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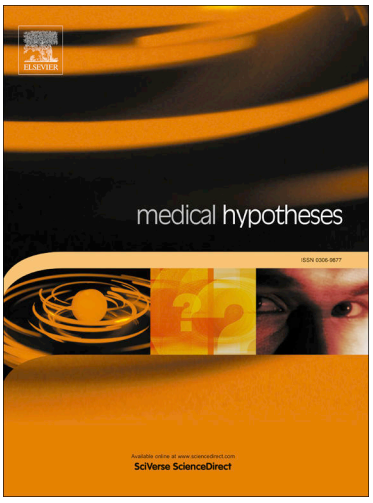

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Fundamental disincorporation and early non-inflammatory microglia alterations: possible bridging phenomena between neurobiology and psychopathology in schizophrenia

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Abstract: Increasing evidence shown that schizophrenia (SCZ) is associated with immunological aberrations. Particularly, microglia, the resident innate immune cells of the brain, have been implicated in SCZ pathogenesis. However, despite the abundance of empirical findings highlighting the role of microglia-mediated neuroinflammation in the neuropathology development in a wide range of central nervous system (CNS) disorders, non-inflammatory abnormalities in this cellular compartment have been associated with neuropsychiatric manifestation of SCZ in some postmortem works and in SCZ animal models. We hypothesize that at least some of these non-inflammatory perturbations represent the core neurobiological features of a "disincorporated" state of microglia in SCZ and indicate a switch from homeostatic coupling/functional response to maladaptive reaction to environmental inputs. This work aims to summarize experimental evidence that support this possible novel conceptual paradigm. This hypothesis needs further confirmations. In summary, the possible role of microglia in the pathogenesis of SCZ is certainly complex with conflicting evidence. Therefore, new conceptual paradigms useful for capturing the fundamental alterations of microglia seem to be needed. Considering the need for experimental confirmation, the ecological idea of microglial disincorporation might be a first step in this direction, sufficiently supported by recent literature.

Keywords: microglia, schizophrenia, disincorporation, immunomodulation, neuroinflammation

Introduction

The emerging microglia role in brain homeostasis and in major neuropsychiatric disorders

During the last decade, novel insights on the diverse function and phenotype of microglia have progressively emerged [1]. Besides their canonical role in immune surveillance, these cells are also indispensable for brain wiring, bidirectional communication with neural cells, and synaptic pruning [2-4]. This broad functional apparatus suggests an important involvement of microglia in brain development and homeostasis [1,4] as well as in several neuropathological conditions, including neuropsychiatric illnesses [5-9]. While microglia-mediated neuroinflammation has been almost unanimously associated with central nervous system (CNS) disorders, emerging evidence, particularly in the pathological context of schizophrenia (SCZ), has pointed to a possible involvement of a unique non-inflammatory microglia phenotype in the development of the disorder [10]. Furthermore, results from some recent studies seem to dictate the necessity of separating the notion of microglia pathological phenotypic shift from the presence of neuroinflammation, since these elements are not necessarily covariant [11]. Finally, the conceptual association of neuroinflammation, microglia activation and psychiatric diagnostic categories cannot be easily established [12].

