester, and even at this stage the fetal storage is limited until birth (Skeaff, 2011). Iodine can also act as an antioxidant (Aceves et al., 2013). On the other hand, excessive intake of iodine increases the risk of developing autoimmune thyroid disease (Luo et al., 2014), meaning that the inflammatory properties of iodine may be more complex and need further study.

Iron is important for blood cell's ability to transport oxygen around the body. It can be found in food as two distinct forms: heme—hemoglobin and myoglobin found in meat and fish—and non-heme—obtained from cereals, fruits, and vegetables (Abbaspour et al., 2014). Nutrients can modulate its absorption; while vitamin C promotes iron absorption, milk and tea inhibit its absorption (Kominiarek and Rajan, 2016). With the increase in blood volume as well as iron-dependent developmental mechanisms during pregnancy (Nnam, 2015), iron intake is key (Means, 2020). Failing to meet the iron needs can cause, in the pregnant woman, inflammation (Gambling et al., 2002) among other detrimental outcomes on pregnancy (O'Connor et al., 1988; Huang et al., 2001; Means, 2020).

Iron intake can modulate inflammatory processes, and when intake of iron is insufficient, animal models (e.g., rodent, fish) have demonstrated that it causes a reduction of anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-11, IL-15, TGF-β) and mediators [e.g., inhibitor of NFκB (IκB) α], as well as upregulation of proinflammatory cytokines (e.g., IL-1β, IL-8, IL-12, IL-17, IFN-γ) and other mediators [e.g., NFκB, IκB kinase (IKK) α/β, eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP)] in the periphery (e.g., gut, placenta) (Gambling et al., 2002; Guo et al., 2019). Therefore, iron intake can modulate inflammatory states of pregnant or breastfeeding women. Iron deficiency is the most common nutrient deficiency (Stoltzfus, 2003). Further assessment particularly during the early life period, including pregnancy and childhood (McCann et al., 2020; Means, 2020) is thus warranted, considering that the severity of iron deficiency may be time sensitive (Gambling et al., 2004).

Zinc is found in red meat, seafood, and grains (Saper and Rash, 2009). Proteins generally promote zinc absorption and bioavailability (Roohani et al., 2013). Zinc-dependent enzymes, factors, or transporters are necessary for a broad range of cellular processes during division, differentiation, and function (Donangelo and King, 2012). Zinc is thus crucial to embryogenesis, fetal growth, and development, as well as milk production (Donangelo and King, 2012; Roohani et al., 2013). Zinc is also an essential mineral for intestinal microbiome flora health. In a mouse model and clinical human studies, zinc deficiency during pregnancy was shown to alter the composition of the intestinal microbiome and gut permeability (Sauer and Grabrucker, 2019), as well as promote systemic inflammation (Wang et al., 2015) and neuroinflammation (Sauer and Grabrucker, 2019). It was suggested that alteration of the gut-brain axis may directly contribute to increasing inflammatory signaling upon zinc deficiency (Sauer and Grabrucker, 2019).

Zinc can act as an inflammatory regulator. Indeed, zinc ions can inhibit signal transduction (e.g., NFκB, IFN-λ3), which in turn prevents cytokine production [e.g., IL-1β, IL-6, monocyte chemoattractant protein 1 (MCP-1), TNF-α] (Jarosz et al., 2017; Read et al., 2017; Olechnowicz et al., 2018). Zinc closely regulates zinc-dependent proteins, including A20 zinc finger protein, metalloproteinase (MMP)2, MMP9, and PPAR-α, that can contribute to inflammatory processes (Jarosz et al., 2017; Olechnowicz et al., 2018). Zinc is also critical for membrane barrier maintenance and function, where a lack of zinc can

damage the membrane barrier (e.g., epidermal, gastrointestinal, pulmonary) hence permitting the entry of pathogens or toxins into the bloodstream. Moreover, zinc promotes cellular adhesion and migration (Jarosz et al., 2017). It is a key nutrient to inflammatory processes and contributes to the maintenance of immune cell homeostasis in steady-state and during pathogen-induced immune challenge (Gao et al., 2018). During pregnancy, low zinc levels increase inflammation as demonstrated by the enhanced expression of IL-6 and astrogliosis in the brain of pregnant mice fed with a zinc-deficient diet for 5 weeks prior to pregnancy and throughout gestation (Sauer and Grabrucker, 2019). This inflammation might also be involved in the development of autistic-like behavior in the offspring (Sauer and Grabrucker, 2019), including increased anxiety, impaired social behaviors (Grabrucker et al., 2014, 2016), attention-deficit-hyperactivity disorder (ADHD), hypotonia, and increased risk of seizure in later life (Grabrucker et al., 2014).

IMPLICATION FOR PREGNANCY AND THE PROGENY

Together, several nutrients can directly or indirectly influence the immune system, thus potentially disturbing pregnancy and fetal development when taken in inadequate quantity or balance. Moreover, pregnancy represents a unique immunological paradox; the maternal immune system tolerates the fetus and circulating fetal antigens, while fetal trophoblast (from the outer layer of the blastocyst or embryo) invades into the maternal uterus to coordinate nutrient delivery. To allow for proper immune tolerance, several mechanisms occur within the placenta: immunosuppression by paracrine signaling, circulation of fetal cells into the maternal circulatory system, secretion of immunosuppressing molecules by trophoblast and low antigen presentation by trophoblasts. Disturbance of the immune tolerance process provokes obstetric complications for the mother and developmental alterations for the progeny (Hsiao and Patterson, 2012).

Furthermore, MIA is a well-known and characterized risk factor of neurodevelopmental disorders like ASD and schizophrenia (Mattei et al., 2014; Fernández de Cossío et al., 2017b; Beversdorf et al., 2018; Bilbo et al., 2018; Prins et al., 2018; Bordeleau et al., 2019). This inflammatory-mediated mechanism is likely behind part of the detrimental effects of an inadequate maternal diet on the offspring.

Maternal Diet and Its Link to Neurodevelopmental Disorders

Genome-wide association studies and linkage studies have shown that the genetic architecture of many neurodevelopmental disorders comprises hundreds of genes affected, but each contributing only small effects to the overall phenotype or alternatively a single genetic mutation with large effects leading to very rare genetic syndromes (Sullivan et al., 2012). These genetic studies have often found common genetic vulnerability across diagnostically different neurodevelopment and neuropsychiatric disorders with convergent disruption of biochemical pathways that sustain synaptic and immune homeostasis (Garbett et al., 2008; Neale et al., 2012; Banerjee et al., 2014; Estes and McAllister, 2015). These pathways can, however, also be disrupted by a number of environmental factors that have now been linked to neurodevelopmental disorders. Some factors include gestational diabetes, maternal age and obesity, autoimmunity, and infection, with the common denominator between these factors being inflammatory processes during pregnancy (Estes and McAllister, 2015). The mother' immune activation during pregnancy appears to be an important risk factor for the neurodevelopmental alterations observed in the progeny that later manifest as behavioral disorders (Boksa, 2010; Knuesel et al., 2014; Ziats et al., 2015).

Pregnancy requires a tight regulation of the maternal immune system: it must allow the implantation and growth of a partially foreign body (the fetus), while simultaneously protecting it against pathogens to ensure the conservation of species (Mor and Cardenas, 2010; Racicot et al., 2014). Diet, even before conception, can affect pregnancy and alter the inflammatory status of the mother during pregnancy, conferring greater risk to the fetus beyond intrauterine developmental stage and throughout the progeny's developmental process. Deficiencies in micronutrients have previously been reported to have adverse outcomes for neurodevelopment. Among them, folate and vitamin B12 are important for DNA methylation, and their deficits were associated to neural tube defects (Molloy et al., 2008). In a meta-analysis examining how nutrition impacts on ASD, ADHD, and intellectual disability, folic acid and vitamin supplementation in the mother during gestation was inversely associated to neurodevelopmental disorders, particularly when supplementation occurred during early pregnancy (Li et al., 2019). Zinc is also important for neuronal development (Anjos et al., 2013) and iodine deficiency has long been known to cause cretinism, a developmental condition that has associated intellectual disability and is preventable (Cao et al., 1994). Notably, deficiency in dietary factors have also been linked to schizophrenia, as shown in three epidemiological studies focusing on specific periods of famine and reviewed in detail in association to other studies on deficient vitamin D, folate, and iron that is associated with an increased risk for this disorder (McGrath et al., 2011). Together, maternal diet has risen as an important risk factor for neurodevelopmental disorders, such as ASD, ADHD, and schizophrenia.

Protein is a macronutrient that has long been associated with impairing brain growth and thus having broad neurocognitive effects (Monk et al., 2013). Another macronutrient: fat, is also known to have important effects on neurodevelopment as components of omega-6 and omega-3 fatty acids (PUFAs) are necessary parts of neural cell membranes, and supplementation during pregnancy in controlled human trials showed better neurocognitive performance in the children (Helland et al., 2003), although supplementation with PUFAs was inconclusive in a meta-analysis looking at nutrition impacts on ASD, ADHD, and intellectual disability (Li et al., 2019). Equally important is the evidence in mice showing that diets high in saturated fat have deleterious consequences on brain development, including decreased hippocampal size (Niculescu

and Lupu, 2009). Importantly, studies revealed an increase in the inflammatory profile in obese/overweight pregnant women, particularly consistent is the increase of proinflammatory cytokine IL-6 and CRP (Pantham et al., 2015) and another recent meta-analysis revealed an increased risk for ASD conferred by overweight and obesity during pregnancy (Li et al., 2016). Another meta-analysis looking at population-based studies on the outcome of maternal weight in pregnancy also revealed increased risk of adult offspring to develop schizophrenia when the mother was obese or had a high BMI during early and late stages of pregnancy (Khandaker et al., 2012).

As with the genetic architecture, all of the individual components of nutritional imbalance can cumulatively contribute to a higher risk of neurodevelopmental disorders. A poor diet during pregancy generally lacks several of the micronutrients discussed as essential for neurodevelopment and for adequately regulating the immune system, as previously described in section "Specific Nutrient Imbalance and Inflammation," while it can simultaneously include an excess of macronutrients, as is the case of "Western diets" that have an inflammatory effect or lead to inflammatory states like overweight and obesity.

Potential Mechanism Behind Pathological Neurodevelopment

Genetic Programming in the Progeny

During development, the epigenome landscape is fully remodeled, therefore creating a time window during which adverse environmental exposure, including maternal diet imbalance, can trigger long-lasting changes on the actively differentiating cells of the offspring (Tarrade et al., 2015). This phenomenon occurring during prenatal and postnatal developmental stages is a process known as genetic programming of the cellular genome, transcriptome, or epigenome (Langley-Evans, 2009). Covalent modification of the chromatin as well as expression of microRNA can participate to fetal genetic or epigenetic programming, which can occur through adaptation mechanisms within the placenta or the developing fetus (Langley-Evans, 2009; Tarrade et al., 2015) (see **Figure 1B**).

The placenta directly contributes to intrauterine embryonic programming. Upon exposure to nutrient imbalance, the placenta adapts to help meet the needs of the growing and developing fetus. Adaptation and programming of the placenta involves alteration of the placental genome, transcriptome, and epigenome (Cox et al., 2015; Wilson et al., 2018) when exposed to various environmental factors, including inadequate maternal diet (Tarrade et al., 2015). Placental adaptation seems to occur in a sex-dependent manner (Rosenfeld, 2015). Male placentas are more dependent on the mother's diet even if the nutrient transfer of their placentas is more efficient, because they possess lower storage capacities than female placentas (Eriksson et al., 2010). On the contrary, female placentas are more sensitive to the maternal environment leading to improved adaptation and lower burden on fetal development (Clifton et al., 2001; Rosenfeld, 2015). For instance, in a mHFD mouse model, placental DNA of female offspring becomes globally hypomethylated at "imprinted" genes involved in cellular, metabolic, and physiological functions (whose expression is determined by the parent and differently expressed depending on whether it was inherited from the mother or the father). In contrast, the placenta of male offspring showed lower methylation levels at steady-state. Together, these findings suggest placental adaptive capacities of offspring exposed to mHFD (Gallou-Kabani et al., 2010). Maternal diet can therefore modulate genetic programming of the placenta early on during development, and it can also influence the inflammatory profile of the placenta thus modifying its function through several mechanisms, rendering the progeny sensitive to neurodevelopmental alterations (Goeden et al., 2016).

In the offspring, maternal nutritional intake has directly been linked to modification of the epigenome landscape, i.e., expression of epigenetic regulators. Although research is expanding on the matter, limited knowledge remains. Nevertheless, a growing body of evidence has been accumulated on the link between maternal dietary consumption of vitamins or fats that highlight the genomic or epigenomic role of maternal nutrients in the offspring. Vitamin B, for instance, directly contributes to methionine synthesis (e.g., involved in DNA, polyamines, amino acids, phospholipids synthesis), which together with ATP forms a methyl group during the one carbon metabolic pathway. Low maternal levels of vitamins B can hence decrease methylation activity and directly contribute to the epigenetic remodeling of the progeny and alter genetic expression (Pepper and Black, 2011; Richmond and Joubert, 2017). Maternal vitamin D has also been associated with DNA methylation in the offspring germline and liver cells, which become hypomethylated in cases of maternal deficiency (Xue et al., 2016). However, these effects are subtle (Xue et al., 2016) and it remains plausible that epigenome-wide studies will reveal more drastic or important effects of vitamin D on the offspring's epigenome signature.

More than the effect of specific nutrients, types of dietary patterns have also been suggested to modulate the offspring's genetic programming. Mediterranean diet—enriched in fish, fruits, and vegetables, and with an increased intake of mono-unsaturated fatty acids (MUFAs)—decreased the risk of child maladaptive and atypical behaviors like ASD, anxiety, and depression in humans (House et al., 2018). This positive behavioral outcome of Mediterranean diet on the offspring was linked to methylation changes of imprinted regions *SEGC endonuclease/paternally expressed gene* (*PEG*) 10 in male and female offspring, as well as *maternally expressed gene* (*MEG*) *3* and *insulin-like growth factor 2* (*IGF2*) in male offspring (House et al., 2018). Dietary supplementation with PUFAs from algal source during pregnancy similarly increased *IGF2*-imprinted methylation (Lee et al., 2014). Other than imprinting on genes expressed paternally or maternally, fatty acids have been proposed to modulate the epigenome of genes involved in the biosynthesis of PUFAs like fatty acid desaturase 1/2 (Mennitti et al., 2015). Genome-wide studies on the blood of preadolescents with regard to their dietary intake demonstrated a significant correlation between methylation and total fat intake or ratio of MUFAs and PUFAs over total consumption of fats

(Voisin et al., 2015). This suggests that in pregnant and nurturing women, fat intake and balance—including type of fats and proportions of other nutrients—may promote epigenetic changes within their progeny. Similarly, diet enriched in saturated and unsaturated fats has been linked to epigenetic changes in the offspring at the periphery (e.g., adipose tissue, heart, liver) (Attig et al., 2013; Keleher et al., 2018; Zhang et al., 2019) and in the CNS (Glendining and Jasoni, 2019). In the brain, mHFD animal model also demonstrated by ChIP-qPCR on the offspring hippocampus that mHFD leads to increased histone 3 K9 acetylation in males as well as decreased methylation of the histone K9 in females (Glendining and Jasoni, 2019). In another study by Grissom et al. (2015), mHFD was associated with the overexpression of epigenome modulator protein, DNA methyltransferase 1 (DNMT1), in the prefrontal cortex of the offspring. Moreover, mHFD mouse model investigating programming effect of mHFD on the offspring prior to pregnancy has demonstrated that hypertrophy and inflammation of adipose tissue, as well as overexpression of genes involved in fat deposition could only be prevented by long-term diet intervention prior to pregnancy (Summerfield et al., 2018), suggesting that offspring programming by the diet is a long-lasting mechanism. Strikingly, as we discussed in section "Carbohydrates and Fats," imbalance of fatty acids as well as mHFD possesses inflammatory properties that seem to coincide with genetic reprogramming.

Limited information is available regarding the specific mechanisms of genetic programming, and how inflammation and epigenetic may be linked together; is the genetic reprogramming influenced by the inflammatory properties of the diet or are the two processes independent of each other? As genetic programming effects of maternal diet can partake in the development of neurodevelopmental disorders in the offspring, it is thus important to understand how inflammation and genes may interact together in the pathogenesis to comprehend how we could guide pregnant woman dietary consumption and limit their detrimental effects.

Neuroinflammation: Microglia and Blood Barrier

As previously discussed, nutrients can influence inflammatory processes in the mother, which in turn can promote inflammation in the progeny. Indeed, animal models of mHFD have described changes of inflammatory mediators (e.g., CD11b, IL-6, NFκB, TLR4) in the brain across the lifespan: neonate (Bilbo and Tsang, 2010; Graf et al., 2016), juvenile (Bilbo and Tsang, 2010), adolescent (Sasaki et al., 2014; Winther et al., 2018; Bordeleau et al., 2020), and adult (Bilbo and Tsang, 2010; Sasaki et al., 2013) stages. These changes in gene expression seem to occur differently between ages, sexes, and brain regions. Similarly, upon exposure to a maternal low *n*-3 PUFA diet, offspring's brain transcriptomic signature revealed increased expression of transcript cluster associated to innate immune response and inflammation in *n*-3 PUFA-deficient mice (Madore et al., 2019). Moreover, both mHFD and maternal low *n*-3 PUFA studies reported changes in microglial density, morphology, mRNA/protein expression, or functions associated with brain development alterations, commonly observed in neuropsychiatric disorders (Bilbo and Tsang, 2010; Rey et al., 2018; Madore et al., 2019; Bordeleau et al., 2020). Although the impact of maternal nutrients other than fats and sugar has not been studied yet in the context of neuroinflammation changes in the offspring, it should be expected that their inflammatory effects in the mother have a ripple effect in the brain of the offspring, contributing to neurodevelopmental alterations. Moreover, microglia—the main immune cells of the CNS—are highly specialized in detecting and deploying inflammatory signals, which are especially sensitive to the inflammatory status during pregnancy that could modulate their physiological roles in brain development (Schwarz and Bilbo, 2012; Tay et al., 2018) (see **Figure 1B**).

Indeed, microglia are key immunocompetent cells that produce and respond to inflammatory cues. During neurodevelopment, these cues can influence microglial role and lead to changes in their modulation of neuronal network wiring and maturation (Tremblay et al., 2010; Paolicelli et al., 2011; Mallya et al., 2019), myelination (Bennett and Barres, 2017; Hagemeyer et al., 2017; Wlodarczyk et al., 2017), and of neurovascular development and maturation (Arnold and Betsholtz, 2013; Lacoste and Gu, 2015), throughout embryonic, fetal, juvenile, and adolescent neurodevelopment. Additionally, microglia undergo different stages of maturation during brain development, and this appears to be programmed *in utero* and dependent on maternal gut microbiota and inflammatory status of the mother (Matcovitch-Natan et al., 2016). Therefore, exposure to inflammatory-modulating environmental factors can modify microglial developmental roles and thus profoundly impact the offspring's brain development.

Simultaneous to brain development, the offspring cerebrovascular system is developing and maturing from embryonic to adolescent stages (Arnold and Betsholtz, 2013). In the brain, several immune cues such as Notch, TNF-α, vascular endothelial growth factor (VEGF), and Wnt5a/11, released in part by microglia, contribute to neurovascular development and maturation (Arnold and Betsholtz, 2013; Lacoste and Gu, 2015). Peripheral and local inflammatory signals within the CNS can disturb its proper neurodevelopment, thus impacting neurovascular organization and function (Pearson-Leary et al., 2017; Van Dyken and Lacoste, 2018). In turn, this can impact on neuronal network function and plasticity (Sloan et al., 2010; Andreone et al., 2015; Lacoste and Gu, 2015) and render the offspring vulnerable to stress (Menard et al., 2017; Pearson-Leary et al., 2017). In fact, Menard et al. (2017) demonstrated that peripheral administration of cytokine IL-6 alone in steady-state was sufficient to induce neurovascular remodeling leading to increased permeability of the blood–brain barrier. Similarly, several other inflammatory mediators produced or modulated by maternal diet discussed above (see section "Specific Nutrient Imbalance and Inflammation") could result in functional and organizational changes in the brain of the offspring (see **Figure 1B**). Although no intensive work has really been done on the matter, a leaky or dysfunctional blood–brain barrier could lead respectively to the recruitment of peripheral immune cells that could infiltrate and modulate the offspring's

neurodevelopment or to an inefficient transfer of adequate nutrients and energy to the CNS.

Gut Microbiome-Mediated Endotoxicity

Linked to its effects on inflammatory states and barrier permeability, HFD is well documented to have an important effect on the gut microbiome. In male mice, HFD alters gut microbiome within 4 weeks (Cani et al., 2008). Specifically, there are decreases for *Bifidobacterium* spp. and *Bacteroides*-related bacteria, as well as *Eubacterium rectale–Blautia coccoides* in mice (Cani, 2012), although a reduction in *Bacteroidetes* levels and an increase in *Firmicutes* and *Proteobacteria* were previously identified in the context of HFD, with or without inducing obesity (Hildebrandt et al., 2009; Ravussin et al., 2012). In addition, HFD can lead to metabolic endotoxemia (Cani et al., 2007), i.e., a sustained low-grade increase in circulating levels of LPS (a component of the outer membrane of Gram-negative bacteria), a lipoprotein acting as an inflammatory agent. While LPS has a physiological variation that peaks after feeding (Cani et al., 2008), persistent low-grade inflammation was observed after a mere 4 weeks of HFD consumption in adult mice (Cani et al., 2012).

The abnormal levels of LPS that infiltrate into the bloodstream after HFD appear to be due to a leaky gut–blood barrier. Indeed, when LPS levels were maintained abnormally high, there was a decrease in genetic expression of gut barrier tight-junction proteins zonula occludens-1 and occludin (Cani et al., 2012), but when treated with antibiotics, there was no disruption to the intestinal barrier, proposing therefore a role for gut microbiota in maintaining its integrity (Cani et al., 2008). Other forms of gut microbiota modulation through the use of pre- and probiotics have been shown to improve the integrity of the gut barrier and prevent metabolic endotoxemia in obese mice (Cani et al., 2009). In pregnancy, the issue of metabolic endotoxemia could be problematic for two main reasons: (1) it was associated with obesity, metabolic syndrome, and type 2 diabetes, all of which are pregnancy risk factors for the offspring, and (2) increased LPS in the bloodstream during pregnancy (even small but constant increases) amounts to an activation of the maternal immune system, which is known to have adverse consequences for the offspring.

Studies in pregnant women revealed that higher pregnancy BMI is linked to altered microbiome composition, with increase of bacteroides and staphylococcus, associated with excessive weight gain during pregnancy (Collado et al., 2008). In rats, bacterial population ratios change over the course of pregnancy and the alterations are exacerbated with the consumption of a HFD, regardless of whether it is accompanied by weight increase (Mann et al., 2017). Importantly, in a systematic review by Nelson et al. (2010), pre-pregnancy BMI was linearly associated to most adverse pregnancy outcomes and complications such as hypertension, preeclampsia and glucose intolerance/gestational diabetes. Not only is the greater BMI linked to metabolic alterations of fat and glucose pathways (Hellmuth et al., 2017), but obese women are observed to have increased circulating levels of the inflammatory mediator IL-6 (Ramsay et al., 2002; Basu et al., 2011) that appear to be linked to increased amounts of circulating endotoxins including LPS (Basu et al., 2011). Another group showed that overweight pregnant women had increased barrier permeability that was associated with higher blood concentrations of LPS (Mokkala et al., 2017). In addition, Laitinen et al. (2009) elegantly demonstrated in a cohort of normoglycemic pregnant woman that changes in glucose metabolism due to pregnancy could be improved by changing the gut microbiome *via* probiotics, with positive effects lasting past 12 months postpartum. This is congruent with experimental work showing that even in genetically obese mice (i.e., Ob/Ob and Db/Db mice), the use of prebiotics delayed and improved glycemic alterations in steady-state adulthood (Fernández de Cossío et al., 2017a; Song et al., 2019).

Together, the body of literature suggests that pregnancy itself produces microbiome, metabolic and peripheral immune changes in the mother that are similar to those observed in overweight and obese persons and which are associated to cardiovascular and glucose homeostasis issues *via* an augmented inflammatory status. The microbiome alterations are exaggerated further in the case of overweight or obesity before and during pregnancy. The gut microbiome can directly contribute to sustaining inflammatory processes in the mother, which in turn, can compromise offspring development, notably by acting on microglia (Castillo-Ruiz et al., 2018; Thion et al., 2018). Still, future investigation is indispensable to understand the molecular and cellular pathway linking maternal diet-gut microbiome to the offspring's neurodevelopment.

Perspectives and Concluding Remarks

In our day-to-day life, disadvantaged socioeconomic status as well as limited access to nutritious food are linked to the development of metabolic disorders associated with malnutrition, including overweight and obesity in pregnancy (Bleich et al., 2008; Martin et al., 2016). A growing body of evidence demonstrates the importance of an adequate quality and quantity of nutrients during pregnancy for the progeny's development. As we discussed in this review, inadequate nutrient intake can create a systemic imbalance in the mother, compromising the developmental environment, thereby inducing an inflammatory state and/or a malabsorptive status. Under these conditions, the offspring might have an increased risk of developing neurodevelopmental disorders, which may be revealed with the occurrence of other environmental challenges later in life. As such, neurodevelopmental disorders have long been believed to mainly originate from genetic predisposition that upon exposure to certain environmental conditions lead to the expression of pathological behaviors. Maternal diet can exacerbate genetic vulnerability when it is deficient in essential nutrients or is grossly unbalanced; however, it can also directly influence fetal genetic programming and expression, and it has been shown to contribute to inflammation in both the mother and progeny with regulatory effects on brain development. As an influencing factor or part of the etiology, maternal diet is an actionable environmental contributor to brain pathology. Therefore, it may be a corner stone in setting the developmental outcomes of the progeny.

The tremendous importance of an optimal maternal diet for proper development and neurodevelopment encompassing cognitive and behavioral outcomes not only emphasizes the need to investigate thoroughly the long-term incidence of maternal lifestyle factors, namely dietary, but also stress exposure, meditation, physical exercise, etc. on the offspring. Moreover, the impact of the quality standard of food production (e.g., food-processing treatment, agriculture exposure, water and soil contamination) as well as water and air pollution should be considered. Epidemiological studies so far have also provided little information about food origin (e.g., GMO labels are not mandatory worldwide), as well as limited knowledge of environment contamination, pollution, use of pesticides, etc. In addition, little is known of how nutrition, metabolism, and inflammation are tied together, which highlights the need for longitudinal studies investigating their joint involvement in the progression, resolution, and modulation of offspring outcomes across life (infancy, childhood, adulthood, and aging).