Schizophrenia: an overview on the complexity of neuroimmunological undepinnings

Among etiological hypotheses, emerging experimental evidence pointed to a relevant role of inflammation as a causal immunity-related factor in the pathogenesis of SCZ [13]. In this regard, inflammatory changes have been observed in serum and cerebrospinal fluid (CSF) of SCZ patients [14-16]. In their systematic review, Rodrigues-Amorim et al.[17] showed that circulating levels of TNF-α, IFN-γ, IL-1β, IL-1RA, IL-2, IL-6, IL-8, and IL-10 differed between SCZ patients and healthy controls in more than half of 99 included studies with a total of 8234 participants. Furthermore, genetic polymorphisms for IL-1β (i.e., rs16944), IL-6 (i.e., rs1800795), and TNF-α (i.e., 1800629) as well as an elevated expression of IL-1β, IL-6, TNFR1, and TNFR2 mRNAs were observed in SCZ subjects. Moreover, the importance of TNF signaling pathway for SCZ development in humans & SCZ-like behaviors in mice has been recently highlighted by Yeo et al. [18]. Notably, the pro-inflammatory cytokine IL-6 was most frequently elevated in SCZ patients, while antipsychotic treatment in drug-naive patients after first-episode psychosis (FEP) led to significant reductions of IL-2 and IL-6 [19]. Elevated level of monocyte chemoattractant protein-1 (MCP-1) was also detected in FEP patients [20]. Furthermore, the importance of symptomatic state- and age-dependent alteration of the brain immune environment in SCZ has been by De Picker et al. in a recent work [21]. In a meta-analysis of 28 studies [17], CSF levels of IL-1β, IL-6, and IL-8 were significantly increased in SCZ patients as compared to healthy controls. Unexpectedly, despite these inflammatory alterations in SCZ, some of the most powerful anti-inflammatory drugs (e.g., corticosteroids and neurosteroids) could not completely resolve the clinical manifestation of the disease [22], and in some cases, might trigger psychotic episodes [23]. This lackluster clinical efficacy of anti-inflammatory medications has called into question the primitive role of inflammation in SCZ pathogenesis.

Despite the highlighted inflammatory changes, some inconsistencies have likewise emerged regarding the unambiguous involvement of microglia in such proinflammatory transitions [11,24]. Moreover, regardless of the necessary expression of proinflammatory phenotypes, microglia have also been proposed as the direct target of some psychiatric medications in SCZ [25-27], prompting the possible contribution of maladaptive microglia-related non-inflammatory response in SCZ development.

The hypothesis

The purpose of this article is not to deny the experimentally supported importance of neuroinflammation associated with proinflammatory microglial changes in SCZ. Rather, it is to properly contextualize both terms. It is perhaps erroneous to view these phenomena as necessarily related and primitive. Instead, the present theoretical work proposes to reconsider them as epiphenomena in the context of a more fundamental pathological development, not necessarily related to inflammation, affecting microglia from the

earliest stages of their ontogeny and leading to progressive disincorporation from the CNS. Therefore, this article aims to provide a synthesis of current findings on both the acquisition of the physiological identity of microglia in the CNS and possible fundamental characters of early microglia dysfunction in SCZ. At the same time, we propose a new concept of microglia disincorporation, especially related to the early noninflammatory changes in microglia, as a potential neurobiological substrate for the early psychopathology of SCZ and as a promising theoretical framework to better understand the further specific stages of the disease [21,28], the involvement of dysfunctional inflammation, and the aberrant pro-inflammatory phenotypes of microglia in the neuropsychiatric manifestations associated with SCZ..

Evaluation of the hypothesis

Microglia ontogenetic development and multilayered identity acquisition

Microglia as the central nervous system resident innate immune cells

Microglia, described as a distinct glial cell type in 1919 by Pío del Río Hortega, are the resident parenchymal myeloid cells of the central nervous system (CNS) [29]. Microglial elements constitute about 5-12% of total cells in the healthy human CNS [2]. During development, primitive macrophages and C-kit+/CD41+ erythromyeloid progenitor of the mesodermic yolk sack are the only embryonic source of human adult microglia, which maintain themselves via in situ proliferation in response to M-CSF and the CX3CR1 ligand (i.e., IL-34) [30,31] without contribution of peripheral monocytes [32] throughout every life stage [33,34]. Of note, apart from the crucial importance of local self-renew [35], the long-lived nature of microglia [36] might exist only at the population level, while microglial subpopulations are dynamically regulated by a rapid turnover, fine-tuned by spatiotemporal inputs [37]. On one hand, these characteristics may explain the great heterogeneity of microglia global pool. On the other hand, it clearly emerges that the colonization of the CSN by the microglial progenitors quite sharply defines the fundamental characters of the microglial populations in each further phase of the individual life.

Microglia are the most morphologically dynamic cells of the vertebrate brain [38]. Ramified microglia were classically defined as “resting or quiescent” as opposed to the “active” amoeboid form. However, recent studies indicated that these cells were in a basal yet highly active state, designed to monitor the neurosynaptic microenvironment [39-41]. Phenotypically, microglial identity is characterized by the expression of several genes and molecular markers (e.g., P2Y12R, TMEM119, and Sall1, and others), with possible distinctions between sexes and among developmental phases and species [42,43]. The main functions of microglia include immune defense, regulation of inflammatory responses, and CNS homeostasis as these cells are capable of detecting the first signs of tissue damage and pathogenic invasion [44]. Data from the murine brain suggest that microglia acquire their functions in a stepwise manner through the activation of distinct transcriptional programs from the embryonic stage to adulthood [45]. In rodents, Dab2, Mcm5 and Lyz2 were expressed by early embryonic microglia while Crybb1, Csf1 and CXCR2 were expressed during synaptic pruning and neurodevelopmental phase. In adult microglia, MafB, CD14 and Mef2 were linked to prevalent immune-homeostatic functions. In humans, besides important immune functions, microglia are involved in normal brain development, supporting the dynamics of (re)structuring of neural networks and maintaining the homeostasis of synapses [46,47]. The majority of these “basic” and interspecifically conserved functional features (e.g., phagocytic clearance and chemotactic response to injuries) are linked to classical immune activity and shared with other cells of myeloid lineage, while other higher order neuro-immune and homeostatic functions seem to emerge in parallel with the evolution of more complex brain structures [48]. In summary, microglial identity appears irreducible to a single dimension of analysis (i.e., morphological, molecular, functional etc.). Rather, this fundamental dynamic and fluid character appears to take the form of a multilayered and complex determination, progressively acquired and continuously reaffirmed during neuroenvironment development and individual ontogenesis [49].

Microglia incorporation – a novel possible conceptual framework

As said, given the complex and fluid notion of microglia identity, simple morphological analysis could lead to an underestimation of the phenotypic and functional continuum underlying microglia heterogeneity and complexity in physiological and pathological conditions [44]. The idea of microglia polarization has been proposed with M0, M1, M2a/b, and M3 phenotypes [50]; however, this "M" subdivision remains excessively discrete, lacking a contextual element of continuity that helps in the interpretation of each discrete form (**Figure 1)**. In attempt to provide foundational insights for this novel conceptual paradigm to characterize microglial dynamics, the concept of "incorporation" or "dynamic coupling" is proposed.

----------Figure 1----------

The term "incorporation" reflects the set of all possible dynamic conditions of phenotypic, morphological, and functional (homeostatic) coupling between microglia and other neuro-glial elements (i.e., the CNS environment) [49], mediated by many structural and soluble factors and intracellular pathways [2]. In fact, multiple microglial subpopulations are detectable within various CNS areas with different phenotypic features (e.g., presence of CD86, CD45, and CX3CR1, and absence of HLA-DR in the subventricular zone and thalamus as compared to other brain regions) [51]. Additionally, the morphological transition of ramified to amoeboid microglia is known to reflect their response to neuropathological or infectious stimuli. Further, cerebellar and hippocampal microglia are associated with a more immune-vigilant state [52], lipid droplet-accumulating microglia (LDAM) and grey matter associated microglia (GAM) are linked to high levels of free radicals release and pro-inflammatory cytokines production (due to their defective phagocytosis), and white matter associated microglia (WAM) are linked to the absence of immune reactivity [53]. Last but not least, heterogeneous microglial subpopulations are identifiable not only in different spatial contexts but also at different time points within the same region [54]. Collectively, these findings point to the importance of environmental cues in continuously and locally shaping multiple aspects of microglial identity.