In a similar way that women about to conceive or that are pregnant need to avoid toxic and infectious elements in the environment, it is also critical for them to pay particular attention to their nutritional intake. Indeed, environmental factors are actionable and nutrition can exert an effect on mechanisms that regulate and interact with genetic expression. If maternal diet can prime through its genetic and inflammatory effects the offspring to develop disorders in later life, then better understanding of dietary needs during fetal and neonatal development could lead us to understand the proper diet for a healthy progeny.

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MB and LFC wrote the main manuscript of the review under the guidance and critical reviewing of MMC and M-ÈT. All the authors read, edited, and approved the final manuscript.

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Structural and Functional Features of Developing Brain Capillaries, and Their Alteration in Schizophrenia

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Schizophrenia affects more than 1% of the world's population and shows very high heterogeneity in the positive, negative, and cognitive symptoms experienced by patients. The pathogenic mechanisms underlying this neurodevelopmental disorder are largely unknown, although it is proposed to emerge from multiple genetic and environmental risk factors. In this work, we explore the potential alterations in the developing blood vessel network which could contribute to the development of schizophrenia. Specifically, we discuss how the vascular network evolves during early postnatal life and how genetic and environmental risk factors can lead to detrimental changes. Blood vessels, capillaries in particular, constitute a dynamic and complex infrastructure distributing oxygen and nutrients to the brain. During postnatal development, capillaries undergo many structural and anatomical changes in order to form a fully functional, mature vascular network. Advanced technologies like magnetic resonance imaging and near infrared spectroscopy are now enabling to study how the brain vasculature and its supporting features are established in humans from birth until adulthood. Furthermore, the contribution of the different neurovascular unit elements, including pericytes, endothelial cells, astrocytes and microglia, to proper brain function and behavior, can be dissected. This investigation conducted among different brain regions altered in schizophrenia, such as the prefrontal cortex, may provide further evidence that schizophrenia can be considered a neurovascular disorder.

Keywords: schizophrenia, blood vessels, claudin-5, neurovascular unit, neurovascular coupling

INTRODUCTION

Affecting 1% of the global population, schizophrenia (SCZ) is a disabling neurodevelopmental disorder that has seen little improvement in treatments over the last decades (Insel, 2010), leaving patients with a low quality of life (Ritsner et al., 2003). SCZ shows very high heterogeneity in the positive (i.e., hallucinations, delusions), negative and cognitive symptoms (i.e., incoherent alogia, affective flattening, anhedonia, learning, memory deficits) experienced by patients, which can be

linked to dysfunction in different brain regions (Norris and Strickland, 2017; Glausier and Lewis, 2018). Many features of this disorder are being investigated and have been reviewed from different perspectives, such as the role of the immune system (Sekar et al., 2016; Hui et al., 2018), dopamine pathways (Weinstein et al., 2017), psychiatric deficits (Bora and Murray, 2014; Catalano et al., 2018) and sex differences (Bordeleau et al., 2019). Known risk factors include genetic variants (Marshall et al., 2017) and environmental factors (e.g., air pollution, stress, infection) (Huttunen and Niskanen, 1978; Gomes and Grace, 2017; Korpela et al., 2020). Another important aspect to consider for proper understanding of the pathogenesis of SCZ is the characterization of postnatal development of the brain and its vasculature, as proper establishment of the neurovasculature via bidirectional communication between endothelial cells (ECs) and central nervous system (CNS) cells (Segarra et al., 2018) is crucial for CNS development.

As the highway of the brain, the neurovasculature serves many roles for brain support by providing ions, oxygen, nutrients, and energy metabolites, while also allowing for communication between the periphery and the brain (McConnell et al., 2017). In homeostatic conditions, cerebral blood flow is regulated by the vasculature based on brain activity, increasing and reducing the flow in regions of high or low need (Peterson et al., 2011). To accomplish these functions, cerebral blood vessels need to develop and mature as an efficient network. Vascularization has been shown to be tightly guided by glial cells, such as microglia and astrocytes (Tata et al., 2015). Previous literature shows evidence of vascular impairments contributing to developmental disorders such as autism (Ouellette et al., 2020), and potentially SCZ (Najjar et al., 2017; Kealy et al., 2020). Although the role of these vascular alterations in SCZ is still not clear, one could hypothesize that vascular changes during development affect the establishment of the blood vessel network, leading brain maturation down a path that eventually results in the symptoms experienced by SCZ patients. This review will underline the current view on the vascular hypothesis through discussing normal postnatal development of the neurovascular unit (NVU) in humans and animal models, the establishment of the neurovascular coupling, as well as the misshaping of this development as a potential contributor to SCZ pathogenesis.

DEVELOPMENT OF THE NEUROVASCULAR UNIT

The NVU is a relatively recent concept (Iadecola, 2017) that refers to the cellular components [e.g., endothelial cells (ECs), pericyte and astrocyte] that contribute to the functional relationship between brain cells and cerebral vasculature (Coelho-Santos and Shih, 2020) with each cell type having their specific molecular signature (Vanlandewijck et al., 2018). This relationship notably allows for neurovascular coupling (NVC) between neuronal activity and blood flow and the establishment of a properly selective blood-brain barrier (BBB) required to protect the brain against homeostatic disturbance from the periphery (Bell et al., 2019; Sweeney et al., 2019).

Neurovascular Coupling During Normal Development

Although still an area of active research, the various cellular elements of the BBB play a role in coupling neuronal activity to vascular tone and cerebral blood flow. Astrocytes can react to glutamatergic synaptic signaling by producing vasoactive compounds that cause pericytes to dilate capillaries (Hall et al., 2014; Mishra et al., 2016; Kisler et al., 2017). Capillary ECs can also detect potassium ionic currents and subsequently propagate a vasodilatory signal to upstream arterioles (Longden et al., 2017). Various neuronal subtypes directly signal to the vasculature by producing vasodilative or vasoconstrictive molecules (Uhlirova et al., 2016), for example nitric oxide release by glutamatergic neurons was proposed to suppress release of the vasoconstrictor 20-hydroxyeicosatetraenoic acid by astrocytes (Hall et al., 2014). This neurovascular coupling (NVC) explains the relationship between neuronal activity and the tight modulation of local oxygen/glucose concentration (Iadecola, 2017) and can provide an indirect measure of metabolic demand, which is altered in certain disorders including SCZ (Zhu et al., 2017).

NVC is also the basis for hemodynamic based non-invasive imaging of brain activity. When neuronal activity elicits an increase in blood flow in a given brain region, the rate of oxygen delivery exceeds the rate at which it is consumed, leading to a localized increase in oxyhemoglobin concentration concomitant with a decrease in deoxyhemoglobin (HbR) concentration (Buxton, 2013). With hemodynamic based functional imaging techniques, this change in oxygenation can be measured and used as a proxy for neuronal (and glial) activity. Among those techniques, functional magnetic resonance imaging (fMRI) and near infrared spectroscopy (NIRS) are the most commonly used for imaging neurovascular development in infants (Kozberg and Hillman, 2016; Hendrikx et al., 2019). In fMRI, changes in HbR concentration create the positive (HbR decrease) or negative (HbR increase) blood oxygenation level-dependent (BOLD) signal (Ogawa et al., 1992; Kim and Ogawa, 2012). Optical functional techniques, such as NIRS and its more invasive equivalent used in rodents, intrinsic optical signals (IOS), also measure HbR as well as oxy- and total hemoglobin concentration changes. Performing MRI in infants is still very challenging because of its sensitivity to motion artifacts (Dean et al., 2014), whereas NIRS offers a portable alternative for measuring functional hemodynamic signals in the cortex at low cost and which can be used in multiple experimental environments, even in schools (Soltanlou et al., 2018; Whiteman et al., 2018).

MRI and NIRS have shown great potential to measure hemodynamic signals longitudinally (Demirci et al., 2008; Yang et al., 2019) with growing literature investigating development as gathered in **Table 1**. This table compares results from previous studies in which task-evoked hemodynamic responses were measured in healthy young children or rodents using fMRI or NIRS/IOS.

TABLE 1 | Summary of 20 years of studies investigating hemodynamic responses at several stages of homeostatic cerebrovascular development.

fMRI studies

References	Species	State	Stimulation	Age	BOLD results
Born et al. (2000)	Human	Asleep/Awake	Visual	48 weeks	↑
				56 weeks	↓
Yamada et al. (2000)	Human	–	Visual	0–7 weeks	↑
				8–22 weeks	↓
Anderson et al. (2001)	Human	Awake	Auditory	40–50 weeks	↑
				50 weeks	↓
Sie et al. (2001)	Human	Sedated	Visual	18 months	↓
Born et al. (2002)	Human	Sedated	Visual	4–71 weeks	↓
Erberich et al. (2006)	Human	Sedated	Somatosensory	28–46 weeks	↓
Colonnese et al. (2008)	Rats	Sedated	Somatosensory	P13 to adulthood	↑
Heep et al. (2009)	Human	Sedated	Somatosensory	Preterm infant (26.5 weeks)	↓
				Term infant (39 weeks)	↓
Arichi et al. (2010, 2012)	Human	Sedated	Somatosensory	Preterm	↑
				Term	↑

Optical imaging studies

References	Species	State	Stimulation	Age	HbO	HbR	HbT	BOLD equivalence
Sakatani et al. (1999)	Human	Awake	Visual	3 years	–	–	↑	–
Hoshi et al. (2000)	Human	Asleep	Visual	4–5 days	↑	↑	↑	–
						None		
						↓		
Zaramella et al. (2001)	Human	Awake/Asleep	Auditory	0–7 weeks	–	–	↑	–
Taga et al. (2003)	Human	Awake	Visual	2–4 months	↑	↓	–	↑
Kusaka et al. (2004)	Human	–	Visual	4–16 weeks	↓	↑	↑	↓
Watanabe et al. (2008)	Human	Awake	Visual	2–4 months	↑	↓	–	↑
Karen et al. (2008)	Human	Asleep	Visual	2–9 days	↑	↓	↑	↑
Liao et al. (2010)	Human	Asleep	Visual	2 days	↑	↓	↑	↑
Kozberg et al. (2013)	Rats	Anesthetized	Somatosensory	P12–P13	↑	↓	↑	↑
				(∼1 year human in humans)	↓	↑	↓	↓
Sintsov et al. (2017)	Rats	Non-sedated	Somatosensory	0–3 months (∼8 years in humans)	↑	↓	–	↑

The up or down arrows indicate an increase or a decrease, respectively, in the value of the measure of blood oxygen level dependent (BOLD) signal, oxyhemoglobin (HbO), deoxyhemoglobin (HbR) and total hemoglobin (HbT) during the activation period in comparison to the resting period. Multiple arrows in the same box signify different responses observed within the group of the study and no change between those two states is identified by "None." Parameters not reported in these studies are identified with a hyphen (-). Equivalence between rat and human ages were estimated based on (Sengupta, 2013).

Overall, these results are difficult to properly interpret. Although it is known that the hemodynamic response is necessary to induce vessel remodeling (Lucitti et al., 2007), the timeline of developmental patterns of the various components of NVC are not all well-defined, making it difficult to know if the varied hemodynamic responses observed are caused by altered neuronal activity in infants or an immature NVU. Second, as was previously noted (Harris et al., 2011), the lack of standardization in imaging parameters and stimulation paradigms adds many confounding variables when looking for consistent trends in results from functional imaging studies. Given the vascular component of SCZ, it can be investigated using techniques reported in **Table 1**. In our review of the literature on the hemodynamic response in SCZ patients investigated using NIRS (Ikezawa et al., 2009; Takizawa et al., 2009; Fujita et al., 2011; Kinou et al., 2013; Pu et al., 2015, 2016; Noda et al., 2017) and fMRI (Barch et al., 2003; Kircher et al., 2004; Tregellas et al., 2004, 2009; Ford et al., 2005; Dyckman et al., 2011; Mayer et al., 2013, 2016; Hanlon et al., 2016), no studies were found during development, a question that should be addressed to better understand NVC deficits in SCZ. The structure of the NVU is also a growing field for SCZ research (Villabona-Rueda et al., 2019).

Development of the Capillary Network

During postnatal development, bidirectional communication between brain cells and the nascent vasculature ensures that capillaries grow side-by-side with the maturing neurons and glial cells so that the latter are provided with sufficient energy

substrates (Paredes et al., 2018). This results in a dense mesh of capillaries matching the metabolic demand of the neurons and glial cells they support (Craigie, 1945; Weber et al., 2008; Lacoste et al., 2014). In rodents, at birth, the capillary bed is sparse, but goes through a rapid expansion in the first few postnatal weeks. Studies examining capillary growth from birth to adolescence in rodents have consistently shown more than a twofold postnatal increase in measures such as vessel density and volume compared to neurons density and branching before the growth stabilizes at postnatal day (P)20 (Keep and Jones, 1990; Wang et al., 1992; Zeller et al., 1996; Harb et al., 2013). A similar increase is seen in postnatal primates, in which relative vascular volume can double between birth and adulthood, reducing the distance between tissue and the vasculature by 32%. This doubling occurs mostly via angiogenesis and partly from the lengthening of existing vessels (Risser et al., 2009). This vascular increase is thought to originate almost solely from the capillary bed, as the network of larger penetrating arterioles and ascending venules is stable throughout postnatal development (Norman and O'Kusky, 1986; Risser et al., 2009). Interestingly, an earlier study in young rats showed that the vascularization of the capillary bed is not a continuous process, but rather occurs in distinct bouts of intense sprouting between P0 and P4, P7 and P8, at P10 and at P14, across the cerebral cortex (Rowan and Maxwell, 1981) but not the cerebellum (Craigie, 1924). The temporal pattern of sprouting was different across cortical layers, but always more intense in the middle layers, peaking within cortical layer 4 at adulthood (Harrison et al., 2002; Blinder et al., 2013).

Angiogenesis in the capillary bed is highly adaptive during early development. In rodents, enhanced sensory stimulation of the whiskers or complex experiences (e.g., vision) in the first postnatal month can increase capillary density in the somatosensory and visual cortices, respectively (Black et al., 1987; Lacoste et al., 2014). On the other hand, both sensory deprivation and hyperstimulation during that period can result in lower capillary density (Lacoste et al., 2014; Whiteus et al., 2014) without measurable changes in neuronal density in the regions analyzed. The pial vasculature for its part does not seem to adapt to sensory stimuli (Adams et al., 2018).

Following an early critical window, the microvasculature becomes less adaptive: for example, Whiteus et al. (2014) showed that the decreases in capillary density observed following chronic hyperstimulation by repetitive sounds, whisker deflection or motor activity in mice neonates (P15) can be restored if the perturbations were stopped after 5 days, but not if they were sustained for 15 days. Chronic hypoxia, which can induce robust angiogenesis in young mice during the second week of life, has also been shown to stop evoking capillary responses in the somatosensory and motor cortices after 3 months of age (Harb et al., 2013).

Development of the Cellular Components of the NVU

The main components of the NVU (**Figure 1**), ECs, exert functions such as the active transport of ions and nutrients through the BBB via membrane transporters whose levels vary during development. Expression of the P-glycoprotein (PGP) efflux transporter, which is hardly detectable at birth, is upregulated throughout the first postnatal month in mice (Daneman et al., 2010). ECs also upregulate the glucose transporter (GLUT) 1 in the second week to reach adult levels by P30 in rats (Harik et al., 1993; Vannucci and Vannucci, 2000).

The second main component of the NVU is astrocytes and their endfeet. In rodents, astrocytes start to be present in the cortex shortly after birth, and their endfeet typically fully ensheath capillaries by P15 (Mathiisen et al., 2010; Gilbert et al., 2019). In parallel, the gliovascular interface undergoes maturation, as protein complexes at the junction between perivascular astrocytic endfeet are assembled between P10 and P15 (Gilbert et al., 2019). The timing of astrocyte appearance in the cortex differs between species. In humans, this begins embryonically (El-Khoury et al., 2006). When astrocytes appear postnatally, the BBB is already functional meaning that astrocytes are not required for BBB function but rather seem to have a role in BBB maintenance later in life (Daneman et al., 2010). In addition, microglia were shown to ensheath the basement membrane of capillaries and contribute to the glia limitans, although their roles in the BBB formation and maintenance remain largely elusive (Bisht et al., 2016; Joost et al., 2019).

In contrast, pericytes coverage of capillaries is already established in neonatal rodents and is vital for BBB establishment, playing a role in proper tight junction orientation (Daneman et al., 2010; Ben-Zvi et al., 2014). Furthermore, during postnatal angiogenesis, pericytes are recruited to induce the formation of new capillaries via platelet-derived growth factor signaling in mice (Lindblom et al., 2003). Pericyte proliferation decreases steadily in mice from birth to P25 in the somatosensory and motor cortex (Harb et al., 2013). Initially, ECs express cluster of differentiation 146 (CD146) in order to upregulate claudin-5 forming the BBB. Expression of CD146 by pericytes promote their migration toward the ECs which in turn release transforming growth factor beta 1, down-regulating endothelial CD146 to reduce the expression of claudin-5 (Chen et al., 2017). Of the many components required for the development of the NVU, claudin-5, the dominant component of tight junctions forming the BBB, is already expressed in capillary ECs at P0 (Ek et al., 2006; Greene et al., 2019). In mice, its production increases more than threefold by P15 before stabilizing, indicating continued postnatal maturation of the BBB (Gilbert et al., 2019). Claudin-5 deficiency, resulting in BBB dysfunction, is causal in animal models of stress and depression (Menard et al., 2017; Pearson-Leary et al., 2017). Furthermore, mutation in claudin-5 is also seen in SCZ human patients (Omidinia et al., 2014) with dysfunction linked to change in other tight junction proteins such as ZO-1 and occludin (Maes et al., 2019; Greene et al., 2020).

Cellular, Vascular, and Genetic Dysfunction in SCZ

SCZ is recognized to be linked to genetic vulnerabilities (Strawbridge et al., 2018; Chen et al., 2019; D'Ambrosio et al., 2019) (also reviewed in Comer et al., 2020a)

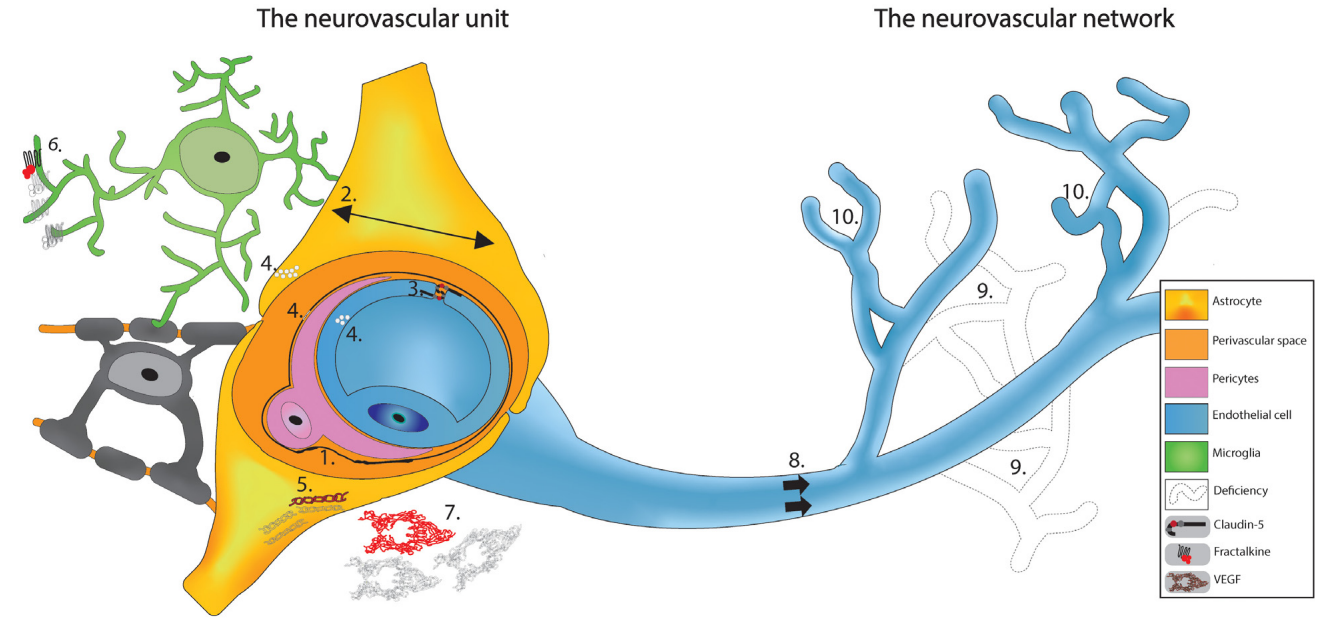

FIGURE 1 | Schematic based on cumulative observations made on SCZ patients in the PFC region. The vasculature appears to have deficits at the neurovascular unit where we see: (1) Thickening and deformation of basal lamina. (2) Increase in the area of the astrocytic endfeet. (3) Haplodeficiency of claudin-5. (4) Cytoplasm vacuolation; and (5) Reduced GFAP labeling. When investigating the vasculature as a network surrounded by glial cells, cumulative evidences shows: (6) Diminished fractalkine signaling. (7) Decreased VEGF expression. (8) Reduced cerebral blood flow. (9) Lower capillaries density; and (10) Abnormal arborization. Altogether, these findings propose a potential vascular signature for SCZ that might explain the neuronal (and glial) functional deficits.

and environmental factors during adolescence and into young adulthood (Pulver, 2000; Gomes and Grace, 2017; Qiu et al., 2019; Barichello et al., 2020). On the vascular level, genetic mutation on the chromosome 22q11 results in the loss of about 40 genes, one gene of interest being claudin-5 (Graw et al., 2012; Tang et al., 2014; Thompson et al., 2017). In mice engineered with a mutation in 22q11, claudin-5 expression is reduced by 75% in ECs, which was reproduced in cell culture (Greene et al., 2018). Furthermore, using MRI in SCZ patients, the 22q11 mutation was associated with decreased brain volumes for both total grey ($g = -0.81$) and total white matter ($g = -0.81$) calculated by a meta-analysis of between-group differences in mean volumes, representing the effect size (g) (Rogdaki et al., 2020). Considering that most investigations on vascular alterations in patients with SCZ are done using *post-mortem* tissue (McGlashan, 2011; Harris et al., 2012; Katsel et al., 2017), it is difficult to have a good idea on the temporal development of those deficits. To our knowledge, no longitudinal studies have been performed on the vascular aspect of SCZ, a question that remains to be addressed in the field. When the NVU and the BBB are altered in SCZ, then the vasculature would be unable to answer neuronal and glial cells engaging in their normal activities. A *post-mortem* study showed cardiovascular disorders as the primary cause of death in SCZ patients (Sweeting et al., 2013). More clinical evidence was extensively reviewed by Najjar et al. (2017). Notably, patients show elevation in CSF albumin (higher ratio of CSF-albumin to P-albumin), IgG, IgM, S100B and in several vascular endothelial adhesion molecules (soluble platelet selectin, serum L-selectin, integrin αIIbβIIIa, receptors on platelets) as well as decreases in vascular endothelial growth factor (VEGF) (Najjar et al., 2017; Melkersson and Bensing, 2018). In living human studies using dynamic contrast-enhanced (DCE)-MRI to study BBB integrity of the hippocampus, investigations pertaining to dementia and related disorders are extensive (Raja et al., 2018; Nation et al., 2019) but have not yet been targeted at the specific case of SCZ.

Vascular Dysfunction in SCZ PFC

Brain imaging in SCZ patients investigating the hemodynamic response has been performed using fMRI (Hanlon et al., 2016). Although data is lacking about the prodromal stage, many vascular correlates of the disease have been identified. The PFC has been the subject of a great number of studies detailing the vasculature in SCZ, but is not the only region implicated. Whole brain analysis using inflow-based vascular-space-occupancy MRI also show significant reduction in arterial cerebral brain volume in temporal cortex grey matter of SCZ patients (Hua et al., 2017). Studies using different MRI sequences found reduced CBF in the frontal lobe (Malaspina et al., 2004), temporal lobe (Kindler et al., 2015), parietal lobe (Scheef et al., 2010) and occipital lobe (Pinkham et al., 2011).

SCZ patients also show morphological and functional alteration in glial cells present in this region, such as microglia (Bordeleau et al., 2019) and astrocytes (Abbink et al., 2019). Dark microglia, classified as such by their electron dense cytoplasm, have been found in numerous pathological conditions including in patients with SCZ and animal models of schizophrenia-like disorder simulated with the viral mimic poly I:C (Hui et al., 2018; St-Pierre et al., 2020). These altered microglia

make extensive interactions with the NVU and have been suggested to take over astrocytic functions in SCZ (St-Pierre et al., 2020). Investigations of astrocytes in SCZ patients revealed larger astrocytic endfeet covering vessels (Uranova et al., 2010). This could be a compensation mechanism for the decreased astrocytic density seen in SCZ patients (Najjar et al., 2017), resulting in missing NVU components (**Figure 1**). There are also myelination deficits in patients with SCZ, implicating another glial cell type, oligodendrocytes (Raabe et al., 2018). A recent review has highlighted the need for NVU integrity to promote oligodendrocyte survival, potentially explaining the myelination deficit in SCZ (Hamanaka et al., 2018).

All three glial cell types appear to be key players in SCZ as covered in reviews focused on the subject (Bernstein et al., 2015). Astrocytes and microglia play key roles in controlling cerebral blood flow in a calcium dependent way as shown in mice (Mulligan and MacVicar, 2004; Mishra et al., 2016; Kleinberger et al., 2017). Overall, defects in the PFC vasculature and alterations in glial cells in SCZ investigations keep emerging, allowing us to both revisit existing and draw new hypotheses on its pathophysiology.

DISCUSSION

The Vascular Hypothesis

Although many of the findings discussed above are recent, the vasculature hypothesis of SCZ is not. As highlighted in a brief history (Meier et al., 2013) based on a century old hypothesis (McGlashan, 2011), symptoms of SCZ could possibly be explained by cerebral microvasculature damages (Hanson and Gottesman, 2005). A possible mechanism is systemic inflammation shown in SCZ patients (Cai et al., 2020) coming from environmental factors (e.g., pollution, stress, nutrition induced gut-brain axis dysbiosis, viral infection, maternal immune activation) and genetic predisposition as the source of perturbation (Comer et al., 2020a). This inflammation is detrimental to the development of the vasculature, possibly already weakened by genetic mutation resulting in cellular damage (Hanson and Gottesman, 2005). The affected cells of the NVC would fail to maintain BBB integrity resulting in leakiness, associated with homeostatic disturbance from the periphery (e.g., inflammatory mediators and cells), and blood flow reduction providing limited oxygen and nutrient supply to the brain, impairing brain maturation. This mechanism is consistent with evidence seen in other disorders such as Alzheimer (Korte et al., 2020) and could explain the higher probability of neurodegenerative disorder in diabetic patients in which many vascular anomalies are observed (Nelson et al., 2016). Alterations in glial cells (mainly microglia and astrocytes) could contribute to this neurovascular fragility (**Figure 1**). Growing evidence place the PFC as central in this hypothesis because multiple investigations on SCZ patients found vascular defects in this particular region, ranging from decreases in claudin-5 (Greene et al., 2018), reductions in VEGF signaling (Fulzele and Pillai, 2009; Huang et al., 2020), a less dense capillary network (Uranova et al., 2013), to oversimplified angioarchitecture (Senitz and Winkelmann, 1991; Uranova et al., 2010), and other ultrastructural defects (**Figure 1**; Webster et al., 2001; Uranova et al., 2010; Ishizuka et al., 2017; Hill et al., 2020). As many key components of NVC are impacted by SCZ, it is not surprising that one of the most consistently observed neurovascular correlates of the illness is hypo-activity in PFC regions and in the left superior temporal gyrus, as revealed by a recent systematic review of both task and resting-state fMRI cross-sectional studies in first-episode SCZ patients (Mwansisya et al., 2017).

Although this hypothesis places the vasculature as a central element of SCZ, it is not clear whether the structural and functional abnormalities in blood vessels are a cause or a consequence of the cortical maturation deficiency. Growing evidence shows that an abnormal pruning of synapses and neurons by microglia potentially causes the cortical deficiency associated with SCZ (Sellgren et al., 2019). This altered removal of synapses is still partially unexplained, although it may result from dysfunctional fractalkine, triggering receptor expressed on myeloid cells 2 or complement signaling (Paolicelli et al., 2011; Hoshiko et al., 2012; Schafer et al., 2012; Filipello et al., 2018), all involved in microglia-mediated synaptic pruning. Complement is a prime suspect as work has shown upregulation of complement 4 protein in SCZ patients' brain (Sekar et al., 2016) and mouse models of SCZ (Comer et al., 2020b). When compared to other neurodegenerative disorders, the SCZ vascular hypothesis has similitudes with the recent vascular hypothesis for dementia (Ting et al., 2016), with differences in the affected regions. For example, vascular dementia is considered to arise from vascular defects in the white matter (Dichgans and Leys, 2017; Iadecola, 2017). For SCZ, beyond defects in the PFC represented in **Figure 1**, recent evidence points in the direction of vascular dysfunction in the brain network responsible for treatment of visual stimuli (Lefebvre et al., 2020), possibly resulting in hallucination.

CONCLUSION

Projects investigating the immune and vascular components of SCZ in the same protocol are required more than ever to shed light on the pathophysiology of SCZ. This should be approached in more causal studies for the vascular hypothesis to take traction in the SCZ field. A potential avenue would be based on previous work suggesting microvascular damages are coming from hypoxia induced factor 1 after lack of oxygenation during prenatal or early postnatal development (Schmidt-Kastner et al., 2012). This could mean inducing the conditional production of hypoxia induced factor 1 in a double hit protocol to potentially reproduce SCZ-like behavior, thus providing an effective model to the field. The models could then be investigated using 2-photon microscopy to measure blood velocity and glial interactions with the vasculature (Letourneur et al., 2014). Another way would be to directly induce hypoxia in animal models, as done for other pediatric conditions (Johnson et al., 2018; Kiernan et al., 2019) and see if this can reproduce a similar outcome as seen in SCZ patients. In both models, investigating

the vascular and the immune dynamic could provide a new understanding leading to novel therapeutic approaches for SCZ.

AUTHOR CONTRIBUTIONS

MC was responsible for planning and managing the review, writing of the introduction, discussion, and schizophrenia section while taking care of the overall revision and formatting of the manuscript, and is also the creator of the figure included in the manuscript. JG was in charge of writing the neurovascular unit section and contributing to the neurovascular coupling of the manuscript and on the literature search included in the figure creation. J-PL was responsible for writing the neurovascular coupling section and creating the table. MD and M-ÈT were in charge of revising the manuscript and contributed to the theoretical and writing part of the manuscript while MD contributed significantly to the organization and design of the manuscript. All authors contributed to the article and approved the submitted version.

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Maternal Immune Activation and Schizophrenia–Evidence for an Immune Priming Disorder

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Schizophrenia is a complex neurodevelopmental disorder affecting around 19. 8 million people worldwide. The etiology of the disorder is due to many interacting genetic and environmental factors, with no one element causing the full spectrum of disease symptoms. Amongst these factors, maternal immune activation (MIA) acting during specific gestational timings has been implicated in increasing schizophrenia risk in offspring. Epidemiological studies have provided the rationale for this link with prevalence of maternal infection correlating to increased risk, but these studies have been unable to prove causality due to lack of control of confounding factors like genetic susceptibility and inability to identify specific cellular and molecular mechanisms. Animal models have proved significantly more useful in establishing the extent to which MIA can predispose an individual to schizophrenia, displaying how maternal infection alone can directly result in behavioral abnormalities in rodent offspring. Alongside information from genome wide association studies (GWAS), animal models have been able to identify the role of complement proteins, particularly C4, and display how alterations in this system can cause development of schizophrenia-associated neuropathology and behavior. This article will review the current literature in order to assess whether schizophrenia can, therefore, be viewed as an immune priming disorder.

Keywords: schizophrenia, maternal, immune, complement, neurodevelopment

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INTRODUCTION

Schizophrenia is a long-term mental health disorder with an etiology characterized by a range of positive symptoms (e.g., delusions and hallucinations), negative symptoms (e.g., loss of motivation) and cognitive deficits (e.g., working memory deficits) during early adulthood (1). Heritability is estimated to be 80% (2, 3), but susceptibility of developing schizophrenia is determined by complex interactions between genes and the environment (4, 5). Risk of developing the clinical phenotype is generally modeled on the "two-hit hypothesis" where the "first hit" is an early priming event disrupting neurodevelopment and establishing increased vulnerability to a "second hit" which tends to be environmental insults occurring later in life (6, 7). Genetic abnormalities are widely accepted as the "first hit," but MIA may also serve this function (6).

Ecological studies were first to show the possible link between MIA via infection during pregnancy and increased schizophrenia risk in offspring. Following the 1957 influenza type A2 epidemic in Finland, maternal viral infection resulted in increased schizophrenia rates per 1,000 live births recorded in Helsinki (8). Other similar studies have attempted to replicate these findings,

with some showing positive associations whilst others finding no association (9). Due to widely contradictory results, MIA animal models have been necessary to establish a causal relationship between MIA and schizophrenia-related abnormalities (10–13). These models have been pivotal in identifying molecular mechanisms that affect normal neurodevelopment in early life (10), most significantly changes to the complement system (4, 14). These changes cause neuronal connectivity and synaptic pruning dysfunction which may contribute to pathological characteristics seen in post-mortem schizophrenic brains (e.g., decreased cortical gray matter thickness), leading to social deficits observed in schizophrenic patients (14–16).

MIA falls under the category of prenatal maternal stress which broadly encompasses maternal infection/inflammation, obstetric complications, nutritional deficiencies and psychosocial stress. In particular, maternal psychological stress is documented to be a well-characterized early priming event resulting in both physiological and behavioral deficits in offspring, such as cardiovascular abnormalities, enhanced anxiety-like behavior and increased risk of schizophrenia and other psychotic disorders (17–19). Increased glucocorticoid secretion due to stimulation of the hypothalamic-pituitary-adrenal (HPA) axis may play a part, resulting in schizophrenia-like phenotypes, as seen with pregnant rats exposed to variable stress paradigms (20). Although maternal psychological stressors can have a large impact on schizophrenia risk in offspring, this essay will be focused on reviewing the evidence linking MIA in particular to schizophrenia in regard to epidemiological studies, MIA animal models and the complement system in order to evaluate whether schizophrenia can be classed as an immune priming disorder.

EPIDEMIOLOGICAL STUDIES

Epidemiological studies have linked MIA to increased schizophrenia risk via infectious agents like influenza, herpes simplex virus 1 (HSV-1) and *Toxoplasma gondii (T. gondii)* (21–23). Ecological studies, primarily based on maternal influenza infection, identified this link, but there were many limitations associated with these. A notable limitation was diagnostic misclassification of influenza, with exposure in early studies being based upon maternal recall or whether the mother was in gestation during the epidemic, meaning around 70% of those in gestation during the 1957 influenza epidemic would have been misclassified as being infected whilst, in reality, being unexposed (8, 22, 24, 25).

A new approach was required to overcome this, leading to an increase in birth cohort studies using serological evidence, as summarized in **Table 1**. These studies used well-characterized birth cohorts and measured biomarkers in individual pregnancies using archived biological specimens obtained during pregnancy or early life of offspring (22). Results showed increased schizophrenia risk associated with maternal influenza infection in the first trimester (9), rather than the second trimester as found in ecological studies (8), and elevated levels of maternal antibodies against *T. gondii* (26, 27, 31, 32), as well as impaired cognitive functions displayed by HSV-1 seropositive offspring (23, 28, 29).

The mechanisms by which infection increases schizophrenia risk are only speculative and may either be infection-specific or work by a common causal mechanism (22). For example, the genome of *T. gondii* contains two genes encoding tyrosine hydroxylase which catalyzes L-DOPA production. L-DOPA is the precursor to dopamine, the dysregulation of which is well-established in schizophrenia pathology, thereby providing a potential infection-specific mechanism for increasing schizophrenia risk (33). However, there is currently limited evidence identifying *T. gondii* infection as a risk factor for schizophrenia, so larger birth cohort studies would be required to establish a more definitive association. Using human neurons derived from human induced pluripotent stem cells may also be useful in studying molecular mechanisms involved in *T. gondii* infection that lead to schizophrenia-associated pathogenesis. On the other hand, infections may act through common mechanisms by causing a pro-inflammatory state, increasing cytokine levels like IL-6 which has been shown to play a key role in behavioral abnormalities (22). A single injection of IL-6 during mid-pregnancy in rodents caused pre-pulse inhibition (PPI) and latent inhibition deficits in adult offspring, translating to sensorimotor gating deficits seen in schizophrenic patients, which can be prevented through administration of anti-IL-6 antibodies (34).

The prospective nature of serological studies has been powerful in establishing a link between MIA and increased schizophrenia risk through the use of qualitative measures of pathogenic markers in prenatal life. However, these studies are limited since they do not have the capacity to identify cellular and molecular mechanisms which affect neurodevelopment. Research in animal models provide a platform to overcome these limitations and potentially establish causality for these associations.

MIA ANIMAL MODELS

Initially, MIA was modeled using experimental mouse models of prenatal exposure to human influenza, where pregnant mice were infused with a mouse-adapted human influenza strain (35). Neuropathological signs were observed in the offspring, such as corticogenesis deficits, decreased hippocampal volume, decreased expression of γ-aminobutyric acid (GABA) markers, e.g., reelin, and behavioral abnormalities linked to schizophrenia (35). These results were extended to rhesus monkeys who showed decreased gray and white matter in cortical and parietal-cortical brain regions of neonates, verifying the relevance of rodent findings since corticogenesis in primates is more advanced and similar to humans (36). Viral MIA immune models were useful since they produced the full spectrum of immune responses caused by infections, but due to limitations associated with viral models such as the need for strict biosafety provisions and decreased control of immune response intensity and duration, animal MIA models moved onto using other immunogens such as polyinosinic:polycytidylic acid (polyI:C) to mimic viral infection, lipopolysaccharides to mimic bacterial

TABLE 1 | Table reviewing serological evidence linking various infections to increased schizophrenia risk.

Infection	References	Method	Population	Findings	Strengths	Limitations
Influenza	Brown et al. (9)	Nested case-control study of the Prenatal Determinants of SZ birth cohort.	A subgroup of 12,094 offspring to mothers receiving obstetric care from the Kaiser Foundation Health Plan in Alameda County, California from 1959 to 1966.	Seven-fold increased SZ risk when exposed to influenza in 1st trimester. Three-fold increased SZ risk when exposed to influenza from mid-1st to mid-2nd trimester.	Prospective study in well-characterized birth cohort. Face-to-face psychiatric diagnostic assessment.	Serum samples frozen for 30+ years which may affect antibody levels. No inclusion of family history of SZ.
T. gondii	Brown et al. (26)	Nested case-control study of the Prenatal Determinants of SZ birth cohort.	A subgroup of 12,094 offspring to mothers receiving obstetric care from the Kaiser Foundation Health Plan in Alameda County, California from 1959 to 1966.	Two-fold increased SZ risk in offspring exposed to higher IgG antibodies against *T. gondii in utero.*	Prospective study in well-characterized birth cohort.	Serum samples frozen for 30+ years which may affect antibody levels. No inclusion of family history of SZ, maternal lifestyle or health. Modest group size.
	Mortensen et al. (27)	Cohort-based, case-control study in Denmark.	A group of 413 individuals registered in the Danish Psychiatric Case Register born from 1981 to 1999.	Increased SZ risk associated with increased IgG antibodies against *T. gondii* in offspring.	Large sample size. Included confounders like family history of SZ/mental health disorder.	Confined only to cases with early onset (18 years or younger), so cannot generalize to other age groups. 28% of SZ cases could not be located in the biobank which could have caused bias in results.
HSV-1	Yolken et al. (28)	Cognitive examination of SZ patients from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) sample.	A group of 1,308 individuals with SZ from 57 sites across all regions of the USA except Alaska.	Impaired visuomotor skills, verbal memory, attention and executive function in patients seropositive for HSV-1 only, not with other human herpesviruses.	Large sample size.	No inclusion of family history of SZ.
	Dickerson et al. (29)	Cognitive examination of SZ patients using the Repeatable Battery for the Assessment of Neuropsychological Status.	A group of 229 SZ patients from several treatment and rehabilitation programs in central Maryland aged 18 to 65 years old.	Impaired cognitive function in patients seropositive for HSV-1 only, not with other human herpesviruses.	Used solid-phase enzyme immunoassays which allow small volumes of serum samples to measure antibodies against a number of different antigens.	No evidence of CNS infection since did not measure levels of herpesvirus antibodies in the cerebrospinal fluid. Cross-sectional study so could not determine timing of initial HSV-1 infection or age of cognitive dysfunction development.
	Buka et al. (30)	Nested case-control study of three cohorts from the National Collaborative Perinatal Project (NCPP).	A group of 200 offspring with psychoses from a sample of pregnant women from Boston, Providence and Philadelphia.	1.8-fold increased SZ-associated psychosis risk in offspring of HSV-2 seropositive mothers. Elevated risk associated with offspring of mothers who did not use regular contraception and had frequent intercourse.	Prospective study in well-characterized birth cohort. Large sample size.	Losses to follow up may have caused bias. Overrepresentation of males and those from a high socioeconomic background in this sample.

SZ, schizophrenia; Ig, immunoglobulin G.

infections and turpentine to mimic local inflammation (10). This article will particularly focus on polyI:C animal models due to the strength of their results, with **Table 2** summarizing the main contributions these models have made to the study of schizophrenia pathophysiology.

In viral infection, double stranded RNA (dsRNA) is an intermediate in single stranded RNA replication or during symmetrical transcription of DNA viruses (10). Recognition of dsRNA by toll-like receptor 3 (TLR3) causes increased pro-inflammatory cytokine expression (10, 13). PolyI:C acts as a synthetic analog of viral dsRNA and mimics the acute phase response to viral infection (10, 13). The most significant finding from polyI:C rodent models would be how gestational timing of MIA exposure can influence the phenotype displayed by offspring (9, 11, 12). Pregnant mice were injected with polyI:C at either gestational day (GD) 9 or GD17 (39, 40, 47), translating to mid-first trimester and early second trimester, respectively, in humans (48). GD9 offspring were found to have phenotypes consistent with positive symptoms such as PPI deficits, latent inhibition deficits, decreased dopamine 1 (D1) and D2 receptor density in the prefrontal cortex (PFC) and increased tyrosine hydroxylase levels. Conversely, GD17 offspring showed phenotypes consistent with negative and cognitive symptoms such as N-methyl-D-aspartic acid (NMDA) receptor alterations, impaired reversal learning, working memory deficits, increased hyperlocomotion to MK-801 (an NMDA receptor antagonist) and decreased hippocampal expression of the N1 subunit of NMDA receptors (12). A limitation of this study is that these results were observed only in the C57 mouse strain. Therefore, further studies were undertaken in rats to provide evidence that these results were generalizable across species (12).

Pregnant Wistar rats were injected with polyI:C at GD10 or GD19 and their offspring undertook behavioral tests such as the acoustic startle response, delayed non-match to position testing and drug-induced locomotor activity assessments (12). Results from the rat study partially replicated findings from mouse studies; offspring exposed to late MIA showed working memory deficits and hyperlocomotion in response to MK-801, and offspring from both groups exhibited hyperlocomotion in response to amphetamine (12). Unlike the mouse study, the rat study showed decreased PPI in male offspring exposed to MIA at any gestational timing (12). This may be because the estrous cycle was not controlled for in female rats, meaning estrogen may have prevented the PPI disruption (12). On the contrary, a later study found female rats exposed to MIA in late gestation also exhibited sensorimotor gating deficits (11). These contradictory results suggest GD10 and GD19 may not be key sensitive periods of gestation for rats. Studies based on different gestational days with larger sample sizes should be undertaken to compare results.

Gestational timing of MIA is of great importance due to neuronal migration in neurodevelopment. A recent study reported decreased cortical and striatal, but not hippocampal, somatostatin (SST) mRNA in polyI:C adult rat offspring (11). This may be related to the distinct origins of these inhibitory neurons and the different timings involved in their migration (11). Cortical SST-containing inhibitory neurons derive from the medial ganglionic eminence (MGE), whilst 40% of hippocampal SST-containing inhibitory neurons derive from the caudal ganglionic eminence (CGE) (11). The MGE has an early dominant period of neurogenesis between GD9.5-GD13.5, whilst the CGE has a later period between GD12.5-16.5 (11). This explains why the hippocampus, and not the cortex, was spared when MIA was inflicted during GD10 and GD19, highlighting the great impact the specific timing of MIA can have on neuronal distribution (11). Further experiments should be conducted with MIA being inflicted during the key neurogenesis period of the hippocampus. If polyI:C offspring expressed characteristics associated with hippocampal dysfunction, such as memory and attention deficits, it would confirm whether MIA affects neurodevelopment through alterations in neuronal migration.

MIA animal model studies have also played a prominent role in identifying a link between complement system dysregulation and increased risk of schizophrenia-like phenotypes. The complement system is a key mediator of immunity, meaning that dysregulation of this system directly implicates the immune system in the etiology of schizophrenia. Complement proteins are involved in neurogenesis, neuronal migration and synaptic formation (4, 14), with C3 knockout mice showing synaptic elimination deficits (49) and impaired migration of neuroblasts (50). Early studies implicating complement system dysfunction in schizophrenia used hemolytic activity assays to measure overall complement component function in the blood (51–53). Results were varied, with some studies finding decreased total hemolytic activity in patients (52), whilst others found no differences between patients and controls (53). Complement system abnormalities are more reliably observed during infection; mothers of schizophrenic patients with increased fetal antibodies against adenovirus and HSV-2 also had higher C1q levels, indicating an early neurodevelopmental role for the complement cascade that may be due to MIA (54). Western blot analysis of C1q in different brain regions of polyI:C adult rodent offspring showed increased C1q expression in the PFC compared to controls (55). Although increased C1q likely contributes to behavioral abnormalities, this hypothesis has not specifically been assessed, meaning further behavioral tests are required to confirm this.

A recent GWAS of schizophrenia confirmed a robust genetic association with the major histocompatibility (MHC) locus, strongly implicating immune dysfunction as a potential pathological mechanism in schizophrenia (56). More specifically, through the use of digital droplet polymerase chain reactions and analysis of single nucleotide polymorphism (SNP) data, overexpression of C4 (originating from the MHC locus), particularly the *C4A* gene, has been linked with increased schizophrenia risk, with *C4A* mRNA being elevated in post-mortem brain tissue from schizophrenic patients (15). Immunohistochemistry on brain sections showed majority of C4-positive cells to be in the hippocampus, and co-immunostaining with pre-synaptic (vesicular glutamate transporters 1 and 2) and post-synaptic (post-synaptic density protein 95) markers showed much of the C4 was localized at synaptic puncta (15). Additionally, C4 knockout mice showed reduced synaptic pruning, suggesting C4 overexpression may contribute to decreased synapse numbers observed in schizophrenia (15). A

TABLE 2 | Main contributions made by polyI:C rodent models to the study of schizophrenia pathophysiology.

References	Gestational day challenge	Main neurobiological findings	Main behavioral findings
Meyer et al. (37)	9	Increases in IL-10 at 1 and 6-h post-challenge. Increases in IL-1β at 12 h post-challenge	Impairments in exploratory behavior, PPI, LI and spatial working memory. Enhanced locomotor response to systemic amphetamine.
Meyer et al. (38)	6, 9, 13 or 17	Increases in IL-10:TNF ratio in GD17 offspring.	GD6, 9 or 13 offspring display LI deficits.
Smith et al. (34)	12.5	-	Co-administration of IL-6 antibody with polyI:C prevents PPI, LI and exploratory/social deficits caused by polyI:C in offspring.
Meyer et al. (39)	9 or 17	GD9 offspring show decreased prefrontal D1 receptors in adulthood. GD17 offspring show decreased hippocampal NMDA receptor subunit expression. Potentiation of locomotor activity to amphetamine and decrease in reelin- and parvalbumin-expressing prefrontal neurons occur independently of polyI:C challenge timing.	GD9 offspring display impaired sensorimotor gating. GD17 offspring display impaired working memory and potentiation of locomotor activity to NMDA receptor antagonists.
Li et al. (40)	9 or 17	GD9 offspring display enlarged lateral ventricles in adulthood. GD17 offspring show 4th ventricle volume expansion.	GD9 offspring display PPI deficits.
Bitanihirwe et al. (41)	17	Sex-specific changes in neurotransmitter levels including: Reduced dopamine and glutamate in the PFC and hippocampus. Reduced GABA in hippocampus. Reduced glycine in the PFC.	Both male and female offspring display deficits in social interaction, anhedonic behavior, alterations in locomotor, and stereotyped behavioral responses to acute apomorphine treatment. Male offspring also display enhanced LI.
De Miranda et al. (42)	16	PolyI:C exposure inhibits embryonic neuronal stem cell replication and population of the superficial neocortex layers by neurons which is dependent on TLR3.	Impaired neonatal locomotor development and abnormal sensorimotor gating responses in adult offspring.
Abazyan et al. (43)	9	Prenatal polyI:C exposure in mhDISC1 mice causes decreased reactivity of the hypothalamic-pituitary-adrenal axis and attenuated serotonin neurotransmission in hippocampus. Reduced enlargement of lateral ventricles. Decreased volumes of amygdala and periaqueductal gray matter. Decreased density of dendritic spines of granule cells in hippocampus. Modulated secretion of cytokines in fetal brains. Altered levels of mhDISC1, endogenous mouse DISC1 and glycogen synthase kinase 3β.	Offspring display anxiety, depression-like phenotypes and altered social behavior. Behavioral effects only observed if mhDISC1 expressed throughout lifespan.
Coiro et al. (44)	12.5	Decreased number and turnover rates of dendritic spines at sites of excitatory synaptic inputs in cortex. Reorganization of pre-synaptic inputs with more spines contacted by both excitatory and inhibitory presynaptic terminals.	Increased repetitive behavior. Treatment with anti-inflammatory drug ibudilast prevents synaptic and behavioral impairments.
Duchatel et al. (45)	10 or 19	GD19 offspring showed increased NeuN+ IWMN density. Offspring exposed to polyI:C at both gestational stages showed increased SST+ IWMN density. 1st study to show MIA increases IWMN in adult offspring in a similar manner to what is seen post-mortem SZ brains.	-
Meehan et al. (12)	10 or 19	Male GD10 offspring show increased D1 receptor mRNA levels in nucleus accumbens.	GD19 offspring display transient working memory impairments. Male offspring exposed at either gestational timings display sensorimotor gating deficits.
Duchatel et al. (46)	10 or 19	Male GD19 offspring show significant increase in C4 gene expression in cingulate cortex.	-
Rahman et al. (11)	10 or 19	Decreases in SST mRNA in cingulate cortex and nucleus accumbens shell, and reduction of parvalbumin mRNA in infralimbic cortex regardless of polyI:C challenge timing. GD19 offspring display decreases in SSTR2 mRNA in cortex and striatum.	-

IL, interleukin; LI, latent inhibition; TNF, tumor necrosis factor; TLR, toll-like receptor; mhDISC1, mutant human-disrupted in schizophrenia 1 gene; NeuN, neuronal nuclear antigen; IWMN, interstitial white matter neurons; SST, somatostatin; SSTR2, somatostatin receptor 2.

limitation of this investigation is that C4 is encoded for by the *C4A* and *C4B* genes in humans, whilst encoded by a single *C4b* gene in mice, meaning these results may not directly translate to humans (15).

The involvement of C4 in synaptic pruning was rigorously studied by Comer et al. through C4 overexpression in mouse models (16). Results show dendritic spine abnormalities in C4-overexpressing mice which is consistent with post-mortem studies of schizophrenic patients (57). Changes in dendritic spines were most prominent in L2/3 of the PFC, but abnormalities have also been observed in other regions like the frontal lobe, temporal lobe and hippocampus (57). Therefore, targeting different areas with *in utero* electroporation (IUE) would be required to see if C4 overexpression decreased dendritic spine density in other areas also (16). Additionally, filopodia play a large role in synaptogenesis (58); decreases in the number of filopodia seen in these mice mean C4 may cause dysregulation of filopodia-dependent synapse formation in early development, resulting in abnormal synaptic elimination and circuitry that is seen in schizophrenia (16). Findings of decreased excitatory synaptic drive show how these structural abnormalities in dendritic spine density result in functional changes in connectivity (16). They also found excessive microglial engulfment of synaptic material was driven by C4 overexpression (16). Previous studies have showed that phagocytosis of synaptic material by microglia to be a necessary aspect of normal brain wiring (59), meaning the complement system may mediate brain circuitry, resulting in pathology when dysregulated. As well as genetic and molecular associations between C4 overexpression and schizophrenia, Comer et al. have found C4 overexpression in the frontal cortex to be sufficient in changing social interactions of both juvenile and adult mice, with results suggesting that overexpression of C4 can lead to long-term changes in PFC circuitry (16).

These results are the first to have shown a direct causal link between increased C4 levels and PFC dysfunction, and how this can lead to hypoconnectivity and abnormal synaptic functioning that drives expression of behavioral phenotypes seen in schizophrenia. Although it is recognized that schizophrenia is caused by a wide range of etiological factors, strong genetic, molecular and behavioral evidence has been provided by Comer et al. that altering the expression of only the *C4* gene is sufficient in mice to cause schizophrenia-like pathology. Future studies should focus on identifying whether changes in C4 expression in other brain areas can cause other schizophrenia-related phenotypes such as memory deficits.

Additionally, Comer et al. identified a critical developmental time period, around E16 in mice, during which PFC circuits are more vulnerable to changes in C4, highlighting the importance in the timing of gestational insults. This is further strengthened by a study using polyI:C MIA rodent models which, through qPCR, found a significant increase in *C4* gene expression in the cingulate cortex of offspring infected at GD19 only. This provides direct evidence, paired with results from Comer et al., that late MIA can cause schizophrenia-like deficits (46). Future studies could utilize transcriptomic and proteomic technology to delineate the molecular pathways underlying the relationship between late gestational MIA and C4 overexpression.

DISCUSSION

After review of epidemiological studies and animal models of MIA, it can be argued that schizophrenia can be regarded as an immune priming disorder, with MIA increasing the vulnerability of an individual to neuropathological effects of other environmental insults. Animal models have provided a wealth of information highlighting interactions between early neurodevelopmental disturbances and psychosis, showing strong face, construct and predictive validity (60). In particular, polyI:C models have been able to model behavioral, neuroanatomical and neurochemical changes seen in schizophrenic patients, including increased mesencephalic dopamine neuronal density and decreased NMDA receptor function in the PFC, consistent with the dopaminergic and glutamatergic hypotheses of schizophrenia (13). The obvious limitation of animal models is that many clinical manifestations of schizophrenia, e.g., hallucinations and delusions, are impossible to detect in animals (60). Therefore, behavioral, anatomical and physiological characteristics must be examined through translational models rather than modeling the whole syndrome (60). Although polyI:C models cannot mimic the full spectrum of immune responses, the relevance of this model in preclinical studies is not undermined since it does not influence its capacity to mimic schizophrenia-like behaviors and neuropathology (13, 60).

Animal models have provided strong evidence for the role of MIA in schizophrenia pathology, emphasizing the importance of gestational timing of infection in neurodevelopment and behavior. More specifically, MIA in late gestation causes increased C4 expression seen in schizophrenic patients. Subsequently, C4 overexpression has been proven to cause hypoconnectivity in the PFC and social deficits that are characteristic of schizophrenia, displaying how MIA is sufficient in causing schizophrenia-like phenotypes. Future studies utilizing modern functional imaging techniques are required to elucidate this relationship in humans and further confirm the contributing factor of C4 overexpression in schizophrenia development.

Although MIA may predispose individuals to schizophrenia, it is neither sufficient, nor necessary by itself for the development of the disease. Instead MIA can be viewed as a "priming" event, increasing vulnerability to the disorder, acting as a "first hit" toward the development of the disorder. The role of complement proteins in MIA is simply one aspect, among many, of immune dysfunction schizophrenia. Furthermore, the effect may also likely to be non-specific. Maternal stress is also a significant factor resulting in increased risk of different psychiatric disorders amongst offspring, potentially due to its effect on immune molecules like cytokines, with elevated maternal pro-inflammatory cytokines being associated with abnormal neurodevelopment (61–63). Taken together, a "multi-hit threshold model" encompassing different aspects of neurodevelopment

would more accurately represent the dynamic interaction between genes and the environment in schizophrenia development (64).

Understanding the neuropathological origins of schizophrenia is important for detecting increased disease risk at an early stage and the development of preventative therapies. Detection of significant immune molecules, such as C4, may allow development of medication that could target these molecules or their receptors. Additionally, the identification of a key window of vulnerability provides a critical time period where such preventative therapies would be most efficacious. However, preventative therapies for neurodevelopmental disorders are controversial and would require extensive evaluation on both a scientific and ethical basis before being implemented in a clinical setting. Nevertheless, further investigation into the immune basis of schizophrenia could provide many potential therapeutic opportunities to improve treatment of this disorder.

AUTHOR CONTRIBUTIONS

ZC prepared the manuscript and constructed the accompanying tables. BL provided guidance with the structure of the manuscript and contributed to revision and proofreading. Both authors contributed to the article and approved the submitted version.

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Differential Macrophage Responses in Affective Versus Non-Affective First-Episode Psychosis Patients

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Increased innate immune activation and inflammation are common findings in psychotic and affective (mood) disorders such as schizophrenia (SCZ), bipolar disorder (BD), and major depressive disorder (MDD), including increased numbers and activation of monocytes and macrophages. These findings often differ depending on the disorder, for example, we previously found increases in circulating inflammatory cytokines associated with monocytes and macrophages in SCZ, while BD had increases in anti-inflammatory cytokines. Despite these differences, few studies have specifically compared immune dysfunction in affective versus non-affective psychotic disorders and none have compared functional monocyte responses across these disorders. To address this, we recruited 25 first episode psychosis (FEP) patients and 23 healthy controls (HC). FEP patients were further grouped based on the presence (AFF) or absence (NON) of mood disorder. We isolated peripheral blood mononuclear cells and cultured them for 1 week with M-CSF to obtain monocyte-derived macrophages. These cells were then stimulated for 24 h to skew them to inflammatory and alternative phenotypes, in order to identify differences in these responses. Following stimulation with LPS and LPS plus IFNγ, we found that macrophages from the NON-group had diminished inflammatory responses compared to both HC and AFF groups. Interestingly, when skewing macrophages to an alternative phenotype using LPS plus IL-4, the AFF macrophages increased production of inflammatory cytokines. Receiver operating curve analysis showed predictive power of inflammatory cytokine concentrations after LPS stimulation in the AFF group versus NON-group. Our results suggest dysfunctional monocyte responses in both affective and non-affective psychotic disorder, with varying types of immune dysfunction depending on the presence or absence of a mood component.

Keywords: psychosis, schizophrenia, bipolar disorder, major depressive disorder, affective, immune, macrophage, inflammation

INTRODUCTION

Psychotic and affective (mood) disorders, including schizophrenia (SCZ), bipolar disorder (BD), and major depressive disorder (MDD) cause significant disability and are a major public health concern (Reddy, 2010; Ferrari et al., 2012). The burden of these neuropsychiatric disorders are likely underestimated, despite their appearance within the top 20 causes of global burden of disease in 2013 (Vigo et al., 2016). The exact cause of these disorders is yet unknown, however findings from genetic and epidemiological studies indicate that the interplay of environmental and genetic components contribute to the development and severity of neuropsychiatric disorders (Uher and Zwicker, 2017). Inflammation and immune dysfunction are common findings in these disorders, with activation of the immune system and increased inflammatory mediators proposed as possible etiological mechanisms (reviewed in Bauer and Teixeira, 2019). Chronic infections, inflammatory conditions and autoimmunity are considered risk factors for psychotic and affective disorders (Benros et al., 2013, 2014; Rosenblat and McIntyre, 2015). Several meta-analyses have consistently found elevations of peripheral and central inflammatory cytokines and chemokines across these disorders (Dowlati et al., 2010; Modabbernia et al., 2013; Munkholm et al., 2013; Goldsmith et al., 2016; Pillinger et al., 2018; Wang and Miller, 2018). As the majority of these cytokines and chemokines are produced predominantly by cells of the innate immune system, an overarching hypotheses that connects the immunological dysfunction of these disorders is aberrant activation of innate immunity. Originally described for SCZ as a "chronically activated inflammatory response system" (Smith and Maes, 1995), this hypothesis proposes that alterations in the numbers and activation states of peripheral monocytes and macrophages, and microglia in the brain, lead to the increased inflammatory mediators commonly seen in psychotic and affective disorders and may be contributing to their etiology by altering brain function (Drexhage et al., 2010a; Beumer et al., 2012).

Monocytes and macrophages, and their counterpart the microglia in the brain, are myeloid cells of the innate immune system. As first responders in the immune response, their activity during activation is critical for orchestrating inflammatory or alternative/anti-inflammatory responses, as they produce mediators that help initiate and sustain adaptive responses (Geissmann et al., 2010). Circulating monocytes are present in humans as a heterogenous population, the majority of which are classical $CD14^{++}CD16^{-}$ monocytes, with intermediate $CD14^{hi}CD16^{lo}$ and alternative $CD14^{+}CD16^{++}$ monocytes making up 10% (Italiani and Boraschi, 2014). When recruited to tissues during immune activation, these cells differentiate into macrophages, which have been recently highlighted in their ability to polarize pro-inflammatory versus anti-inflammatory responses under various circumstances. Two states exist in the simplistic model of macrophage polarization: "M1" are the classically activated inflammatory macrophages, producing pro-inflammatory cytokines such as interleukin (IL-1)-β, IL-6 and tumor necrosis factor (TNF)-α to increase the inflammatory response. "M2" macrophages are alternatively activated anti-inflammatory cells, involved in wound healing and restoring homeostasis after inflammatory events by secreting anti-inflammatory cytokines and growth factors that help reduce and prevent further damage from inflammatory immune responses and promote recovery. In reality, these cells polarize along a spectrum, not strictly M1/M2 as these represent extremes of the spectrum and M2 has further subcategories that vary in function depending on the type of stimuli. However, macrophages can be polarized *in vitro* by stimulating with interferon (IFN)-γ or IL-4 to drive them toward M1 versus M2 phenotypes, respectively (Italiani and Boraschi, 2014).

Alterations in numbers and function of monocytes and macrophages have been repeatedly identified in these disorders, with varying degrees of dysfunction depending on the disorder(s) studied. Increases in circulating monocytes and cerebrospinal fluid monocytes were found repeatedly in SCZ and BD (Zorrilla et al., 1996; Rothermundt et al., 1998; Nikkila et al., 1999; Barbosa et al., 2014). When comparing gene expression profiles of monocytes from SCZ and BD patients, two clusters of highly correlated genes were identified in BD, one involving inflammatory mediators and the other involving motility and adhesion. However, in SCZ only the pro-inflammatory cluster was seen, and the motility/adhesion cluster was downregulated (Drexhage et al., 2010b). Elevated inflammatory gene expression in the monocytes of BD patients during mood episodes, compared to during the euthymic state was also found (Becking et al., 2015). More recently, an increased M1/decreased M2 gene expression signature was identified in peripheral blood mononuclear cells (PBMC) from BD patients but not from patients with SCZ, including increased *il-6* and *ccl3* (encodes for macrophage inflammatory protein [MIP]-1α) (Brambilla et al., 2014). Other studies have identified increased responses of stimulated monocytes from mood disorder patients. Knijff et al. (2007) found significantly altered pro-inflammatory responses in monocytes from BD patients compared to controls after lipopolysaccharide (LPS) stimulation, including increased IL-6. Conversely, when monocyte-derived macrophages from SCZ patients were stimulated with LPS or IL-4, no differences in functional responses at the gene-expression level were found, indicating a normal response to stimulation in SCZ macrophages (Ormel et al., 2017).

Additional support for the hypothesis that myeloid cells are dysfunctional in neuropsychiatric disorders are the repeated findings of increased inflammatory cytokines associated with these cells in SCZ, BD, and MDD. For example a meta-analysis of 30 BD cytokine studies showed significant increases in inflammatory cytokines and soluble receptors with variations in expression depending on manic or depressive episode (Modabbernia et al., 2013). IL-6 was elevated in acutely ill patients when comparing across studies of SCZ, BD, and MDD, which decreased in SCZ and MDD but not bipolar mania after treatment (Goldsmith et al., 2016). Elevated IL-6 mRNA and protein are proposed biomarkers for SCZ and MDD, respectively (Mössner et al., 2007; Chase et al., 2016). These cytokines typically associate with innate inflammation, supporting myeloid dysfunction in these disorders.

Taken together, findings of increased inflammatory cytokines and myeloid cell activation suggest that there is a relationship between innate immune activation and psychotic and affective disorders. Given this, we sought to identify differences in activation of monocyte-derived macrophages from first episode psychosis (FEP) patients with and without mood disorders. We also sought to compare macrophage responses to those of healthy controls. By stimulating macrophages toward M1 and M2 phenotypes, we aimed to identify variations in production of inflammatory cytokines and chemokines associated with monocytes, macrophages, and microglia to support the hypothesis of innate immune cell activation and dysfunction in psychotic and affective disorders, and to potentially address discrepancies seen in previous studies.

MATERIALS AND METHODS

Study Participants

We recruited a total of 48 participants between the ages of 14 and 37 years old, including 23 healthy controls and 25 first episode patients with a psychotic disorder. Diagnoses included 10 SCZ, 1 psychosis not otherwise specified, 8 BD, and 6 MDD. Psychosis participants were outpatients within one year of onset of symptoms. Trained clinicians assessed patients using the Structured Clinical Interview for the DSM-IV-TR (SCID)-I/P (First et al., 2002) and symptoms were rated on several scales including the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1983), Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984), and Brief Psychiatric Rating Scale (BPRS) (Lukoff et al., 1986). The majority of patients in both groups were on monotherapy antipsychotic medication. Exceptions included two individuals in the AFF group that were taking more than one antipsychotic, five unmedicated participants the AFF group and two unmedicated participants in the NON-group. One patient in the AFF group was also taking antidepressants in addition to an atypical antipsychotic. When this patient was omitted from the analyses, it did not alter outcome therefore the patient was included. Antipsychotic dosage was converted to chlorpromazine (CPZ) equivalent dose to assess relative antipsychotic potencies. Participants were excluded for positive urine toxicology at the time of testing, for alcohol or drug abuse/dependence within three months of assessment, and/or who had a Wechsler Abbreviated Scale of Intelligence (WASI) IQ score that was below 70. In addition, healthy controls (HC) were excluded for presence of any Axis I or Axis II disorder, or psychotic disorder within first degree family members. Diagnoses was later confirmed at a 12-month assessment. The University of California, Davis Institutional Review Board approved this study.

Cell Isolation

Peripheral blood was collected from each participant in an acid-citrate-dextrose Vacutainer tube (BD Biosciences, San Jose, CA, United States) and processed within 12 h of collection. The blood was centrifuged for 10 min at 2,100 rpm, then plasma was collected and stored at −80°C. The remaining blood was diluted 1:2 with Hanks Balanced Salt Solution (HBSS; Gibco, Gaithersburg, MD, United States) then carefully layered over a Ficoll-Paque gradient (Pharmacia Biotech, Piscataway, NJ, United States) and centrifuged at 1,700 rpm for 30 min at room temperature. PBMC were collected from the interface layer and washed twice with HBSS. Cell viability was determined by trypan blue exclusion. The cells were resuspended at a final concentration of 1×10^6 cells/mL in tissue culture medium consisting of RPMI-1640 (Gibco) supplemented with 10% low endotoxin heat inactivated fetal bovine serum (Omega Scientific, Tarzana, CA, United States), 100 IU/mL penicillin, and 100 IU/mL streptomycin (Sigma, St Louis, MO, United States).

Macrophage Growth and Stimulation

Freshly collected PBMC were plated in RPMI with 100 ng/mL recombinant human macrophage colony stimulating factor (rh-M-CSF, R&D Systems; Minneapolis, MN, United States) in Corning Ultra-Low Binding 100 × 20 mm dishes at an approximate density of 10 million cells in 10 mL volume. Media and rh-M-CSF were replenished after three days, and cultured for a total of 1 week, at which time plates were washed with HBSS to remove all but adherent macrophages. Adherent cells were then incubated for 5 min on ice with Cell Stripper (Corning, Manassas, VA, United States) and then removed by vigorously washing plates. Macrophages were plated at 2.5×10^4 cells/well and cultured at 37°C with 5% CO_2. The cells were stimulated in duplicate either with RPMI alone, or RPMI plus 10 ng/mL LPS (Sigma-Aldrich; St. Louis, MO, United States), or 50 ng/ml IFNγ (R&D Systems) and 10 ng/mL LPS, or 40 ng/mL IL-4 (R&D Systems) and 10 ng/mL LPS. After 24 h, plates were centrifuged at 2,100 rpm for 10 min and supernatants were collected and stored at −80°C until analysis.

Cytokine and Chemokine Analysis

To assess macrophage inflammatory responses, supernatants from stimulated macrophage cultures were quantified for IL-1β, IL-6, IL-12p40, IL-12p70, and TNFα. Chemokine production was assessed by measuring monocyte chemoattractant protein (MCP)-1 (C–C chemokine ligand [CCL]2), MIP-1α (CCL3), and MIP-1β (CCL4). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-10 were measured to identify production of growth and repair mediators. These measurements were made using a high sensitivity Multi-Plex bead set (Millipore, Saint Charles, MO, United States). Samples were run in duplicate. Based on manufacturer's recommendations, supernatants were incubated with antibody-coupled fluorescent beads overnight at 4°C in a humidified box. Plates were then washed. Biotinylated detection antibodies were then added to each well and incubated at room temperature for 1 h, followed by streptavidin–phycoerythrin and an additional 30 min incubation. The samples were analyzed using a flow-based Luminex^{TM} 100 suspension array system (Bio-Plex 200; Bio-Rad Laboratories, Inc.). Unknown sample cytokine concentrations were calculated by Bio-Plex Manager software using a standard curve derived from the known reference cytokine standards provided in each kit. The sensitivity of this assay allowed the detection of cytokine concentrations with the following limits

of detection: IL-12(p40) (12.3 pg/mL), IL-12(p70) (0.9 pg/mL), IL-1β (0.7 pg/mL), IL-6 (0.4 pg/mL, TNFα (0.2 pg/mL), MCP-1 (1.2 pg/mL), MIP-1α (6.6 pg/mL), MIP-1β (3.2 pg/mL), GM-CSF (2.3 pg/mL), and IL-10 (0.3 pg/mL). Values below the limit of detection (LOD) were replaced with one-half the LOD.

Statistical Analysis

Shapiro–Wilk test determined that the majority of the cytokine data were not normally distributed. Outliers were removed using ROUT with a coefficient Q set to 1%. Kruskal–Wallis tests were used to analyze differences across the three groups and Mann–Whitney *U* tests were then used for pair-wise analyses, with Holm–Sidak method to correct for multiple comparisons. To evaluate whether an inflammatory macrophage profile may discern between individuals with affective and non-affective psychosis, we ran binary logistic regression on cytokine levels that were log2 transformed to improve the goodness of fit as measured by the Hosmer–Lemeshow statistic, where a non-significant probability value indicates good fit (Grund and Sabin, 2010). Predictive power was evaluated by receiver operating characteristics analysis. To correct for the potential impact of antipsychotic medication on cytokine production, supplementary analyses were performed comparing cytokine concentrations between the AFF and NON-group by conducting both Mann–Whitney *U* tests in medicated versus non-medicated patients, and one-way analysis of covariance (ANCOVA). In order to meet assumptions required for ANCOVA, cytokine concentrations were rank transformed across groups, then separate ANCOVAs were performed for each cytokine including antipsychotic dose as a covariate, based on procedures described previously (Lesh et al., 2018). Statistical analyses were carried out using analyses software SPSS Statistics Version 26 (IBM, Armonk, NY, United States) and GraphPad Prism v7.0e (GraphPad Software, San Diego, CA, United States). A two-tailed alpha of $p < 0.05$ was considering statistically significant.

RESULTS

Demographics and Clinical Characteristics

Table 1 summarizes study participant demographics and clinical scores. We recruited 48 participants which included 25 FEP participants and 23 healthy control (HC) subjects. FEP participants were further categorized based on the presence or lack of a primary affective disorder into affective (AFF), and non-affective (NON) groups. All participants were between the ages of 14 and 37, and there were no statistically significant differences in age between groups. The NON-group was male-skewed, however significant differences across groups were not evident based on Fisher's exact test. Parent education level was similar across all three groups, however participant education level was significantly lower in both the AFF ($p = 0.0121$) and NON ($p = 0.0016$) groups compared to the HC group. BPRS and SAPS scores between both AFF and NON-populations did not differ, however the AFF group scored significantly lower on SANS ($p = 0.0435$) compared to the NON-group. WASI scores did not differ across groups. No significant differences were seen in dose of antipsychotic medication using CPZ equivalent doses for estimation, and cytokine concentrations were not significantly different in medicated versus non-medicated patients, based on Mann–Whitney U tests of significant cytokines in AFF and NON-groups (all $p > 0.05$). Supplementary ANCOVA analyses of rank-transformed significant cytokines with antipsychotic dosage equivalent (CPZ) as a covariate had no impact on the results (**Supplementary Table S1**).

Basal Cytokine and Chemokine Production (Media Alone)

We first assessed baseline macrophage production of inflammatory cytokines, chemokines and growth/anti-inflammatory factors after 24-hour culture in RPMI medium. Kruskal–Wallis tests revealed significant differences between HC, AFF, and NON-groups for production of IL-12p40 ($p < 0.01$) and IL-6 ($p < 0.05$). After correcting for multiple comparisons, Mann–Whitney *U* tests revealed significantly reduced production of inflammatory cytokines IL-12p40 ($p = 0.0069$) and IL-6 ($p = 0.0191$; **Table 2**, **Figure 1**) in the AFF group compared to HC. No significant differences were seen when comparing IL-12p70, IL-1β, TNF-α, MCP-1, MIP-1α, MIP-1β, GM-CSF, or IL-10.

Cytokine and Chemokine Production After LPS Stimulation

After stimulating macrophages with LPS for 24 h to measure cytokine production after immune activation, Kruskal–Wallis tests revealed significant differences in IL-12p40 ($p < 0.01$), IL-1β ($p < 0.01$), IL-6 ($p < 0.001$), TNF-α ($p < 0.01$), MIP-1α ($p < 0.05$), and MIP-1β ($p < 0.05$) across the three groups. After correcting for multiple comparisons, Mann–Whitney *U* tests revealed significant decreases in IL-12p40 ($p = 0.0009$), IL-1β ($p = 0.0248$), IL-6 ($p = 0.0003$), TNF-α ($p = 0.0057$), and MIP-1β (p=0.0418) were seen in the NON-group compared to AFF (**Table 3**, **Figure 2**). The NON-group also had decreases in IL-12p40 ($p = 0.0272$), IL-1β ($p = 0.0078$), IL-6 ($p = 0.0008$), TNF-α ($p = 0.0072$), and MIP-1β ($p = 0.0282$) compared to HC.

Cytokine and Chemokine Production After LPS Plus IFNγ Stimulation

After stimulating with LPS plus IFNγ to induce a classically activated "M1" phenotype, Kruskal–Wallis tests showed significant differences in IL-12p40 ($p < 0.05$), IL-6 ($p < 0.01$), MCP-1 ($p < 0.05$), and MIP-1β ($p < 0.01$) across the three groups. After multiple corrections, Mann–Whitney *U* tests showed significantly decreased production of IL-12p40 ($p = 0.0143$) and IL-6 ($p = 0.0033$) in the NON-group compared to the AFF group (**Table 4**, **Figure 3**). IL-12p40 ($p = 0.0213$), IL-6 ($p = 0.0116$), and TNF-α ($p = 0.0452$) were also lower in the NON-group compared to HC. When comparing chemokines, MIP-1β was significantly decreased in the NON-group compared to both the AFF ($p = 0.0102$) and HC ($p = 0.0021$) groups.

TABLE 1 | Demographic characteristics and clinical scores.

				P values		
	CTL (*n* = 23)	**AFF (*n* = 14)**	**NON (*n* = 11)**	**CTL vs AFF**	**AFF vs NON**	**CTL vs NON**
Age: median	22.4	19.4	19.7	0.246	>0.9999	0.4433
Range [minimum, maximum]	[14.5, 36.8]	[15.7, 24.2]	[15.5, 35.2]			
IQR	20.2–24.4	17.1-23.4	16.3-24.0			
Gender (%male/female)	65/35	57/43	91/9	0.7321	0.0900	0.2137
Subject education: median (IQR)	14 (14–16)	12 (10–14)	11 (9–13)	*0.0121*	>0.9999	*0.0016*
Parental education: median (IQR)	14.5 (13–16)	15.3 (12–17.25)	13.5 (12.5–16)	>0.9999	0.8997	0.8735
SANS: median (IQR)	–	6 (6–13)	12 (9–18.75)	–	*0.0435*	
SAPS: median (IQR)	–	2 (0–5)	2 (0.25–7)	–	0.8213	–
BPRS: median (IQR)	–	41 (34–59)	42 (35.5–55.5)	–	0.874	–
WASI: median (IQR)	113 (105–120)	112 (98–118.5)	103 (78–114)	>0.9999	0.6324	0.1448
Antipsychotic dose (CPZ mg: median (IQR)		150 (116.7–300)	133.3 (83.3–225)	–	0.4993	–
Antipsychotic medication: (*n*)	–					
Aripiprazole		2	2			
Chlorpromazine		0	1			
Lurasidone		0	1			
Olanzapine		2	1			
Quetiapine		1	0			
Risperidone		2	4			
More than one atypical		2	0			
Unmedicated		5	2			

CTL, control; AFF, affective; NON, non-affective; IQR, interquartile range; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; BPRS, Brief Psychiatric Rating Scale; WASI, Wechsler Abbreviated Scale of Intelligence; CPZ, Chlorpromazine equivalent dose.

TABLE 2 | Concentration of cytokines/chemokines at baseline (media only).

	HC	**AFF**	**NON**	**Adjusted *P* values**		
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**HC vs AFF**	**AFF vs NON**	**HC vs NON**
IL-12p40	5.23 (2.93–6.67)	1.835 (0.7375–2.93)	1.89 (0.3125–4.135)	*0.0069*	0.8079	***0.0624***
IL-12p70	2.25 (1.66–2.91)	1.765 (0.7425–2.178)	1.665 (0.515–2.855)	0.1689	0.9999	0.3083
IL-1β	0.79 (0.64–1.08)	0.81 (0.42–0.97)	0.55 (0.29–1.86)	0.5525	0.9206	0.7451
IL-6	2.62 (1.71–3.5)	1.2 (0.8–2.16)	2.16 (0.7–3.5)	*0.0191*	0.4569	0.4552
TNF-α	9.52 (5.19–12.1)	6.95 (5.1–23.19)	5.775 (2.75–8.943)	0.8758	0.3260	0.2780
MCP-1	908.7 (413.8–1,663)	409.3 (315.9–781.6)	548.6 (202.8–1,009)	0.1752	0.9362	0.2540
MIP-1α	41.12 (5,492–9,912)	21.37 (6,281–19,945)	12.37 (4,494–14,958)	0.1839	0.6939	0.5319
MIP-1β	10,189 (9,269–12,945)	9,458 (8,377–13,438)	7,387 (3,362–10,462)	0.9998	0.9999	0.9998
GM-CSF	3.74 (2.16–4.7)	2.59 (1.58–5.5)	3.52 (1.055–4.97)	0.8187	0.8187	0.8187
IL-10	13.48 (8.23–19.51)	9.56 (7.56–14.65)	10.96 (7.448–26.92)	0.6961	0.8520	0.852

Median and interquartile ranges (IQR) of measured cytokines. Significant P values italicized.
Bold indicates P values that were significant prior to multiple correction testing.

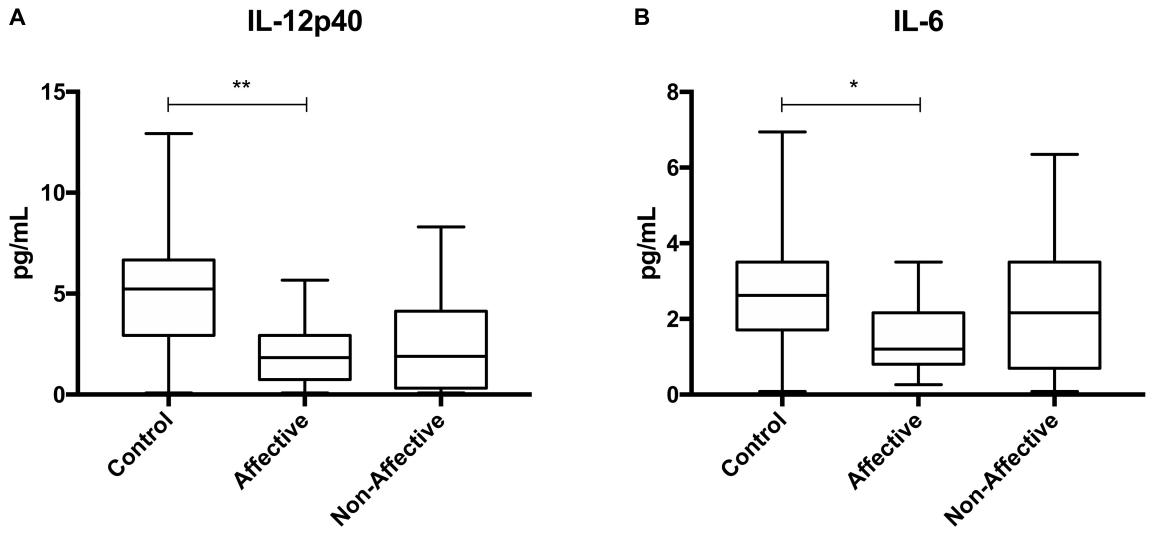

FIGURE 1 | Baseline (media only) cytokine/chemokine levels. Basal macrophage cytokine and chemokine production after culturing for 24 h in RPMI. Concentrations in pg/mL of IL-12p40 **(A)** and IL-6 **(B)** were significantly lower in the AFF group compared to the HC group. Box and whiskers graphs depict median, upper and lower interquartile ranges, $*p < 0.05$, $**p < 0.01$. HC $n = 23$, AFF $n = 14$, and NON $n = 11$.

TABLE 3 | Concentration of cytokines/chemokines after LPS stimulation.

	HC	AFF	NON	Adjusted *P* values		
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**HC vs AFF**	**AFF vs NON**	**HC vs NON**
IL-12p40	964 (669.2–1,997)	1,652 (1,037–2,356)	499.4 (311.8–907.2)	*0.0487*	*0.0009*	*0.0272*
IL-12p70	70.5 (39.4–116.2)	108.5 (59.67–228.7)	44.38 (32.35–94.13)	0.1304	***0.0527***	0.5155
IL-1β	10.16 (4.395–15.23)	8.005 (4.393–12.49)	3.22 (2.468–4.885)	0.5056	*0.0248*	*0.0078*
IL-6	1,898 (1,147–3,044)	2,430 (1,230–3,966)	524 (336.8– 784.4)	0.3268	*0.0003*	*0.0008*
TNF-α	8,242 (5,453–9,612)	7,444 (4,956–10,200)	2,982 (2,393–5,680)	0.9617	*0.0057*	*0.0072*
MCP-1	13,117 (9,294–23,301)	9,498 (8,258–17,013)	8,489 (4,281–17,646)	0.3194	0.5357	0.3033
MIP-1α	7,425 (5,492–9,912)	16,523 (6,281–19,945)	5,374 (4,494–14,958)	***0.0837***	***0.0837***	0.6960
MIP-1β	10,189 (9,269–12,945)	9,458 (8,377–13,438)	7,387 (3,362–10,462)	0.5535	*0.0418*	*0.0282*
GM-CSF	120.1 (107.6–188.7)	179.5 (94.59–265.9)	114.7 (67.85–171.3)	0.3283	0.2332	0.6695
IL-10	259.6 (150.7–492)	199.4 (130.7–366.3)	185.4 (93.95–325.1)	0.7928	0.7928	0.7928

Median and interquartile ranges (IQR) of measured cytokines. Significant P values italicized.
Bold indicates P values that were significant prior to multiple correction testing.

Cytokine and Chemokine Production After LPS Plus IL-4 Stimulation

After stimulating macrophages with LPS and IL-4 to induce an alternatively activated "M2" phenotype, Kruskal–Wallis tests showed significant differences in IL-12p40 ($p < 0.01$) and IL-12p70 ($p < 0.05$) across the three groups. For pairwise comparisons, Mann–Whitney *U* tests showed significantly increased production of IL-12p40 with increases in IL-12p70 and IL-6 trending to but not reaching statistical significance (**Table 5**, **Figure 4**).

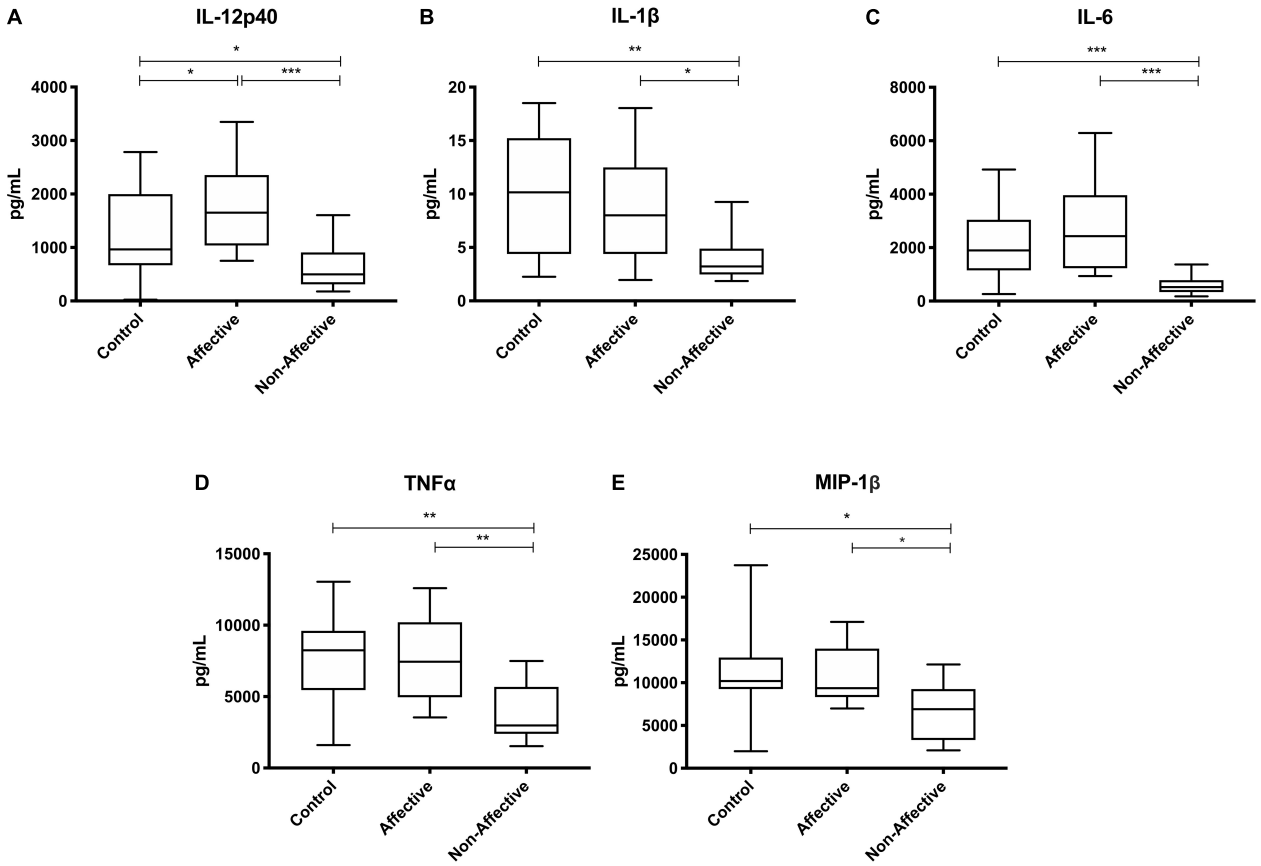

FIGURE 2 | Cytokine/chemokine levels after LPS stimulation. Macrophage cytokine production after culturing for 24 h in RPMI with 10 ng/mL LPS. Macrophages from the NON-group produced decreased concentrations of IL-12p40 **(A)**, IL-1β **(B)**, IL-6 **(C)**, TNF-α **(D)**, and MIP-1β **(E)** compared to the AFF group after stimulation with LPS. NON-macrophages also produced reduced concentrations of IL-12p40 **(A)**, IL-1β **(B)** IL-6 **(C)**, TNF-α **(D)**, and MIP-1β **(E)** compared to HC. Box and whiskers graphs depict median, upper and lower interquartile ranges, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. HC $n = 23$, AFF $n = 14$, and NON $n = 11$.

Logistic Regression With Receiver Operating Characteristic (ROC) Curve for Discriminating Affective Versus Non-Affective Patients

Considering the consistent differences in inflammatory macrophage responses after LPS stimulation between the AFF group compared to the NON-group, we investigated whether these differences had the potential to predict whether psychosis participants would fall into the AFF group or NON-group using binary logistic regression analysis and receiver operating characteristic (ROC) curve output. We first log2 transformed the concentrations of each cytokine to improve goodness of fit (Grund and Sabin, 2010). Unadjusted univariate binary logistic regression analysis of the log2-transformed cytokine concentrations revealed that higher levels of IL-12p40, IL-1β, IL-6, TNF-α, and MIP-1β increased odds of being in the AFF group compared to the NON-group ($p < 0.05$); however, confidence intervals were wide (**Supplementary Table S2**). ROC analysis indicated that when the log2 concentrations of these cytokines and chemokines were used individually to discriminate between groups, the area under the curve (AUC) ranged from 0.7730 to 0.9571. The most predictive of the cytokines and chemokines measured to discriminate between the AFF and NON-groups were IL-6 and IL-12p40, followed by IL-1β yielding AUC of 0.9571 ($p = 0.0002$), 0.9154 ($p = 0.0008$), and 0.8000 ($p = 0.0139$), respectively (**Figure 5**).

DISCUSSION

In order to advance our understanding of innate immune dysfunction in psychotic disorders, our analysis focused on identifying differences in inflammatory cytokines associated with macrophage activation in individuals with either affective or non-affective psychosis. Our approach measured cytokines during a range of stimulatory conditions to identify dysfunction over different possible phenotypes of macrophages, including toward M1 and M2. We found that monocyte-derived macrophages from the NON-group consistently produced lower levels of innate cytokines under inflammatory conditions compared to both HC and AFF. Additionally, the AFF group had lower cytokine production at baseline compared to controls,

TABLE 4 | Concentration of cytokines/chemokines after LPS plus IFNγ stimulation.

	HC	AFF	NON	Adjusted *P* values		
	Median (IQR)	Median (IQR)	Median (IQR)	HC vs AFF	AFF vs NON	HC vs NON
IL-12p40	5,443 (3,657–7,664)	5,386 (4,264–6,587)	1,690 (1,425–4,394)	0.9224	*0.0143*	*0.0213*
IL-12p70	848.3 (653–1,253)	1,195 (1,017–1,396)	416 (305.9–1,483)	0.2922	***0.1356***	0.2922
IL-1β	13.18 (7.76–16.06)	12.96 (7.225–18.72)	5.61 (4.11– 13.48)	0.9324	0.2710	0.2710
IL-6	3,463 (1,834–4,104)	4,032 (2,528–4,429)	940.6 (735.6–1,846)	0.2312	*0.0033*	*0.0116*
TNF-α	11,134 (8,477–11,926)	9,991 (8,597–11,772)	8,542 (3,179–10,645)	0.9597	0.1779	*0.0452*
MCP-1	14,821 (9,095–29,783)	9,101 (7,607–10,435)	8,634 (3,869–11,603)	***0.0611***	0.8201	***0.0611***
MIP-1α	9,277 (5,492–9,912)	11,442 (6,281–19,945)	6,747 (4,494–14,958)	0.4534	0.4201	0.4534
MIP-1β	10,189 (9,269–12,945)	9,458 (8,377–13,438)	7,387 (3,362–10,462)	0.6536	*0.0102*	*0.0021*
GM-CSF	73.7 (53.77–108.5)	94.88 (68.12–227.5)	83.1 (53.77–108.4)	0.3146	0.3544	0.9450
IL-10	117.3 (70.44–148.8)	117.7 (73.72–168.1)	108.6 (71.37–162.9)	0.8677	0.9459	0.9850

Median and interquartile ranges (IQR) of measured cytokines. Significant P values italicized.
Bold indicates P values that were significant prior to multiple correction testing.

however, after inflammatory activation, they showed more robust responses when compared to the NON-group. Under conditions of alternative activation, AFF macrophages produced higher amounts of inflammatory cytokines compared to controls. These data support an overall dysfunctional innate immune response both affective and non-affective psychosis groups.

Our findings of decreased responses in the NON-group and increased responses in the AFF group are not completely unexpected. Our results support previous work that looked at gene expression in PBMC and identified an increased M1/decreased M2 signature in BD, but not in SCZ (Brambilla et al., 2014). Two previous studies also identified increased expression of clusters of inflammatory genes in monocytes from affective and psychotic patients, with some overlap but also some distinct differences between BD, SCZ, and MDD. All groups showed increases in inflammatory gene expression however the SCZ group had downregulation of transcription factors for adhesion and chemotactic molecules (Drexhage et al., 2010b; Carvalho et al., 2014). It is important to note that these cells were not stimulated, so their results only provide insight as to their behavior at rest. A recent study by Ascoli et al. (2019) found that inflammatory macrophage responses after M1/M2 skewing diminished with disease progression in BD, where "early stage" BD macrophages more closely resembled HC macrophage responses, but in later-stage disease the responses were attenuated. Our patients were not considered chronically ill, all were within a year of first symptoms and our findings that macrophages in the AFF group respond similarly to HC after LPS and M1 stimulation supports this research.

Chronic activation of macrophages has been a proposed etiological mechanism of SCZ for decades (Smith and Maes, 1995), and many studies have indicated circulating pro-inflammatory cytokines associated with these cells are increased in SCZ, BD, and MDD (Dowlati et al., 2010; Modabbernia et al., 2013; Upthegrove et al., 2014; Pillinger et al., 2018), Under certain circumstances such as with LPS stimulation, when macrophages are previously exposed to LPS they can become refractory to this stimuli. This could be the mechanism behind the decreased macrophage activity seen in the NON-group (Biswas and Lopez-Collazo, 2009). This does not explain responses similar to HC after LPS and M1 stimulation in the AFF group, however, some cytokine studies have proposed that inflammation is not consistently present in AFF disorders, rather they vary depending on state of disorder (Munkholm et al., 2013; Goldsmith et al., 2016), therefore it is possible that AFF cells are not experiencing the same levels of chronic activation as seen in SCZ. These differential responses suggest that a different type of immune dysfunction may be involved in AFF disorders.

The increased inflammatory responses in the AFF macrophages after alternative activation was not expected. Production of IL-6 and IL-12 is generally associated with classically activated macrophages. In the presence of IL-4, macrophages typically skew to the M2 phenotype; however, it is important to note there is high heterogeneity in M2 macrophages and the specific phenotype driven by IL-4 exposure is M2a,

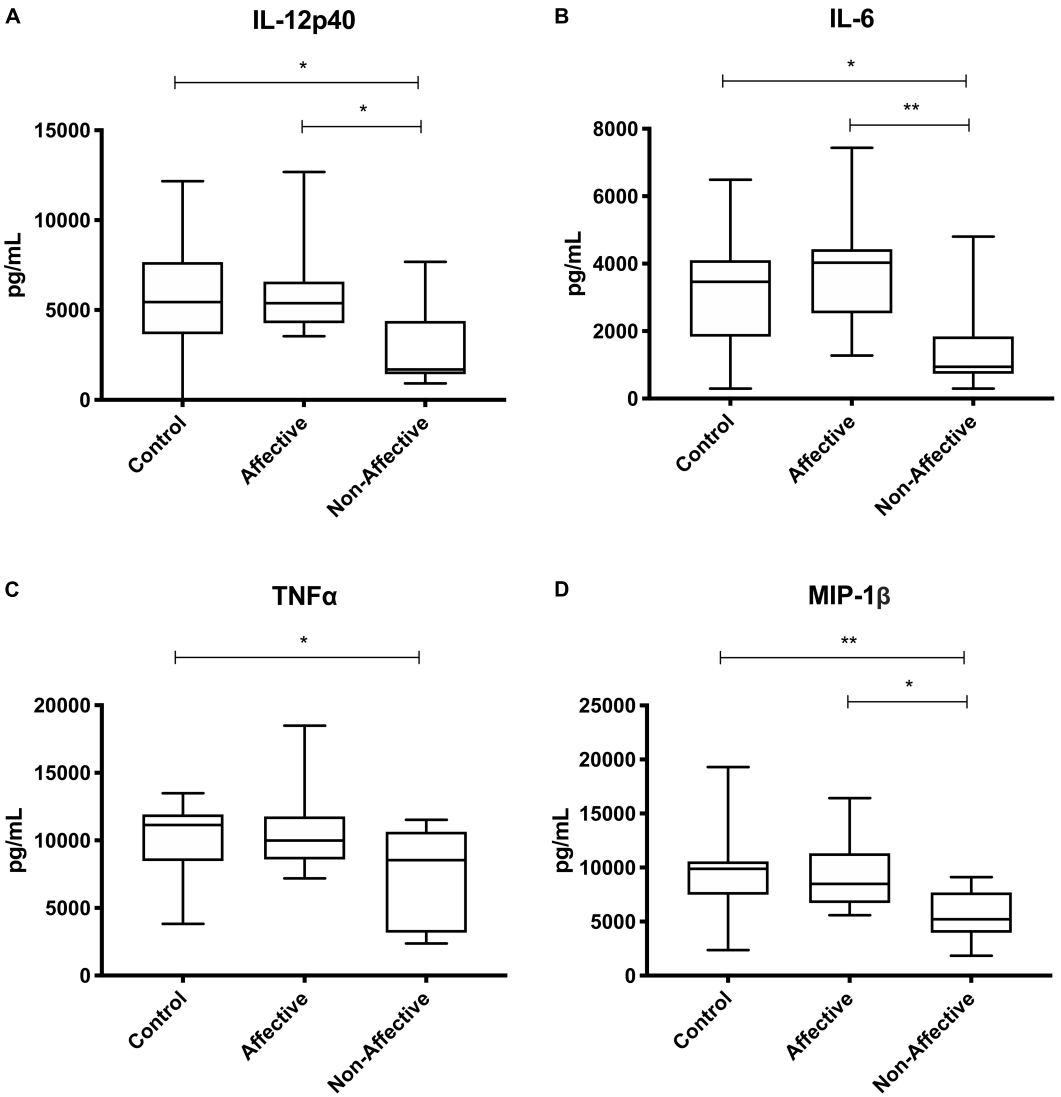

FIGURE 3 | Cytokine/chemokine levels after LPS plus IFNγ stimulation. Macrophage cytokine and chemokine production after culturing for 24 h in RPMI with 10 ng/mL LPS plus 50 ng/ml IFNγ. Macrophages from the NON-group produced decreased concentrations of IL-12p40 **(A)**, IL-6 **(B)**, and MIP-1β **(D)** compared to the AFF group. The NON-group had decreases in IL-12p40 **(A)**, IL-6 **(B)**, TNF-α **(C)**, and MIP-1β **(D)** compared to the HC group after stimulation with LPS plus IFNγ. Box and whiskers graphs depict median, upper, and lower interquartile ranges, $^{*}p < 0.05$, $^{**}p < 0.01$. HC $n = 23$, AFF $n = 14$, and NON $n = 11$.

commonly seen activated in T-helper cell type 2 (Th2) parasite and allergic responses (Luzina et al., 2012). The atypical response to IL-4 in the AFF macrophages may provide a clue that there could be different types of immune dysfunction involved in affective versus non-affective psychotic disorders. For example, cytokine meta-analyses show elevated IL-4 in BD but decreased IL-4 in FEP and SCZ (Modabbernia et al., 2013; Goldsmith et al., 2016). Increased IL-4 mRNA was also previously identified in BP patients compared to SCZ (Brambilla et al., 2014), and affective disorders may accompany a higher risk of allergic diseases than psychotic disorders (Tzeng et al., 2018). We recently measured plasma cytokines in a cohort of FEP patients and found elevated plasma IL-2, IL-6, and IFNγ in SCZ patients, however, only IL-10 was elevated in BD compared to healthy controls (Lesh et al., 2018). These differential findings when comparing disorders could potentially be useful for distinguishing between AFF and NON FEP patients early in the diagnostic process, similar to the predictive ROC curves we provided.

Cytokines are critical mediators of the immune response, and often function as messengers to elicit inflammatory or anti-inflammatory responses from other cells (Turner et al., 2014). These cytokines are tightly regulated within the central nervous system (CNS), as appropriate amounts are necessary for neurodevelopment, homeostasis, synaptic pruning and plasticity (Deverman and Patterson, 2009). Circulating inflammatory cytokines such as IL-1β, IL-6, and TNF-α are able to cross the blood-brain-barrier, and are known to influence behavior, therefore they have the capacity to directly influence the activity

TABLE 5 | Concentration of cytokines/chemokines after LPS + IL-4 stimulation.

	HC n = 13	AFF n = 9	NON n = 5	Adjusted P values		
	Median (IQR)	Median (IQR)	Median (IQR)	HC vs AFF	AFF vs NON	HC vs NON
IL-12p40	591.7 (499.9–840)	1,489 (975.3–2,843)	767 (360.8–2,338)	*0.0024*	0.4137	0.5214
IL-12p70	105 (60.06–179.5)	231.6 (182.6–798.7)	132.4 (60.74–252.3)	***0.0588***	0.1414	0.659
IL-1β	6.44 (3.195–12.81)	11.52 (3.75–13.33)	3.475 (2.548–8.568)	0.6494	0.6494	0.6494
IL-6	1,056 (671.3–1,472)	2,214 (1,289–3,574)	1,166 (509.3–2,137)	***0.0588***	0.2561	0.968
TNF-α	5,874 (3,714–8,727)	9,264 (4,123–10,671)	6,778 (5,245–7,469)	0.6887	0.6922	0.7181
MCP-1	9,131 (6,717–15,481)	18,951 (6,899–28,525)	12,820 (9,090–18,900)	0.6378	0.7440	0.7440
MIP-1α	6,305 (5,492–9,912)	20,000 (6,281–19,945)	9,517 (4,494–14,958)	***0.1002***	0.5338	0.2282
MIP-1β	10,189 (9,269–12,945)	9,458 (8,377–13,438)	7,387 (3,362–10,462)	0.9989	0.9999	0.9989
GM-CSF	121.1 (68.34–173.3)	174.7 (97.09–386)	127.3 (96.98–216.9)	0.6188	0.8618	0.8618
IL-10	564.7 (277.5–892.8)	197.8 (141.6–1,579)	271.9 (141–2,406)	0.9956	0.9956	0.9956

Median and interquartile ranges (IQR) of measured cytokines. Significant P values italicized.
Bold indicates P values that were significant prior to multiple correction testing.

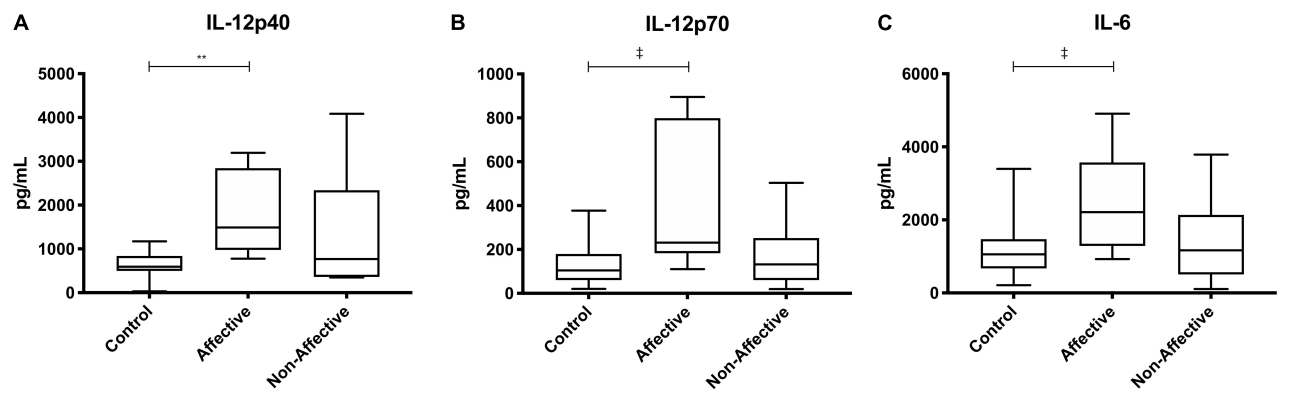

FIGURE 4 | Cytokine/chemokine levels after LPS plus IL-4 stimulation. Macrophage cytokine and chemokine production after culturing for 24 h in RPMI with 10 ng/mL LPS and 40 ng/mL IL-4. Macrophages from the AFF group produced higher concentrations of IL-12p40 **(A)** with trending increases in IL-12p70 **(B)** and IL-6 **(C)** compared to the HC group. Box and whiskers graphs depict median, upper and lower interquartile ranges, $^{**}p < 0.01$. ‡Indicates comparisons that were significant prior to multiple correction testing. HC n = 14, AFF n = 7, and NON n = 6.

of resident immune cells such as the microglia (Yarlagadda et al., 2009). Although not technically the same cells due to differing ontogeny, the yolk-sac derived microglia are myeloid cells that have similar gene expression and cytokine production profiles as monocytes and macrophages (Takahashi et al., 2016). Microglia play a major role in neurogenesis, and are responsible for eliminating excess neuronal precursors in the cerebral cortex during this process (Cunningham et al., 2013). Additionally, they are crucial for the maintenance of synapses by constantly surveilling and interacting with all regions of the neuron including dendritic processes, which they refine through pruning (Paolicelli et al., 2011), and failure of this process leads to impaired connectivity and altered behavior in mice (Zhan et al., 2014). Perivascular and meningeal macrophages (also yolk-sac derived) as well as choroid plexus macrophages (which can be replenished from circulating monocytes) also have the potential to skew to an M1 phenotype when inflammatory cytokines are present in the milieu (Goldmann et al., 2016;

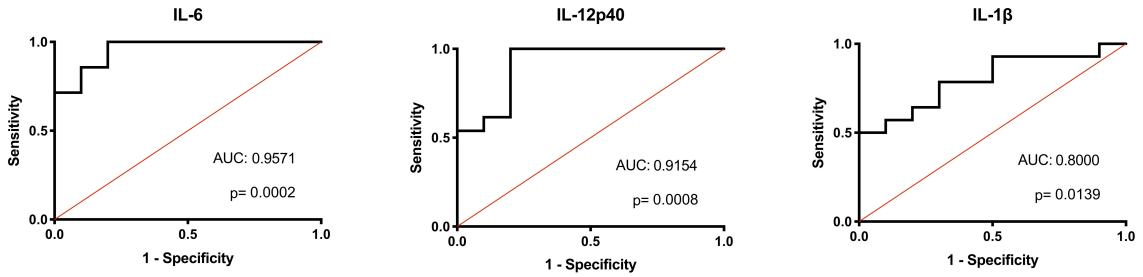

FIGURE 5 | Binary logistic regression with ROC curve output. Binary logistic regression with ROC curve output of the AFF group plotted against those in the NON-group. A receiver operating characteristic (ROC) curve analysis indicated that IL-IL-6, 12p40, and IL-1β levels were significant predictors of affective psychoses (area under ROC curve, 0.9571, 0.9154, and 0.8000, respectively). The diagonal defines random event.

Prinz et al., 2017). During neuroinflammatory events, it is possible for infiltrating monocytes to enter the CNS (Minogue, 2017), further contributing to neuroinflammation.

Dysfunctional activation of microglia and neuroinflammation have been proposed to contribute to pathological mechanisms in psychiatric disorders, and these can occur under conditions of systemic inflammation and macrophage activation (Perry et al., 2007; Takahashi et al., 2016). Considering the important role that microglia play in neuronal survival and maintenance of synapses, it has been suggested that failure of this maintenance or dysregulation of microglia may play a role in cortical gray matter thinning during aging (Vidal-Pineiro et al., 2020). Excess cortical thinning is common pathology seen in psychiatric disorders, for example, meta-analyses of voxel-based morphometry to measure gray matter volume found that the dorsal anterior cingulate, right insula, and left insula gray matter loss are consistently seen across a diverse group of psychiatric conditions that included SCZ, BD and depression, however, MDD patients had greater gray matter losses in the hippocampus and amygdala while gray matter increases were seen exclusively in the striatum of SCZ spectrum patients (Goodkind et al., 2015). In a previous study identifying differences in plasma cytokines in SCZ and BD, researchers found an inverse relationship between inflammatory cytokines and gray matter thickness in SCZ, but not in BD (Lesh et al., 2018). Antipsychotic medication has been associated with gray matter loss, however, one study found progressive loss of cortical thickness despite no use of antipsychotic drugs in subjects at high-risk for developing a psychotic disorder who later converted to psychosis, suggesting that this phenomenon is part of the pathophysiology of these disorders (Cannon et al., 2015). Differences in microglial phenotype and function could be contributing to these differences, and investigation of post mortem brain tissue as well as *in vivo* positron emission tomography (PET) studies have provided some evidence of microglia activation in these disorders (Bayer et al., 1999; van Berckel et al., 2008; Doorduin et al., 2009; Dean et al., 2010; Rao et al., 2010; Fillman et al., 2013; Haarman et al., 2014; Setiawan et al., 2015; Bloomfield et al., 2016; Trepanier et al., 2016; Holmes et al., 2018; Richards et al., 2018; Setiawan et al., 2018). However, findings have not always been consistent and complexity due to variations in methodology complicate the overall picture of neuroinflammation in SCZ, BD, and MDD. A theme that has emerged during investigation of neuroinflammation is that increases may be associated with the state of disease, contributing to variations in findings (reviewed in Hughes and Ashwood, 2020).

There are several limitations to our study that must be taken into account when interpreting results. Our sample sizes for both the AFF and NON-groups were relatively small, and this was most apparent during regression analysis which suggested that higher concentrations of inflammatory cytokines after LPS stimulation are associated with the AFF macrophages. However, the wide confidence intervals indicate these data need to be replicated in larger cohorts for confirmation of this analysis. Moreover, patients were assessed within one year of diagnosis, not necessarily during active first-episode, and therefore may have variability in inflammatory and behavioral status based on the possibility that the state of disease may influence level of immune activation. Many of the psychotic subjects were taking medications including anti-psychotics which have been shown to alter cytokine production in psychotic patients (Noto et al., 2015), however, these findings are not consistent across studies (Theodoropoulou et al., 2001). Despite these limitations, our study had several advantages over previous cytokine studies in neuropsychiatric disorders. It is important to note that circulating cytokines can have significant individual variation, and simple measurement of plasma cytokines does not provide information about the type of immune cell(s) producing the cytokines. By differentiating circulating monocytes into macrophages, we are able to identify dysfunction specific to this cell set and therefore provide functional information about how macrophages might be behaving during various states of immune activation. Additionally, by culturing and differentiating monocytes into macrophages over a seven-day period prior to stimulation, we likely minimized the effect of any medications on the behavior of these cells.

CONCLUSION

It is clear from an abundance of research that immune dysfunction is present and likely plays a role in neuropsychiatric disorders. In summary, we report differential monocyte

dysfunction in psychotic disorders with and without a mood component. Our work expands on previous studies that identified differences and similarities in macrophage responses across SCZ, BD and MDD, and suggest that affective psychoses may have a different type of immune dysfunction than primary psychotic disorders, which may involve elevated IL-4 and dysfunctional M1/M2 skewing. It is possible other circulating cell types may be responsible for the chronic inflammatory findings in these disorders. Future studies could attempt to screen other circulating immune cells for similar differential patterns, or characterize macrophages further through flow cytometric analysis and transcriptomics. This could help identify if there are differences across groups in ability of macrophages to differentiate, which may be contributing to the differences in cytokine production seen in our study. By better characterizing immune dysfunction in these disorders we can identify targets for therapeutics that might be beneficial for affective and non-affective psychotic disorders.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of California, Davis Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HH and EM-K contributed to the design of the study, performed the data analyses, contributed to the data interpretation, and drafting of the manuscript. HH, HY, and EM-K processed the blood, and ran the assays on the macrophage cultured samples. TL and CC contributed to the interpretation of the data, and revisions of the manuscript. PA designed the study, and made substantial contributions to interpretation of the data, and drafting and revisions of the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fncel.2021.583351/full#supplementary-material

Supplementary Figure S1 | Macrophage cytokine and chemokine production after culturing for 24 h under four conditions: RPMI alone, RPMI with 10 ng/mL LPS, RPMI with 10 ng/mL LPS plus 50 ng/ml IFNγ, or RPMI with 10 ng/mL LPS and 40 ng/mL IL-4. Box and whiskers graphs depict median, upper, and lower interquartile ranges, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. ‡Indicates pairwise comparisons that were significant prior to multiple correction testing.

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MINI REVIEW
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Cytokine Imbalance in Schizophrenia. From Research to Clinic: Potential Implications for Treatment

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Cytokines are one of the most important components of the immune system. They orchestrate the brain's response to infectious and other exogenous insults and are crucial mediators of the cross-talk between the nervous and immune systems. Epidemiological studies have demonstrated that severe infections and autoimmune disorders, in addition to genetic predisposition, are risk factors for schizophrenia. Furthermore, maternal infection during pregnancy appears to increase the risk of schizophrenia, and proinflammatory cytokines may be negatively involved in the neurodevelopmental process. A cytokine imbalance has been described in the blood and cerebrospinal fluid of schizophrenia patients, particularly in the T helper type 1 [Th1] and type 2 [Th2] cytokines, albeit the results of such studies appear to be contradictory. Chronic stress, likewise, appears to contribute to a lasting proinflammatory state and likely also promotes the disorder. The aim of this mini-review is to investigate the roles of different cytokines in the pathophysiology of schizophrenia and define how cytokines may represent key molecular targets to regulate for the prevention and treatment of schizophrenia. How current antipsychotic drugs impact cytokine networks is also evaluated. In this context, we propose to change the focus of schizophrenia from a traditionally defined brain disorder, to one that is substantially impacted by the periphery and immune system.

Keywords: serum molecular target, inflammatory cytokines, T helper type 1, CNS and Immune system cross-talk, molecular targets

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INTRODUCTION

Schizophrenia, a chronic and often debilitating mental disorder, impacts ∼1% of the world's population and generally occurs in late adolescence or early adulthood. It is characterized by a symptomatology that includes the presence of auditory, visual, tactile and olfactory hallucinations, delusions, confusion, impaired concentration that includes cognitive scarcity, lethargy, and movement disorders (1). Although genetic vulnerability and environmental stressors during the early stages of life are fundamental for the progression of schizophrenia, inflammation is considered a primary causative/contributing/mediating factor in the onset of schizophrenia (2–4). Disturbances of the immune system and its complex interactions with the nervous system may hence contribute to the pathogenesis and pathophysiology of schizophrenia (5, 6). As a

consequence, a bidirectional interaction between the immune system and the brain has aroused an increasing interest in the role of the immune system in neuropsychiatric diseases. Abnormal blood lymphocyte parameters, such as the numbers of total T lymphocytes, T helper cells, an increased CD4/CD8 ratio (7), and a decreased mitogen-induced lymphocyte proliferation have been reported (8), and the presence of select antibrain antibodies have been detected in the serum of schizophrenic patients (9).

An interesting current debate concerns the relationship between different immune factors and the pathophysiology of schizophrenia, and particularly cytokine dysregulation in patients with schizophrenia. It is well-known that cytokines are key messengers in the cross-talk between the central nervous system (CNS) and immune cells. In the light of this, several studies have hypothesized that in patients with schizophrenia and severe mood disorders, the proinflammatory cytokine microenvironment is likely involved in the pathogenesis and pathophysiology of schizophrenia and the ensuing psychopathological symptoms (10–14).

An increasing number of studies indicating a role of inflammation and immunity in the pathogenesis of symptoms of schizophrenia have provided evidence that systemic inflammation can exert a profound influence on the brain that leads to changes in mood, cognition, and behavior. In this regard, the peripheral immune system-to-brain communication pathways have been studied extensively in the context of other neuroinflammmatory diseases in which inflammatory cytokines are, likewise, considered to play a critical role (15–17). Several hypotheses have been formulated to both highlight and account for the involvement of immune cells and cytokines in schizophrenia. One hypothesis was based on the macrophage-T lymphocyte theory, according to which cytokines such as interleukin-1 (IL-1), IL-2, tumor necrosis factor-α (TNF-α), interferon-α (IFN-α), and IFN-γ that are produced by chronically activated macrophages and T lymphocytes, are the key mediators of schizophrenia (18). A further hypothesis is the Th2 hypothesis, according to which Th2-mediated immune responses are the most important events in schizophrenia (19). There is evidence to indicate that altered cytokines and gray matter abnormalities, such as the cortical thickness of the bilateral Broca's area and temporal gyrus, are associated with schizophrenia (20). In this light, it is possible to speculate that abnormal levels of cytokines could potentially be used as a disease indicator and may provide a diagnostic or prognostic biomarker in schizophrenia (21, 22).

ROAD MAP

The focus of this mini-review is to evaluate whether and how alterations in peripheral cytokines may impact schizophrenia. In undertaking this task, we first define what cytokines are and where they derive from. We next review their involvement in schizophrenia, particularly in relation to studies that have quantified elevations and declines of select cytokines in the periphery or CNS. In this regard, there are numerous studies providing data on different cytokine combinations, with several providing apparently conflicting results in relation to change direction that potentially arise from disease stage and patient state. Medications may, likewise, modify cytokine levels, and we therefore review studies assessing them in subjects treated with antipsychotics. Although first-line therapy, numerous patients do not adequately respond to antipsychotics, and we hence evaluate studies incorporating other medications with a focus on immunomodulatory agents that more directly impact cytokine levels. Finally, we provide a synopsis of where the field is heading and how recent advances, such as sampling exosomes enriched for brain origin, may aid our understanding of cytokine imbalances in schizophrenia and their targeting as a treatment approach.

CYTOKINE OVERVIEW

Cytokines, low molecular weight proteins, affect nearly every biological activity, including embryonic development, disease pathogenesis, specific and non-specific immune responses, cognitive function, as well as progression of the degenerative processes of aging. These effects are mediated by impacting the cells that secrete them (i.e., autocrine action), neighbor them (i.e., paracrine action), and/or are remote from them (i.e., endocrine action). In this regard, the same cytokine can act on many different cell types (i.e., pleiotropic action), or a similar function can be instigated by different cytokines and the same cytokine may have overlapping actions and may regulate several different immune functions (i.e., redundant action). Cytokines can also act synergistically or antagonistically by activation or inhibition of its target cells to generate additional cytokines to, thereby, amplify or dampen an inflammatory response. Although cytokines are generated by numerous cell types, such as fibroblasts and endothelial and epithelial cells, the predominant producers are the white blood cells and, in particular, T cell subsets and macrophages.

In this regard, CD4+ T helper cells are traditionally considered the cytokine-generating cells and, according to their pattern of cytokine production, Th cells are classified as Th1, Th2 (23), Th17 (24), and Th9 cells (25). In addition, two subpopulations of CD8+ effector T cells; specifically, types 1 and 2 cytokine-producing subsets have been identified (26) and, in both physiological and pathological processes, cytokines may be produced in and by peripheral nerve tissue by resident and recruited macrophages, mast cells, endothelial cells, as well as Schwann cells.

Cytokines are classified as proinflammatory and anti-inflammatory. The time-dependent pro- and anti-inflammatory balance determines the outcome of an inflammatory response. As an example among many, elevated levels of proinflammatory cytokines, such as IL-6, IL-1, IL-17, and TNF-α have been demonstrated in the cerebrospinal fluid (CSF) and in demyelinating plaques of patients with multiple sclerosis (MS), suggesting a pivotal role in the pathogenesis of this and other neurodegenerative disorders (27, 28). The synthesis and release of cytokines in response to a variety of stimuli supports their interaction/binding to receptors that, in turn, signal a

TABLE 1 | Several cytokines, cytokine receptors, signaling, and source.

Cytokine	Receptor family	Signaling	Source
IL-1	Immunoglobulin superfamily receptors	NF-κB, IRAK, MyD88, TRAF6	Many cells, expecially monocytes/macrophages; epithelial and endotelial cells; fibroblasts; astrocytes
IL-2	Class I cytokine receptors	JAK1, JAK3, STAT5	Th1, NK cells
IL-4	Class I cytokine receptors	JAK1, JAK3, STAT6	Th2, mast cells, other cells
IL-6	Class I cytokine receptors	JAK1, STAT3	Macrophages, fibroblasts, T cells
IL-10	Class II cytokine receptors	JAK1, TYK2, STAT3	Th2, Treg
IL-12	Class I cytokine receptors	JAK2, TYK2, STAT4	Macrophages, NK cells, DCs, B cells
IL-17	Immunoglobulin superfamily receptors	MAPKs, PI3K, NF-κB.	Th17
IL-18	Immunoglobulin superfamily receptors	IRAK, MyD88, TRAF6, NF-κB	Many cells, expecially macrophages, keratinocytes
IFNγ	Class II cytokine receptors	JAK1, JAK2, STAT1	Th1, NK cells
TNFα	TNF receptors	NF-κB; JNK, ERK, p38	Activated myeloid, T and other cells
TGFβ	Receptor serine kinase family	MAPK	Treg, macrophages, other

response to their target cells—whether proximate or distant—that then leads to a change in cell function or activity. Such cytokine receptors are linked to multiple signaling pathways within the cytoplasm and nucleus, leading to transcriptional and post-transcriptional activation of multiple factors. In this regard, select transcription factors, such as nuclear factor κB (NF-κB), activator protein-1 (AP-1), and nuclear factor of activated T cell (NFAT), are crucial in cytokine production (**Table 1**).

The expression level of receptors can be induced by some cytokines and may, thereby, modify target cell responsiveness. As a consequence, cytokine interactions can be considered a "cytokine network" in light of the numerous potential feedback mechanisms between the various cytokines as well as their targets. Depending on the cytokine concentrations available, target and receptor expressions, the activation of different signal transduction pathways occurs that will lead to a different gene expression in response to different cytokines that share some biological effects. This adavantagiously provides the potential combinations of cytokines to orchestrate multiple different actions to maintain homeostasis in response to a broad array of challenges during health, but, conversely creates a complex picture that is difficult to interpret in the presence of disease and, particularly a mental disorder such as schizophrenia. Furthermore, different secretory pathways characterize the cytokine secretion process based on a cytokine's function and cell type. In this regard, many immune cells store cytokines in granules that allow their rapid release in response to receptor signaling from toll-like receptors (TLRs), Fc receptors, cytokine receptors, and complement receptors, among others (29, 30). Secretion may occur *via* the constitutive or non-conventional secretory pathways, with cytokines such as IL-2, IL-3, IL-6, IL-10, IL-12, and TNF-α being constitutively secreted, while others such as IL-1β, IL-1α, IL-33, and high-mobility group box 1 (HMGB1) being unconventionally secreted.

In synopsis, the synthesis and release of cytokines is an important part of the immune response and can act in a homeostatic protective manner or, when secreted inappropriately or excessively, can be involved in chronic conditions such as a generalized systemic inflammatory response or a neurodegenerative/neuropsychiatric disorder. As cytokines have such potent actions, it has become increasingly clear that dysregulation of cytokine generation and release, as well as cytokine signaling can contribute to human disease and lead to pathogenic effects.

CYTOKINES IN SCHIZOPHRENIA

Our increasing understanding of the functioning of the immune system has strengthened psycho-neuroimmunological theories hypothesizing that schizophrenia is a systemic syndrome, involving both the nervous and immune systems, in which abnormalities in immune system and cytokine functioning have a pivotal role (**Figure 1**). Recently, evidence linking schizophrenia to autoimmunity has been highlighted; autoimmune-related antibodies anticardiolipin, antinuclear, anti-DNA, antihistone, and anti-NMDA receptors have been reported present in the serum of schizophrenia patients. Gene polymorphisms of several cytokines are associated with the development of the schizophrenia syndrome. In patients with schizophrenia, presence of gene polymorphisms of proinflammatory cytokines, such as IL-1β and IL-6, have been linked to high serum levels of these cytokines. An emerging literature suggests that prenatal and postnatal exposure to pathogens may contribute to the etiopathogenesis of schizophrenia *via* the actions of cytokines. In fact, cytokines produced in response to infection are not only involved in the inflammatory response but also in the development and function of the CNS. During prenatal infections, maternally produced cytokines may cross the placenta and blood-brain barrier and drive behavioral, neurochemical, psychophysiologic, and histologic abnormalities ultimately found in schizophrenia patients (31). An imbalance between T helper (Th) 1, Th2, Th17, and Treg cells and cytokines produced,

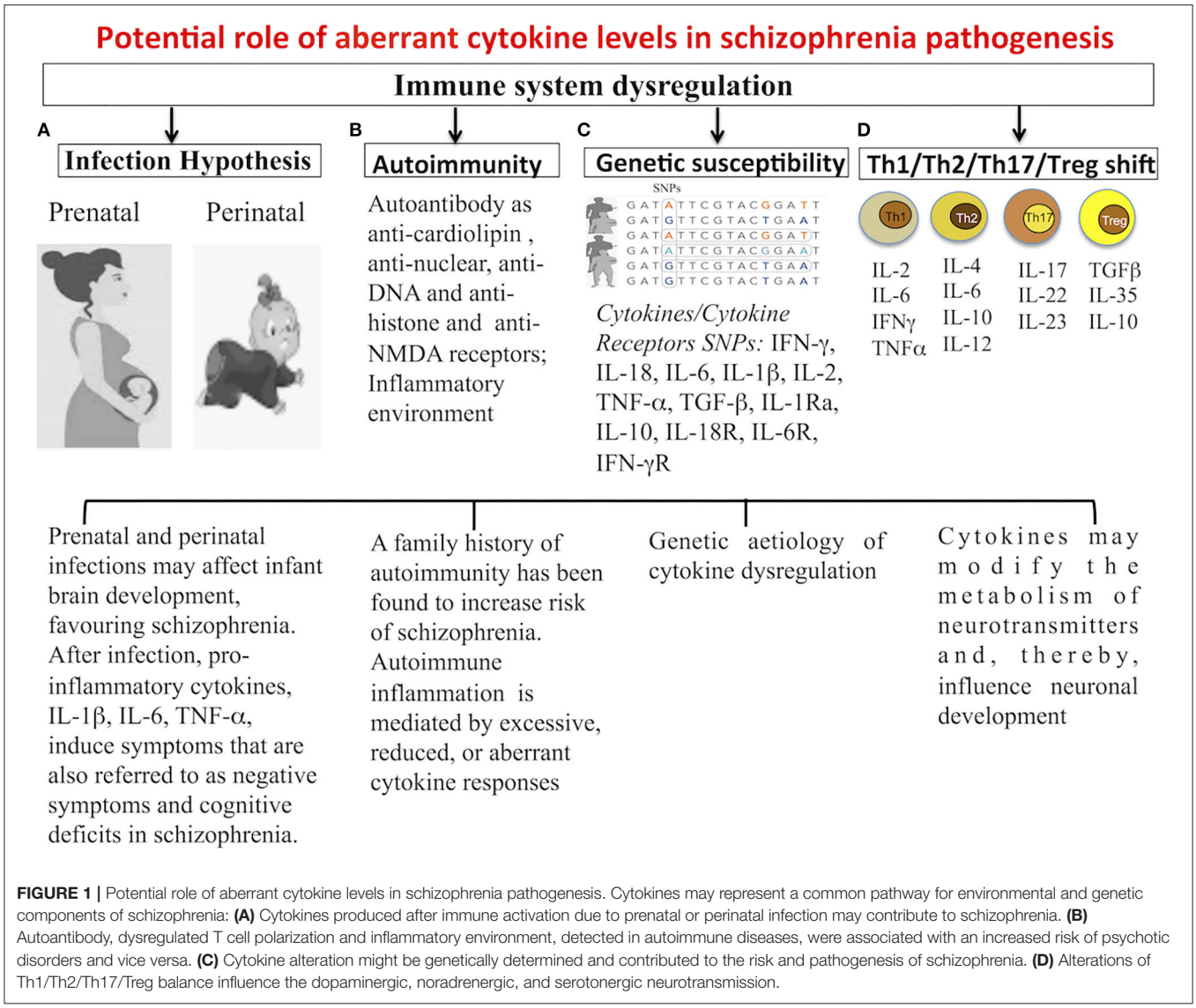


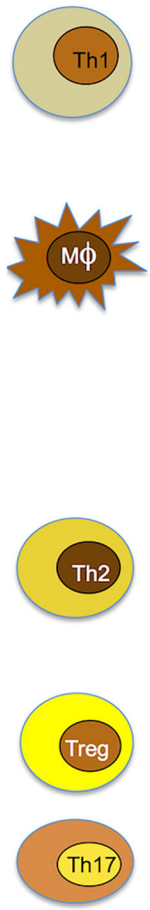
FIGURE 1 | Potential role of aberrant cytokine levels in schizophrenia pathogenesis. Cytokines may represent a common pathway for environmental and genetic components of schizophrenia: **(A)** Cytokines produced after immune activation due to prenatal or perinatal infection may contribute to schizophrenia. **(B)** Autoantibody, dysregulated T cell polarization and inflammatory environment, detected in autoimmune diseases, were associated with an increased risk of psychotic disorders and vice versa. **(C)** Cytokine alteration might be genetically determined and contributed to the risk and pathogenesis of schizophrenia. **(D)** Alterations of Th1/Th2/Th17/Treg balance influence the dopaminergic, noradrenergic, and serotonergic neurotransmission.

represent the essential component of immune dysregulation in schizophrenia.

Peripheral Cytokines

Numerous studies have investigated alterations in peripheral cytokine levels in schizophrenia. Among these, the study of Smith and Maes proposed that in schizophrenia, chronically activated macrophages and T lymphocytes produce cytokines, such as TNF-α, IL-1, IL-2, IFN-α, and IFN-γ, that have a key role in this disorder's development (18). On the basis of this study, the relationship between schizophrenia and cytokine levels was evaluated, and two meta-analyses were performed to shed light on the relationship of abnormal cytokine levels with schizophrenia. Data from 62 studies, analyzed IFN-γ, IL-4, IL-2, soluble IL-2 receptor (sIL-2R), IL-1β, IL-1 receptor antagonist (IL-1RA), TNF-α, IL-6, soluble IL-6 receptor (sIL-6R), and IL-10 in schizophrenia. Increased levels of IL-1RA, sIL-2R, and IL-6 were evident *in vivo*, whereas *in vitro* IL-2 was decreased, and no significant differences were observed for the other cytokines. This, thereby, provided the first evidence to consider the occurrence of an inflammatory syndrome in schizophrenia (32). A meta-analysis by Miller et al. (33) investigated cytokines in schizophrenia at various disease stages and treatment conditions in an analysis of 40 studies. Patients were separated into three groups: drug-naïve first-episode psychosis, acute relapse of psychosis, and schizophrenia patients who were stable medicated outpatients with treatment-resistant psychosis. Results showed that patients with first-episode psychosis and those with acute relapse of psychosis had significantly elevated levels of IL-1β, IL-6, TNF-α, IFN-γ, and IL-12, and patients under antipsychotic treatment showed a significant decline in IL-6, IL-1β, and IFN-γ and a rise in IL-12 and soluble IL-2 receptor.

Taken together, these findings highlight that cytokine alterations in schizophrenia may vary with clinical status



	Increased	Decreased	Unchanged
IL-2	Petrikis et al. 2015	Potvin et al., 2008	
IFN-γ	Miller et. Al, 2011; Ding et al.2014	Reale et al.,2011; Na et al.,2007; Al-Asmari et al., 2014; Das et al., 2014	Di Nicola et al., 2013
TNF-α	Upthegrove et al., 2014;Falcone et al, 2015; Goldsmith et al., 2016; Miller et al., 2011; Wei et al., 2018; Zhu et al.,2018	Lv et al.,2015; Zhu et al.2018; Tian et al,2014	Potvin et al, 2008
IL-1β	Upthegrove et al., 2014; Falcone et al., 2015; Goldsmith et al., 2016; Lesh et al, 2018; Di Nicola et al., 2013; Zhu et al.,2018	Balõtšev et al. 2017; Zhu et al., 2018	Potvin et al., 2008
IL-6	Potvin et al, 2008;Miller et al., 2011; Goldsmith et al., 2018; Frydecka et al.,2018;Lesh et al., 2018		Hope et al., 2009; Wei et al.,2018
IL-4	Eftekharian et al. 2018; Falcone et al., 2015	Goldsmith et al., 2016; Balõtšev et al. 2017; Noto et al. 2015	Upthegrove et al, 2014; Potvin et al., 2008; Wei et al., 2018; De Witte et al, 2014; Kartalci et al., 2016
IL-10	Noto et al., 2007, 2015, 2019; Fu et al., 2019	Goldsmith et al., 2016	Di Nicola et al., 2013; Petrikis et al., 2015; Boerrigter et al.,2017; Dahan et al, 2018
TGF-β	Goldsmith et al., 2016; Ergün S., 2018	Kartalci et al., 2016	Kapelski et al., 2016
IL-17	Ding et al., 2014;Li et al., 2016; Yu-Ting et al.,2017;Chenniappan et al. 2020	Dimitrov et al. 2013; Borovcanin et al., 2012	Fang et al, 2018

FIGURE 2 | Immune cells and related cytokines. Immune cells and related cytokines that modulate immunity. Level variation of most investigated cytokines implicated in schizophrenia. IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

and antipsychotic treatments. Thus, in light of significant heterogeneity across studies, due to patient numbers, different methods of cytokine measurements, use of different diagnostic systems and factors such as age, gender, and smoking habits, concomitant infectious, endocrine, or cardiovascular diseases and obesity, results are highly heterogeneous and report elevated, decreased, as well as unaltered levels of cytokines. Hence, they must be interpreted with caution (**Figure 2**).

Numerous cytokines, particularly IL-1β, IL-6, IL-18, and TNF-α, are released from microglia and astrocytes and can be quantified in brain, CSF, and plasma. Whereas the CNS is generally considered sheltered from the peripheral immune system by the blood-brain barrier (BBB), serum cytokines can reach the CNS under normal physiological conditions; for example, by saturable transport (34, 35) or BBB damage (36) or *via* the circumventricular organs that lack a classical BBB (37), and thus a peripheral contribution to CSF cytokine levels should not be excluded.

CNS Cytokines

Largely due to the lack of a matched control group of healthy volunteers, relatively few studies have analyzed differences in the level of cytokines within CSF, and only a restricted number of cytokines have been evaluated. In the CSF of patients with drug-naïve schizophrenia, levels of IL-1β were found elevated (38), whereas levels of IL-6 and IL-8 were reliably detectable and unchanged, and levels of IL-2, IL-4, IL-5, IL-10, granulocyte–macrophage-colony stimulating factor, IFN-γ, and TNF-α were at the level of detection and appeared unchanged. In contrast, levels of IL-1β were found reduced in the CSF and serum of treated schizophrenic patients, compared with healthy controls, IL-2, IL-6, and TNF-α were determined unchanged, and soluble IL-2 receptor was found decreased in CSF and highly elevated in serum Barak et al. (39).

Although the exact role of cytokines in schizophrenia and the correlation between clinical status, disease duration, symptom gravity, and cytokine remain to be fully clarified, evaluation of their level variations in CSF and plasma offers further support

to an immunological component in schizophrenia pathogenesis (**Table 2**).

TABLE 2 | Relationship between levels of key cytokines and severity of clinical symptoms.

High	
IL-6 and IL-4	Longer disease duration
IL-6 and IL-1β	More severe positive symptoms
IL-6, TNF-α, IL-1β, IFN-γ, IL-4, TGF-β	Exacerbated negative symptoms
IL-6, IL-17, TGF-β	Increased PANSS score
IL-6	Worse cognitive abilities
Low	
IL-2, IL-17	Exacerbated negative symptoms
TNF-α and IL-10	Worse cognitive abilities

IL-6

IL-6 can be considered a "state marker" of schizophrenia. In this regard, the levels of IL-6 and sIL-6R are elevated in the serum of patients with schizophrenia (40, 41). IL-6 is reported raised in subjects with at-risk mental state (ARMS) and might be a marker of transition from ARMS to schizophrenia (42). IL-6 levels are reported to be high in first-episode psychosis and acutely relapsed patients and to normalize with antipsychotic treatment. An association between treatment-resistant schizophrenia and an elevated level of IL-6 has been described (43) and between the IL-6 level and illness duration (44). Increased IL-6 levels in the CSF of schizophrenia patients in a study by Sasayama et al. further supports the occurrence of inflammatory activity in the CNS in schizophrenia (45). Recently, Arabska et al. (46) reported that serum levels of IL-6 and TNF-α in schizophrenia patients were not significantly different with respect to healthy subjects. Significantly higher plasma levels of IL-6, IL-10, and TNF-α were, however, detected in schizophrenia patients treated with olanzapine or clozapine, as compared with normal controls (47).

IL-β

Several studies have suggested that IL-1β plays an important role in the etiology and pathophysiology of schizophrenia. Although studies investigating peripheral levels of IL-1β in schizophrenic patients have provided largely inconsistent results, Gilmore et al. proposed the involvement of IL-1β in the possible link between prenatal exposure to infection and schizophrenia (48). An increased release of IL-1β by peripheral monocytes before treatment, and then normalization by antipsychotic medication, has been described in patients with schizophrenia (49). Barak et al. (39) detected significantly lower IL-1β in CSF, other than in serum, of schizophrenic patients compared with controls. In contrast with these results, Söderlund et al. reported that CSF IL-1β concentrations were markedly elevated and hypothesized the activation of the brain immune system in first-episode schizophrenia (38). They suggested that the increase of IL-1β may be normalized or downregulated along the disease course or during prolonged antipsychotic treatment. Thus, whether or not an increase of IL-1β is congenital, acquired during the prodromal phase or absent until the time of first psychotic episode has not yet been clarified. Acute schizophrenics are reported to have significantly elevated IL-1β concentrations in serum, without correlation with age or duration of illness (50). However, serum levels of IL-1β, corrected for age, gender, body mass index, and smoking have been described as no different in patients with schizophrenia and controls and among subtypes of schizophrenia (51). Schizophrenia has been attributed to a dysfunction of brain dopaminergic and glutamatergic circuits, and it is known that IL-1β can induce the conversion of rat mesencephalic progenitor cells into a dopaminergic phenotype (52). In relation to glutamate neurotransmission, the action of IL-1β may include both excitatory and inhibitory components, potentially acting on intercellular brain signaling or altering the expression of genes encoding the enzymes regulating glutamate neurotransmission. However, whether or not the activation of IL-1β is causally related to the development of schizophrenia or is a consequence of associated dopaminergic/glutamatergic dysfunction remains to be established.

IL-2

Several studies have described altered peripheral levels of IL-2 when compared with healthy controls, as well as a reduction in IL-2 by leukocytes after mitogen stimulation, and such studies also reported that higher levels of IL-2 were indicative of less bad symptomatology and a better cognitive performance. If true, IL-2 may have a key role in the pathophysiology of schizophrenia (53–55).

IL-2R, a key signaling component expressed on T lymphocytes, has been reported to be over-expressed in schizophrenia patients (56), and soluble IL-2R levels are described to be increased in both treatment-naive and treatment-free patients with schizophrenia, as well as in acute and chronic disease patients (57, 58). A study by Bresee and Rapaport (59) suggests that serum-soluble IL-2R levels may represent a biomarker for patients with treatment-resistant psychosis, and a positive correlation between the severity of symptoms and the IL-2R underlines its involvement in schizophrenia.

IL-8

Other than IL-2 and IL-6, serum levels of IL-8 have also been described as increased in patients with schizophrenia, and correlations between serum IL-2 or IL-8 concentrations at baseline and the therapeutic outcome have been reported (60). Serum levels of IL-8 was statistically significantly elevated in patients with the diagnosis of paranoid schizophrenia, as compared with the control group (61, 62). Such an IL-8 increase may be related to the activation of monocytes and macrophages, as IL-1 and TNF-α released from these cells have been shown to be elevated in schizophrenia. IL-8 expression has also been reported to be significantly increased in the brain tissue of schizophrenia patients, compared with controls, as evaluated by qPCR and immunohistochemistry assay, and confirmed by western blotting. Such elevated levels of IL-8, IL-6, and TNF-α imply that schizophrenia might represent an autoimmune neuropsychiatric spectrum disorder that may emerge during an autoimmune CNS disease (63). Furthermore, there may be

connections between the overexpression of IL-8 in patients with schizophrenia and cancer (64). Indeed, an elevation of cytokines may provide insight as to why many patients jointly may have schizophrenia and autoimmune diseases (65, 66).

IL-3

Genetic studies have shown that the IL-3 and IL-3 receptor alpha subunit (IL-3RA) genes are located near genetic markers associated with schizophrenia (67, 68). With regard to IL-3, studies have reported abnormal levels in chronic schizophrenia patients (69–71). Fu et al. reported that IL-3 levels were significantly decreased in first-episode drug naïve (FEDN) schizophrenic patients, with respect to healthy control subjects and chronic treated schizophrenic patients (72). Additionally, and consistent with previous studies showing significantly higher levels of IL-3 and IL-3-like activity (IL-3-LA) released by peripheral blood mononuclear cells in chronic-medicated patients, IL-3 levels were significantly higher in schizophrenic chronic medicated patients compared with control subjects (70, 71). Such higher IL-3 levels may be associated with disease progression as well as with antipsychotic treatment. A significantly positive association between the Positive and Negative Syndrome Scale (PANSS) general psychopathology subscore and IL-3 was also described in chronic medicated patients with schizophrenia. In contrast, no significant association between IL-3 and any clinical psychopathology in FEDN patients with schizophrenia was observed. As a consequence, it has been speculated that reduced IL-3 levels in FEDN patients might be associated with both neuronal apoptosis and abnormal early development of the CNS, which may potentially be implicated in the pathogenesis of schizophrenia.

IL-10

Activated macrophages, regulatory T cells, Th2 lymphocytes, and Th3 cells involved in mucosal immunity and protection produce IL-10 (33) that can potentially inhibit the expression of Th1-related cytokines, such as IFN-γ, IL-2, and TNF-α to, thereby, dampen the immune and inflammatory response. Previous studies have found that IL-10 and IL-10 receptors are synthesized in the brain, including by microglia and astrocyte (73). Thus, these may be considered to be an important modulator of the inflammatory response within the CNS (74). Studies performed to understand the relationship between IL-10 and schizophrenia have provided contradictory results. In some studies, an elevation in IL-10 serum levels was detected in patients with schizophrenia (75–78) but other studies failed to replicate this (79, 80). In yet other studies, a decline in serum IL-10 levels was observed in paranoid schizophrenia (62), or in patients with schizophrenia at late stage (81), and no significant differences were detected in IL-10 gene expression levels in peripheral blood cell samples obtained from patients with schizophrenia vs. healthy controls (82). In schizophrenia, elevated peripheral IL-10 levels were associated with the loss of microstructural white matter integrity, supporting the opinion that inflammation is associated with schizophrenia playing a key role in the pathology of microstructural white matter (72).

IL-12 and IL-23

IL-12 and IL-23 are generated by inflammatory myeloid cells and influence the development of Th1 and Th17 cell responses. IL-12p40 is a component of IL-12 and IL-23 and is induced in excess over the other subunits of IL-12 and IL-23. Plasma levels of IL-12 were investigated in patients with schizophrenia and, likewise, have produced contradictory results (83, 84). A putative role for IL-12p40 as a potential marker in schizophrenia has been proposed, and it has been suggested that its dysregulation may be involved in the pathogenesis of the disorder (85).

IL-17 and TGF-β

Th17 cells are known to generate the pro-inflammatory cytokine IL-17, which has been implicated across various immune and inflammatory processes (86). Interestingly, recent studies have shown that Th3 and Th17 cells are activated in schizophrenia, and that treatment with antipsychotics mitigated such activation (86, 87). Th3 cells exert their action primarily by secreting transforming growth factor beta-1 (TGF-β1) that plays an immune regulation role by exerting potent anti-inflammatory and immunosuppressive effects to dampen pro-inflammatory cytokine synthesis, natural killer cell activity, and growth of T and B cells. However, TGF-β can, under appropriate conditions, provide proinflammatory functions through its stimulatory effects on inflammatory Th17 cells and, in addition to IL-6, can stimulate IL-17 production. In this regard, it is notable that schizophrenia has been associated with the enhanced expression of TGF-β receptors (88) and the release of TGF-β (87). Data from the literature remains controversial, however. A significant elevation in TGF-β serum levels has been reported in patients with schizophrenia during relapse and first-episode psychosis, as compared with a control group, suggesting that TGF-β may provide a marker for acute exacerbation of schizophrenia (33). However, this has not been confirmed in other studies that could not detect differences between patients with schizophrenia and controls (89). TGF-β signaling has been associated with schizophrenia, as suggested by data from a pathway analysis of a genome wide association study (90). A hyperactivity of TGF-β signaling pathways in schizophrenia may represent a neuroprotective mechanism (91) by promoting the survival of midbrain dopaminergic neurons (92), and increasing neurogenesis within the subventricular zone (93).

A hypothesized role of an IL-17 pathway inbalance in schizophrenia has been strengthened by a study that found an increased activation of Th17 cells in patients with recent onset schizophrenia (94). Borovocanin et al. reported decreased levels of IL-17 in schizophrenia patients (95), and found a significant decrease in Th17 cells. Recently, the percentage of IL-17-producing lymphocytes in peripheral blood of patients with stable schizophrenia and the possible correlation of IL-17 systemic levels with proinflammatory cytokines and cognitive scores was analyzed by Borovocanin et al. (96).

A trend toward a decline in plasma levels of IL-17 was reported by Ding et al. in first-episode schizophrenia patients after 4 weeks of risperidone treatment (86), and Diitrov et al. described a significant reduction in IL-17 levels in chronic schizophrenia patients, as compared with controls (97). In contrast, an

increased production of IL-17 following antipsychotic treatment was reported by Himmerich et al. (98). Hence, results on IL-17 in psychosis may be affected by antipsychotic treatment. A recent meta-analysis with drug-naïve first-episode psychosis and healthy control subjects evidenced the absence of significant differences of IL-17 levels between these two groups, suggesting that IL-17 may not be involved in the pathological mechanism underpinning schizophrenia (99) albeit this conclusion derives strictly from the analysis of only drug-naïve FEP patients.

IL-18

IL-18, a member of the IL-1 family of proinflammatory cytokines, plays an important role in the Th1 response (100) and, hence, its abnormality in schizophrenia would support the activated macrophage theory. In this regard, IL-18 reppresent a link between the immune and nervous systems (101, 102). As IL-18 and its CNS receptors mediate brain neuroinflammation, modulating homeostasis and behavior (102), several studies have been conducted to clarify the involvement of IL-18 in schizophrenia.

Elevated levels of IL-18 were detected in serum of schizophrenic patients by Tanaka et al. (100), possibly as a result of high levels of constitutively or lipopolysaccharide (LPS)-induced IL-18 released by peripheral blood mononuclear cells of schizophrenic patients, as compared with healthy controls (103). Significantly higher IL-18 levels were detected in the serum of chronic schizophrenia patients, with respect to first-episode patients and normal controls, by Xiu et al. (104). Additionally, significant positive associations between IL-18 and the PANSS were observed in chronic patients, whereas no significant association was found between IL-18 and clinical psychopathology in FEP patients. Finally, in chronic patients, there were no differences in IL-18 levels between patients treated with typical or atypical antipsychotics or with dose or duration of treatment (104). The observed elevated IL-18 levels further support the previously described link between autoimmunity and schizophrenia, which also is consistent with the macrophage and T lymphocyte theory.

Genes, such as IL-18, IL-18BP, IL-18R1, IL-18RAP, IL-12B, and IL-12, which mediate IL-18 functions were analyzed by a pathway-oriented approach in schizophrenic patients. Five single nucleotide polymorphisms (SNPs) in four genes were associated with schizophrenia (105). The most prominent among these was rs2272127 at IL-18RAP, which is also associated with herpes simplex virus 1 (HSV1) seropositivity in patients.

Although the IL-18 signaling pathway remains to be fully clarified, its potential role in the pathophysiology of schizophrenia remains speculative rather than proven (106).

IFN-γ

As IL-18 acts on the immune system—inducing IFN-γ production from Th1 and NK cells—and as IFN-γ is, in turn, the major activating cytokine of macrophages, the involvement of IFN-γ in schizophrenia has also been studied. In this regard, IFN-γ elevations have been frequently noted in patients with schizophrenia and, above all, in first-episode schizophrenia. A correlation has been observed between the spontaneous production of IFN-γ and Positive and Negative Syndrome Scale G subscore. Additionally, a relationship between IFN-γ and the percent whole-brain gray matter suggests that it contributes to the pathophysiology in schizophrenia. Furthermore, a significant reduction of IFN-γ expression in peripheral blood mononuclear cells obtained from schizophrenic patients is in accord with a deficiency in the Th1-dependent immune response as well as with reduced IFN-γ protein levels, as described by Arolt et al. in treated patients (107). Notably, Kim et al. reported that antipsychotic treatment normalized elevated IFN-γ levels (108). Finally, the study by Jemli et al. (109), in part, validates the hypothesis of excessive proinflammatory cytokines in the physiopathology of schizophrenia.

CYTOKINE POLYMORPHISMS IN SCHIZOPHRENIA

It has been proposed that a genetic predisposition for schizophrenia has a multifactorial character. While the qualitative and quantitative evaluation of cytokines in biological fluids can provide functional evidence related to disease manifestation, genetic investigations can provide insight into the biological mechanisms underlying susceptibility to disease. Therefore, several studies have been performed to investigate associations between SNPs located within genes encoding cytokines implicated in schizophrenia.

Allelic or genotypic association between the IL-1β C-511T polymorphism and schizophrenia was reported by case-control as well as family studies (110, 111). Other studies have reported no significant association between the IL-1β C-511T or IL-1β C3954T SNP and schizophrenia (112), which disagrees with a meta-analysis in a specific population that reported a moderate association of IL-1β C-511T and C3954T polymorphisms with schizophrenia in Caucasian samples, suggesting that the genetic effect of IL-1β on schizophrenia may be ethnicity dependent (113). The association between the IL-1β GTCC haplotypes G-31A, C-511T, C-1473T, and C-373T and IL-1β C-373T SNP (rs4848306) and schizophrenia in the Polish population was observed by Kapelski et al. (114). Furthermore, schizophrenia in a Japanese population study was significantly associated with IL-1β SNPs (C-373T, C-1473T, and C-511T) (115). Additionally, a study by Sasayama et al. in the Japanese population found an association between the IL-1β A5810G SNP and susceptibility to schizophrenia (116).

Several studies have reported an association between the −174G/C polymorphism in the IL-6 gene and schizophrenia that influences disease risk (117, 118). The study of Paul-Samojedny et al. showed a trend toward a significant difference in genotype distribution and allele frequency between paranoid schizophrenia patients and healthy controls (119), but this was not confirmed in other studies (120–122). The study by Zakharyan et al. demonstrated that the IL-6 −174G/C polymorphism is associated with increased plasma IL-6 in schizophrenia patients and constitutes a risk factor for the disorder (118), and a study by Frydecka et al. indicated that elevated IL-6 in schizophrenia patients is not due to genetic variation (123). However, the IL-6 −174G/C polymorphism may affect the severity of positive symptoms and

cognitive impairments observed in schizophrenia and might be the consequence of IL-6 serum levels, thereby, highlighting that schizophrenia is a progressive disorder with low-grade inflammation, essential for its pathophysiology that is then followed by cognitive decline.

The study of Sun et al. reported a signicant association of rs2228145 C allele (Ala allele) of IL-6r with schizophrenia (124). In contrast, Liu et al. found that genetic variants of IL-6 and its receptor are not associated with schizophrenia in Taiwan patients (122). Recently, Kapelski et al. (114) have evaluated the association of functional polymorphisms in several interleukins and interleukin receptor genes and have reported a signicant association of rs2228145 and rs4537545 with schizophrenia. Rafiq et al. (125) found that a common variant of the IL-6r gene increases IL-6r and IL-6 levels, in accord with results of Chan et al. (126) showing that individuals that are homozygous for the minor allele of rs4537545 and its exome SNP exm-rs4537545 had the highest IL-6r levels, as compared with individuals homozygous for the major allele, which had the lowest IL-6r levels. They also reported that SNP, rs7553796 located within the IL-6r gene was associated with increasing levels of the IL-6r protein in blood in homozygous schizophrenia patients.

The association between schizophrenia and IL-8 rs4073, rs2227306, and rs1126647 nucleotide variations was evaluated in a Tunisian population. The rs1126647 polymorphism showed a risk for the development of schizophrenia with a T allele and TT genotype, in particular in the paranoid subtype of schizophrenia. In addition, the rs1126647 SNP appears to predispose more particularly females to the paranoid form. The rare haplotypes TTT, ACT, and TCT haplotypes at rs4073-rs2227306-rs1126647, each comprising the risk allele rs1126647T, were related to the higher risk of paranoid schizophrenia, whereas only the TCT arrangement constitutes a risk factor for general schizophrenia (127).

Sun et al. have reported that IL-10 gene polymorphism is associated with reduced IL-10 production as well as with the susceptibility and the incidence of schizophrenia (128). IL-10 rs1800896, a SNP, is located upstream of the IL-10 gene. The A allele of rs1800896 reduces the production of IL-10, and the known antioxidant actions of IL-10 (129) would therefore be expected to be reduced, which may make long-term treated patients on antipsychotics more vulnerable to the development of tardive dyskinesia (130). Additionally, it has recently been reported that IL-10-592A/C (rs1800872) polymorphisms interact with catechol-o-methyltransferase Val158Met (rs4680) polymorphisms to detrimentally impact cognitive function in schizophrenic patients (131), which is important in the light of cognitive deficits being core symptoms of schizophrenia (132, 133).

The study of Liu et al. (134) evaluated two single promoter polymorphisms, 137 G/C (rs187238) and 607 C/A (rs1946518), of IL-18 in the light of prior studies indicating their potential association with immune-related diseases, including Crohn's disease, diabetes, rheumatoid arthritis, and potentially also Alzheimer's disease, and found that they were not associated with schizophrenia in a Chinese population.

The study of Suchanek-Raif et al. **(author?)** (135) evaluated the potential association between four TNF-α single nucleotide polymorphisms [1031 T/C (rs1799964), 863 C/A (rs1800630), 857 C/T (rs1799724), and 308 G/A (rs1800629)] and schizophrenia in a Caucasian population. Results showed that 1031 T/C, 863 C/A, and 308 G/A are associated with schizophrenia, sex specifically. In fact, high risk of schizophrenia for men is associated with the C/C genotype of 863 G/A SNP. For the −308 G/A SNP, the G/A genotype and A allele were associated with a risk of schizophrenia in women. In contrast, an evaluation of 1031 T/C, 863 C/A, 857 C/T, and 308 G/A SNPs in Caucasian patients with schizophrenia, performed by Tan et al. (136), did not show any significant differences in the SNPs evaluated. Other GWA studies have not found the association between (1031 T/C and 308 G/A) promoter polymorphisms in the TNF-α gene and schizophrenia risk (137). Xiu et al. (138) have suggested an important role for cytokine signaling in mediating the severity of cognitive dysfunction in schizophrenia and reported that the TNF-α 21031T/C polymorphism may not play a role in the predisposition of schizophrenia itself but may be implicated in the cognitive deficits of schizophrenia. In a case-control study, Suchanek-Raif et al. (139) demonstrated that, for the TNFR2 gene, the genetic variants of rs3397, rs1061622, and rs1061624 are linked with a higher risk of developing schizophrenia and a more severe course in men. In contrast, the genotypes with a polymorphic allele for rs3397 SNP appear protective for women. Furthermore, the rs1061624 SNP, in families of people with schizophrenia, might modulate the beginning of the disease process.

Studies aimed at investigating the association between schizophrenia and polymorphisms in genes encoding cytokines shown that the TGF-β1+869T>C gene polymorphism was associated with schizophrenia, especially in females in the context of a TGF-β and estradiol interaction, and the risk of schizophrenia was more than twofold higher in carriers of a T allele (CT+TT genotypes), in comparison with individuals with a CC genotype (140). Also, a significant association between the +874A/T polymorphism of IFN-γ and paranoid schizophrenia has been described, and the minor allele of this polymorphism was correlated with an increased expression of IFN-γ (109). On chromosome 5q, several schizophrenia-related SNPs and haplotypes both in and near the IL-3 cytokine gene have been identified (141). Finally, with the knowledge that T cell networks have consistently been linked to the pathogenesis of schizophrenia, the functional role of the Th17 pathway in schizophrenia was assessed and involved evaluation of the G197A single nucleotide polymorphism associated with production of IL-17A. No association between the IL-17A gene polymorphism and schizophrenia was noted (142).

ANTIPSYCHOTIC TREATMENT AND CYTOKINES

Antipsychotic therapy is indispensable in the treatment of schizophrenia. However, phenotypic traits related to drug

response, such as responders, delayed responders, or resistant patients, are potentially endophenotypes associated with cytokine profile aberrations. Spreading and/or differentiation of monocytes, T cells and B cells can be modulated by antipsychotic treatment, as well as by a cytokines' and cytokine receptors' gene expression (143, 144). Antipsychotic treatment has been reported to reduce levels of IL-4, IL-6, IL-17, and IL-27 in First Episode Psychosis (FEP) patients (145). In addition, in schizophrenic patients receiving therapy with risperidone, olanzapine, or clozapine, the basal and LPS-induced production of RANTES and IL-18 has reported to be increased (103). A meta-analysis on the effects of antipsychotic treatment evidenced a reduction of select pro- and anti-inflammatory cytokines, such as IL-1β, IL-6, TNF-α, IFN-γ, and TGF-β (33). In a different study, treatment with olanzapine and risperidone reduced serum levels of the anti-inflammatory cytokines IL-1RA and IL-10, while not substantially affecting the expression of seven other evaluated cytokines (146). A study by Song et al. (147) reported that in drug-naïve first-episode schizophrenia, IL-1β levels measured time dependently during 6 months of risperidone treatment decreased initially but then rose gradually over time, and IL-6 and TNF-α levels changed significantly over the treatment course but eventually returned to baseline levels after 6 months. This suggests that risperidone treatment initially provided an anti-inflammatory effect that declined with treatment, possibly due to a drug-induced weight gain side effect. In further studies, still, the levels of anti-inflammatory cytokine IL-10 in peripheral blood were increased by treatment with risperidone (148) and chlorpromazine (149).

In vitro studies, likewise, suggest that antipsychotics can potentially modulate the inflammatory response. Haloperidol, a typical antipsychotic, has been reported to normalize increased IL-2 levels (60) and inhibit mitogen-stimulated IL-2 production (150, 151). In LPS-stimulated peripheral blood mononuclear cells, IL-4 and IL-10 levels were increased by haloperidol, and conflicting results have been found in relation to the production of IFN-γ, which in cell cultures may rise (152) or decline—depending on the study conditions (150, 151). Clozapine, risperidone, and quetiapine are common atypical antipsychotics. Quetiapine increased IL-4 levels in LPS-stimulated peripheral blood mononuclear cells, as well as IL-10 production in poly(I:C)-stimulated peripheral blood mononuclear cell cultures—which clozapine and risperidone treatment also significantly increased. All the antipsychotics reduced IFN-γ levels significantly in LPS- and poly(I:C)-stimulated cultures (153). In yet other studies, clozapine has been reported to modulate IL-2, IL-2R, TNF-α and soluble TNF receptors p55 and p75 (154, 155). In schizophrenic patients, risperidone treatment lowered plasma levels of the proinflammatory cytokines TNF-α and IL-6, increased anti-inflammatory cytokine IL-10, and did not impact IL-4 concentrations (156, 157). In closure, a study by Erbağci et al. (51) demonstrated that cytokine concentrations in patients responsive and non-responsive to risperidone were not significantly different.

CYTOKINE-BASED THERAPEUTIC APPROACHES

In general, the current treatment of schizophrenia involves the use of antipsychotics as the first line of therapy (158). This is often followed by non-steroidal anti-inflammatory drugs (NSAIDS), in addition to vitamins and herbal products targeting the immune system and the inflammatory processes (159). It is known that the inflammatory phenotype should be defined and delineated at the individual level both for research and clinical purposes, in order to evaluate an individual treatment for a patient. A recent meta-analysis study has described the effects of antipsychotics and NSAIDS therapy on cytokine levels, with a reduction in the expression levels of proinflammatory ones, such as IL-18, IL-1β, IL-6, and IL-8 (160, 161).

The wide range of pathological phenotypes underlying schizophrenia and the high possibility of adverse effects during medication supports the need to study new treatment strategies that are currently under investigation. Antibody immunotherapy, tetracycline antibiotics, antirheumatic drugs, neurosteroids, antioxidants, and statins have been reported as possible treatment strategies (159). Hence, in this regard, it is interesting to evaluate the literature as to whether or not they may impact cytokines and cytokine receptors. Clinical trials to evaluate the effects of cytokine modulators have to be careful when considering the pleiotropic and manifold activity of the cytokines (160). Although not yet studied in schizophrenia, some monoclonal antibodies directed toward cytokines have been identified and are either clinically available or in the experimental phase of drug development. In particular, IL-6, TNF-α, and IFN-γ might represent new therapeutic targets.

IL-6 as a Potential Target

IL-6 is implicated in the development, onset, and progression of several mental disorders and may also be involved in treatment responses. As described by Potvin et al. levels of IL-6 appear to be increased in patients with schizophrenia, both in *in vivo* and *in vitro* models (32). As reported by Behrens et al. (162), it is established that schizophrenic patients have reduced CNS antioxidant defenses, with notable declines in glutathione, a tripeptide that is found in high concentrations in most cells and that is critical to the detoxification of reactive oxygen species and numerous other radicals. Glutathione has been found to be reduced (−27%) in the CSF from drug-naïve patients with schizophrenia, in postmortem studies (−41%) of patients with schizophrenia vs. normal control subjects, and polymorphisms in the genes for key components of glutathione generation [glutamate cysteine ligase modifier subunit (gclm) and the catalytic subunit for glutamate cysteinen ligase (gclc)] are linked to the risk for schizophrenia. Using a subanesthetic ketamine model of schizophrenia in rodents, Behrens et al. demonstrated that IL-6 induces a loss of parvalbumin interneurons that results in a deficiency in inhibitory circuits and the development of a schizophrenic phenotype (162). Other studies have demonstrated that aberrations in parvalbumin interneurons are implicated in schizophrenia and that elevated IL-6 levels in CNS disrupt

working memory. Based across this information, clinical trials using a humanized anti-IL-6 receptor monoclonal antibody, Tocilizumab developed and approved for rheumatoid arthritis, were undertaken in five patients with schizophrenia, treated for 4 weeks in an open-label trial. Results showed no significant changes in psychopathology scores, but an improvement in cognition, based on Brief Assessment of Cognition in Schizophrenia (BACS) (163). A more recent randomized, double-blind clinical trial of Tocilizumab was published in 36 clinically stable, moderately symptomatic schizophrenic patients (PANSS >60). The drug was well-tolerated; however, there were no significant effects on positive and negative symptoms and cognitive deficits (164). These somewhat disappointing results may reflect the poor brain penetration of Tocilizumab as well as the schizophrenic patient population evaluated, as greatest elevations in IL-6 levels are generally found in first-episode and acute relapsed subjects, rather than in chronically treated schizophrenia patients—as were evaluated in the Tocilizumab trial.

The anti-IL-6 mAbs, Sirukumab and Siltuximab, have been shown to be effective in reducing depressive symptom severity in patients with rheumatoid arthritis and multicentric Castleman disease (165). In this light, they hold promise in subjects with major depressive disorder and, although their actions on CNS cytokine levels remain to be elucidated, they may be worth evaluating in schizophrenics patients too (166–168).

TNF-α as a Potential Target

Beyond IL-6, TNF-α is associated with schizophrenia-negative outcome, and thus can be considered a novel drug target for schizophrenia therapy. Antibodies targeting TNF-α, Infliximab, and Golimumab, have been investigated in several clinical trials in patients with major depression. Infliximab activity has been explored by evaluating inflammatory markers (169), sleep parameters (170), and gene expression signatures (171), and these studies supported a depressant response reduction. Bekhbat et al. measured the Infliximab-induced antidepressant response in 26 patients with treatment-resistant depression and demonstrated that subjects with high inflammation and elevated levels of lipids and cholesterols were more responsive to the antidepressant actions of Infliximab. These results support a role of inflammation as a major driver of metabolic dysfunction in patients with depression and provide a framework for the evaluation of TNF-α blocking strategies in schizophrenia (172).

IFN-γ as a Potential Target

IFN-γ has also been considered a target for cytokine-based immunotherapy in schizophrenic patients. In the light of studies reporting that serum levels of IFN-γ and *in vitro* IFN-γ production after stimulation were lower in samples obtained from unmedicated schizophrenia patients vs. healthy controls (173, 174), a pilot study was undetaken in two treatment-resistant schizophrenia patients in which IFN-γ-1b was administered subcutaneously and psychopathology was assessed weekly with the PANSS (175). In the face of numerous caveats in this small open-label trial, both patients showed a marked clinical improvement, with impressive declines in PANSS total score and good tolerability of the treatment over the relatively short study (6–7 weeks) (175).

IL-12/IL-23 as Potential Targets

Evaluation of the antidepressant activity of immunomodulatory drugs has also involved investigation of anti-IL-12/IL-23 antibodies. As reported in an interventional clinical trial on psoriatic patients, Ustekinumab treatment (that binds IL-12 and IL-23) for 12 weeks, decreased not only psoriasis symptoms but also depression symptoms, augmenting quality of life (176).

Anticytokine-Targetted Therapy Synopsis

Collectively, clinical trials with anticytokine therapy have, thus far, been chiefly performed in major depression disorders or in patients afflicted with physical illnesses, such as psoriasis and rheumatoid arthritis (i.e., conditions for which the drugs were primarily developed), and only two studies directly relate to schizophrenic patients, due—in large part—to the ethical limitations of treating patients except as an adjuvant therapy. Results obtained from studies, to date, generally show an improvement in depressive symptoms in the case of adjuvant treatment and with reasonable tolerability, suggesting the possibility of future clinical evaluation in schizophrenic patients. This approach hence represents a promising avenue that warrants further preclinical and clinical research. However, it is necessary to clarify how immunotherapies can best combine with and/or replace available antidepressant treatments, to better define a personalized therapy to reduce side effects and improve the quality of life of patients.

CONCLUSION

Cytokines are ubiquitous molecules that act as key messengers for and between immune cells and help to maintain a delicate and intricate balance in the immune system and optimize the continuous cross-talk between the CNS and periphery. In untreated schizophrenia patients, a Th1/Th2 imbalance, with dampened levels of Th1-related cytokines and compensatory elevated Th2-cytokine levels, has been consistently observed—suggesting that the inflammatory process is aberrant and plays a central role in the pathophysiology of schizophrenia. Studies documenting that cytokines are potent rate-limiting signals and that some anticytokine therapies have had promising clinical success open new perspectives on the therapeutic capacity of targeting cytokines/cytokine receptors in neurological disorders. Like other prior promising treatments, cytokine/cytokine receptor-focused therapeutic approaches are not a "magic bullet" capable of improving every individual. Techniques will be required to rationally select out potential responders based on sound scientific principles, which too will require evaluation. In this regard, non-invasive blood sampling, for example, can readily provide the small sample amounts needed to evaluate blood levels of cytokines and represent an attractive way to identify and quantify disease biomarkers. From these same blood samples, extracellular vesicles (also known as exosomes) can be collected and enriched for neuronal and astrocytic origin to provide a window to cytokine/cytokine

receptor levels in brain (177–179). Although much research remains to be undertaken, cytokine-based therapies have hugely impacted our understanding and treatment of a broad range of autoimmune disorders and cancers (180) and are among the current top 20 selling drugs worldwide (181). Their pleiotropic actions in brain and potential utility in neurological disorders are only relatively recently being seriously considered. In this regard, identifying the cytokine profiles that precede a psychotic episode could direct strategies for relapse prevention. In addition, biological markers measured during the prodromal phase could have clinical importance in determining diagnosis, further treatment strategies, and prediction of disease progression and treatment response. We have much to gain from better understanding how cytokine imbalances can impact schizophrenia as well as other mental disorders for which current medication is largely inadequate and new treatment strategies are sorely needed.

AUTHOR CONTRIBUTIONS

MR and EC designed the work, conducted the literature review, and wrote an initial draft of the article. NG participated in discussions and wrote and revised the manuscript. All authors have revised and approved the final manuscript.

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OPINION
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False Dogmas in Schizophrenia Research: Toward the Reification of Pathway Phenotypes and Pathway Classes

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INTRODUCTION

Conceptualizations of schizophrenia have always been controversial and subject to considerable argument. Many views prevail as to the nature of schizophrenia, including schizophrenia as: a brain disorder; a unitary disease, via diagnosis derived from DSM (American Psychiatric Association, APA) or ICD (World Health Association, WHO) classification systems; being comprised of positive and negative symptoms; an incurable brain disease, with a progressive deterioration that is determined by a dementing or neuroprogressive course. All of these perspectives have been challenged at some time, and none are universally accepted as descriptive of the processes underpinning schizophrenia phenotypes. Our work indicates that none of these conceptualizations of schizophrenia are supported by recent findings, which are detailed below and in **Table 1**. Our work showed that these false conceptualizations of schizophrenia had a negative impact on the development of more accurate diagnostic criteria, new pathways and new drug targets of schizophrenia phenotypes.

Schizophrenia: Not Only a Brain Disorder

A number of influential psychiatrists, including Nancy Andreasen, David Pickar, E. Fuller Torrey, and Robert Yolken, have helped to conceptualize schizophrenia as an incurable brain disease, thereby being similar to classically defined brain diseases, such as multiple sclerosis, Alzheimer's disease and Parkinson's disease. However, just as with such classical brain diseases (4–6), there is a growing realization that schizophrenia pathophysiology is more "holistic" in nature, being intimately linked to systemic processes, especially activation of immune-inflammatory, oxidative and nitrosative stress (IO&NS) pathways and increased gut permeability (2, 3, 7). Consequently, the early developmental pathoetiology of schizophrenia is associated with alterations in the regulation and interactions of systemic processes (8, 9). Furthermore, maternal immune activation as a consequence of bacterial and viral infections may cause schizophrenia-like symptoms in the offspring and these effects are mediated by IO&NS pathways and lowered neuroprotection (10). The first episode of psychosis (FEP) is typically accompanied by a cytokine storm indicative of immune-inflammatory response system (IRS) activation, coupled to an elevated compensatory immune-regulatory response system (CIRS), indicative of a "mixed" immune response (11). Such immune dysregulation may arise from viral/bacterial infections or complement activation (11). Typically, FEP shows a relatively increased IRS/CIRS activation ratio, as do many defined phenotypes including acute episodes, chronic schizophrenia, deficit schizophrenia, and treatment resistant schizophrenia (11, 12), with low-CIRS patients having poorer clinical outcomes (11, 12).

TABLE 1 | False dogmas in schizophrenia research and the way forward.

False dogmas	New findings	The way forward
1. Schizophrenia is a brain disorder	No, it is a systemic disorder with peripheral IO&NS pathways causing the central phenome of schizophrenia.	Measurement of peripheral IO&NS pathways affecting neuronal circuits, as indicated by cognitive deficits in memory and executive functions and (functional) brain imaging techniques.
2. Schizophrenia is a unitary disease	No, there are distinct phenotypes, including deficit vs. non-deficit, partial vs. non-responders to treatment, FES and MES with and without worsening.	Stop publishing findings based on "schizophrenia," as the findings using these case definitions are difficult to interpret. Consider the different pathway phenotypes.
3. The DSM/ICD case definitions of schizophrenia are the gold-standard to diagnose schizophrenia.	No, those case definitions are not only unreliable, but their dogma-like nature also prevents deductive and inductive remodeling of the case-definition.	The DSM/ICD committees should stop making ex-consensus diagnostic criteria and should consider pathway phenotypes and classes cross-validated using machine learning and the nomothetic network approach (1).
4. Schizophrenia is comprised of positive and negative symptoms	No, the symptomatome comprises many more symptom domains and a single latent trait, which reflects severity of the phenotypes, underpins these different manifestations and therefore is the cause of its manifestations.	Consider the different domains in the phenome of the schizophrenia phenotypes, namely psychosis, hostility, excitation, mannerism, negative symptoms, psychomotor retardation, formal thought disorders, affective symptoms, fatigue and physiosomatic symptoms. Extract the first latent vector, which reflects OSOS.
5a. Schizophrenia is accompanied by distinct cognitive impairments.	No, a general factor, which is essentially unidimensional, underpins all those different cognitive impairments.	Compute a general cognitive decline (G-CoDe) score as the first latent vector extracted from different cognitive scores.
5b. Patients with schizophrenia may show a gradual deterioration which may be assessed by a decline in cognitive scores.	No, this deterioration should be defined based on worsening in behavioral, cognitive, physical, and psychosocial domains.	Compute a behavioral-cognitive-physical-psychosocial (BCPS)-worsening score defined as a latent vector extracted from OSOS, G-CoDe, and health-related quality of life assessments, thereby combining all phenome (symptomatome and phenomenome) feature sets into one worsening index.
5c. Schizophrenia is a dementing or neuroprogressive disorder.	No, the findings show a far more complex picture. A serious deficit can be present following a first episode psychosis. FES/MES may present with or without worsening. The worsening in FES/MES, is associated with IO&NS pathways, and severe worsening largely overlaps with the deficit syndrome in MES or FES.	Consider staging pathway phenotypes, namely FES and MES with and without BCPS worsening.
5d. Many patients show complete remission as assessed with remitter case definitions based on scale-derived cut-off values or a number of items on a rating scale score being rated mild or better.	Probably not. Using the adequate machine learning techniques, not one of the partial responders to treatment could be allocated to the normal control class modeled using pathway phenotypes. These partial remitters show residual psychotic symptoms, neurocognitive deficits and active IO&NS pathways.	Complete remission, partial remission and non-remission should be modeled using SIMCA with pathway phenotypes (IO&NS pathways combined with neurocognitive tests) as modeling and discriminatory variables. The case definition of "complete remission" should be based on a SIMCA model, namely authentication of cases as belonging to the model of normal controls constructed using pathways and cognitive tests.
6. The way forward?	The novel bottom-up, data-driven, computer science-derived, nomothetic psychiatric approach as proposed by Maes et al. (2, 3) and Stoyanov and Maes (4).	This approach provides a route toward a novel model of schizophrenia based on all features sets, a new network-based definition of schizophrenia phenotypes and pathway classes. The latent variable scores delineate an idiomatic feature profile, which is unique for each patient and may be employed for individualized treatments targeting the most disordered feature sets of the nomothetic model.

IO&NS, immune-inflammatory and oxidative and nitrosative stress; FES and MES, first and multiple episode schizophrenia; SIMCA, soft independent modeling of class analogy.

Consequences arising from the increased pro-inflammatory cytokines in FEP, include indoleamine 2,3-dioxygenase (IDO) induction, which drives tryptophan down the tryptophan catabolite (TRYCAT) pathway (13, 14). TRYCATs such as xanthurenic acid and picolinic acid, elevated pro-inflammatory cytokines/chemokines (e.g., CCL11), reactive oxygen and

nitrogen species, and LPS (arising from breakdown of the paracellular and vascular gut barriers) are neurotoxic (2, 3). As such, alterations in neurotoxic activity may be intimately linked to changes in central and systemic neuronal and glia activity, thereby contributing to dysregulated neuronal patterning. Consequently, many of the cognitive deficits that can be associated with people classed with schizophrenia may be intimately linked to the interactions of systemic and central processes (2, 3).

Schizophrenia: Not One Disease, but Distinct Phenotypes

Although regarded as a heterogeneous group of conditions (15, 16), the DSM and ICD classification systems consider schizophrenia as a unitary disease comprised of variable subgroups (such as paranoia, undifferentiated), with the subgroups coming and going across various DSM/ICD versions over time. The lack of diagnostic criteria based on biomarkers coupled with clinical features is a general problem for these classification systems (2, 4, 17).

One classical view is that negative symptoms are specific for schizophrenia and especially for deficit schizophrenia, although negative symptoms display a continuous distribution from healthy individuals to patients with deficit schizophrenia (18). Nevertheless, using soft independent modeling of class analogy (SIMCA), stable schizophrenia presentations could be divided into two qualitatively distinct phenotypes, namely deficit and non-deficit schizophrenia (14). SIMCA showed that deficit schizophrenia is defined by IO&NS pathways, neurocognitive deficits in episodic and semantic memory, increased severity of symptoms including psychosis, hostility, excitation, mannerism, negative symptoms (PHEMN), psychomotor retardation, and formal thought disorders, and a lowered quality of life (19). As well as such continuous variable differences, deficit schizophrenia is also associated with more qualitative differences, including lowered natural immunoglobulin (Ig)M responses to oxidative specific epitopes (OSEs), antioxidants and antibacterial defenses (2). Cognitive deficits may be combined with systemic pathway biomarkers into a neuro-immune brain-circuit axis phenotype, which may be employed as a tool for making the diagnosis of deficit schizophrenia (20).

SIMCA also shows that treatment partial responders, vs. non-responders, form qualitatively distinct classes when using cognitive and systemic pathways, with high accuracy (21). Partial, vs. non-responders may also be differentiated by increased IL-6, endogenous opioid system biomarkers and inhibition of the Wnt signaling pathway (21). Schizophrenia phenotypes may also be differentiated by affective symptoms, fatigue and physiosomatic (formerly psychosomatic) symptoms, all of which are associated with immune/cytokine/TRYCAT/O&NS/gut-brain pathways, clearly indicating the achievability of pathway-phenotype differentiation of people classed with schizophrenia phenotypes (22, 23).

The Schizophrenia Case Definitions of DSM and ICD Are Counterproductive

DSM and ICD are also criticized for providing case definitions of schizophrenia that are based on descriptive psychopathology and de-contextualized narratives of the disorder (4), with problems arising from: (1) lumping qualitatively distinct classes (deficit vs. non-deficit; partial vs. non-responders to treatment) into one category; and (2) top-down determination prior to physiological and neurocognitive investigation (4), with any such measures seen as mere concomitant data. As a consequence of the poor pathway-phenotype basis to classification and treatment in people presenting with schizophrenia, DSM/ICD case definitions show poor reliability/validity, and little consistency across DSM/ICD updates (4).

It is incomprehensible that most biological and molecular research reports define these top-down DSM/ICD case definitions as independent variables, whilst using biomarkers and even causome, protectome and cognitome features as dependent variables. Consequently, while causal reasoning indicates that those features are explanatory variables and schizophrenia is a higher-order concept consisting of these features, researchers continue to use inadequate model assumptions, often confounded by the use of inappropriate statistical tests (3, 4). As such, an inappropriate statistical analysis arises from bestowing primacy to DSM/ICD classifications.

Positive and Negative Symptoms: an Inappropriate Division

Another top-down dogma in psychiatry's classical conceptualization of schizophrenia is viewing such presentations as comprised of positive and negative symptoms, defined as the addition and loss of processes and behaviors, such as hallucinations and social isolation, respectively (15, 24–26). However, we have shown that a single latent unidimensional trait underpins such diverse presentations as PHEMN symptoms, psychomotor retardation, and formal thought disorders with good convergent validity, internal consistency reliability, and predictive relevance that follows a reflective model (27, 28). This unidimensional trait, or latent phenomenon, reflects overall severity of schizophrenia (OSOS). With increasing illness severity the quantitative differences in OSOS become more pronounced thereby shaping a qualitatively distinct class (19) especially in patients with a lowered CIRS protection (2).

Neuroprogression or Total Recovery?

Kraepelin's "dementia praecox" has led to a long-standing belief that schizophrenia is a progressively deteriorating disorder. More recent terminology sees this as neuroprogression, indicative of a deterioration through a series of stages (29). However, some findings have always been incompatible with this, indicating that almost complete recovery may not uncommonly occur (30). Nevertheless, the concepts "cognitive decline," "progressive deterioration" and "complete recovery" were never well-defined.

A classical view is that schizophrenia is accompanied by many cognitive impairments including in executive functions, semantic and episodic memory, attention, and spatial working

memory (31). Nevertheless, we have shown that a common core underpins these neurocognitive deficits which should be denoted as "general cognitive decline" (G-CoDe). Our recent work indicates that "progressive deterioration" or "worsening" in schizophrenia phenotypes should be comprised of OSOS, G-CoDe, and psychosocial and general health domains (22). Pathways underpinning the worsening in first episode schizophrenia (FES) include elevations in indicants of paracellular gut and vascular barrier breakdown, with heightened levels of bacterial translocation (perhaps especially *Klebsiella pneumoniae*), complement C1q activation, and lowered antioxidant defenses (22). However, in multiple episode schizophrenia (MES), worsening is predicted by the number of episodes as well as heightened IO&NS pathways (22). The greatest deterioration largely occurs in people who would be classed with deficit schizophrenia. Such data would suggest that there is a readily measurable pathway substrate to the association of 'cognitive, social and general health domains' with worsening in people classed with schizophrenia that may strongly overlap with the biological underpinnings of deficit schizophrenia.

Classically, the case definitions of complete remission are based on scale-derived cut-off values or eight items of the PANNS being rated mild or better (32). Nevertheless, we showed that complete remission should be defined using SIMCA whereby a SIMCA model of healthy controls is constructed based on the neuro-immune biomarker values and cognitive scores of heathy controls (21). Consequently, using SIMCA, cases considered to be apparent responders to treatment may be projected into this SIMCA model and be allocated or not to this healthy control class (21). Cases that are allocated to the control class are "authenticated" as complete responders, whereas rejection to this normal class membership indicates that the patient did not achieve complete remission (21). Importantly, we found that using SIMCA none of the treatment partial responders could be authenticated as belonging to the SIMCA model of heathy controls (21). By inference, none of the treated schizophrenia patients could be considered as a complete remitter (21).

DISCUSSION

As indicated above, the classical dogmas of DSM/ICD classifications, including in the definition of schizophrenia, are widely regarded as inadequate.

1) Schizophrenia is clearly not simply a brain disease but a systemic neuro-immune and neuro-oxidative stress disorder just like other neurologic diseases, including multiple sclerosis, Parkinson's disease and Alzheimer's disease. Breakdown of paracellular and vascular pathways may lead to BBB breakdown thereby interfering with neuronal circuits which underpin the neurocognitive deficits and symptoms of schizophrenia phenotypes.
2) Schizophrenia is not one unitary disease but comprises different qualitatively distinct phenotypes including deficit and non-deficit schizophrenia, partial and non-responders to treatment, MES and FES with and without worsening. The failure to use such schizophrenia phenotypes complicates comparisons across studies, resulting in a plethora of "mixed results" that adds to the confusion and seemingly intractable nature of schizophrenia. Research based on DSM/ICD criteria suffers from false negative (not exposing phenotype-specific pathways) and false positive (a pathway being specific for only one phenotype is generalized to schizophrenia) results.
3) The top-down nature of DSM/ICD case definitions of schizophrenia are not only unreliable, but their dogma-like nature also prevents deductive (as incontrovertible) and inductive (as top-down) remodeling of the case-definition (4). Importantly, these models do not pass Karl Popper's critical rationalism tests, being non-progressive (not based on all available knowledge), unchangeable (ex consensus-based committees decide), and unfalsifiable (top-down manner precludes refutation) (4). The utilization of DSM/ICD criteria is inhibiting a pathway-based understanding and treatment of schizophrenia phenotypes.
4) The classical bidimensional concept of positive and negative symptoms is another dogma that is not supported by the findings. This is intimately intertwined with counterproductive debates as to the dimensional vs. categorical (distinct phenotypes) conceptualizations of schizophrenia (14, 19). "Schizophrenia" comprises different subtypes which are modeled by pathway phenotypes, which increase in severity along a continuum and give rise to qualitatively differences among those phenotypes (2, 14, 19).
5) Conceptualizing schizophrenia-like presentations as having a neuroprogressive or dementing course seems inadequate. A serious deficit can be present following FES and FES/MES may present with no or minimal deterioration (22). Complete remission should be modeled using SIMCA rather than with case definitions based on scale-derived cut-off values or eight items of the PANNS being rated mild or better (32). Based on SIMCA results, we conclude that complete recovery is probably never achieved, as patients show residual psychotic symptoms, neurocognitive deficits and active immune-inflammatory pathways (21). Our findings extend Bleueler's view that patients may return to a normal functioning, albeit with scarring. Of clinical note, patients showing a partial treatment response, irrespective of first or multiple episode(s), still show heightened activation of treatable pathophysiological processes, the recognition and treatment of which may improve the condition.

The Way Forward: Bottom-Up Nomothetic Networks

Psychiatry can only make progress when the gold-standard top-down approach is abandoned and is replaced by the novel bottom-up, data-driven, computer science-derived, nomothetic psychiatric approach (2–4). This approach first builds a theoretical framework which is based on state-of-the-art knowledge and causal reasoning and assembles the building blocks (feature sets) of schizophrenia into one framework, comprising the causome and protectome and their disbalance computed as a risk to resilience ratio (2, 3), the adverse outcome pathways, namely the different pathways that may

cause the illness, the brainome (including the connectome), and the phenome. The phenome comprises the cognitome, symptomatome, and phenomenome (2–4). This framework can be tested and cross-validated using Partial Least Squares (PLS) pathway analysis which combines factor and multiple regression analysis and combines the significant indicator and feature sets into a causal model of schizophrenia. Using this nomothetic network approach, we were able to objectivate the abstract description of schizophrenia and realized a more concrete concept, a phenomenon named "reification of the clinical diagnosis." Based on the latent variable scores of all feature sets, new categories were exposed using unsupervised pattern recognition methods (2, 3). These novel categories should be cross-validated using supervised learning techniques including soft independent modeling of class analogy (SIMCA) in independent samples. This method is useful to profile phenotype classes (by delineating the features of the novel categories), produce cost-effective classifiers, authenticate or reject class-memberships, and compute the distance between classes, which may help to evaluate quantitative vs. qualitative differences (21, 27).

The above provides a route toward a novel model of schizophrenia based on all features sets. This approach discloses a new network-based definition of schizophrenia phenotypes and pathway classes as well as new treatments of the schizophrenia phenotypes (2, 3, 22). Moreover, the latent variable scores delineate an idiomatic feature profile, which is unique for each patient and may be employed for individualized treatments targeting the most disordered feature sets of the nomothetic model (4).

AUTHOR CONTRIBUTIONS

All the contributing authors have participated in the manuscript and approved the final version of the manuscript.

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