Various types of functional microglial-neuro-glial coupling may exist under the influence of different environmental factors. In this regard, the subset symbiotic couplings refers to exchanges of molecules and interaction during homeostasis and development, while the further subset of possible productive and reactive coupling occurs during infection and tissue regeneration after injury (**Figure 2**). All types of homeostatic coupling are collectively resumed under the continuous concept of incorporation. The character of functionality here evoked refers to the obtained optimization of the dynamics expressed by the system in which microglia cells are included. A sort of meronomic relation subsists between each functional microglia forms and the ideal concept of incorporated microglia. In turn, each functional type of coupling is linked to specific expressions of microglial multilayered identity and continuously regulated by a number of factors from the CNS environment. For example, neuronal activity appears capable of either attracting or averting microglial contact by variations in noradrenergic tone [55], while CD200/CX3CL1 or CD47 could instruct microglia to remain in a surveillance or phagocytic state via microglial CD200R/CX3CR1 or CD172a signaling, respectively [56-58]. Apart from classical cell-cell structure interactions, several cytokines, chemokines, and metabolites could also modulate neuro-glial coupling dynamics [59-61]. For example, TGF-β or glutamate (normally released during synaptic activity) could directly or indirectly evoke cell-type-specific and context-dependent transcriptional programs in microglia [62,63]. Further, some microglia-intrinsic pathways (i.e., PU.1, IL-34, IRF8, and CSF-1) have been identified as the necessary elements for the initial expression of microglia incorporation [64].

----------Figure 2----------

Microglia disincorporation as a contributor to neuropathological development

When abnormalities in fundamental neuro-glial interactions occur, microglia progressively enter a state of disincorporation. Each of the mentioned mediators of functional coupling between microglia and CNS could potentially be negatively implicated in the fundamental phenomenon of disincorporation. It is possible, however, that many more mediators will be identified in the near future. Moreover, disincorporation is simultaneously defined both as an event and as a process. For example, during neuropathological development, the release of various neurotransmitters [65,66] or other mediators as, for example, extracellular vesicles [67,68], could lead to microglial disincorporation. In this first meaning the term disincorporation generically refers to a breaking event, concerning a previous form of functional coupling between microglia and neuro-

glial environment. The equilibrium point of the coupling in the presence of perturbations is always dynamically moved within a certain oscillation range. A primitively defined homeostatic reserve (i.e., see further sections about genetics and priming) is associated with a specific threshold of tolerance to coupling perturbations. When the threshold is exceeded due to a perturbation of sufficient entity (disincorporation-event) the oscillation window of the aforementioned equilibrium point is permanently redefined in a maladaptive sense (i.e., with a progressive reduction of the homeostatic reserve of the renewed coupling). It's important to emphasize the distinction between this concept and the generic inflammatory/activated state of microglia because a productive inflammatory phenotype might exist in the absence of disincorporation (e.g., functional response to brain trauma/injuries). However, an inflammatory phenotype could also be acquired by disincorporated microglia. The main difference between the two inflammatory types (productive vs. disincorporated) of microglia is the inability/defective ability to return the homeostatic state in the latter.

Disincorporation as a process entails several possible forms of further maladaptive interaction, including the initial destabilization event independent from the presence of inflammation, the progressive stepwise weakening of a functional coupling, and eventually, a disincorporated state with the coexistence of inflammation. We propose that the earlier forms of disincorporation might represent a specific neurobiological substrate for at least a subgroup of early manifestations of SCZ.

Disincorporated microglia as a bridging neurobiological phenomenon for early development of SCZ psychopathology

Possible experimentally validated markers of microglia disincorporation in SCZ

Given the role of microglia in coordinating online neuronal function/activity [69,70] and synaptic functional plasticity [71-73], microglia disincorporation may represent a fundamental neurobiological condition reflective of elementary Basic Symptoms (BSs) [74] from the Basic Symptoms paradigm [75-78] in SCZ, representing, at the same time, a disturbance in basic neural information processing. Indeed, the BSs model proposes some transphenomenal elements (i.e., bridging the neurobiological and experiential dimensions) that can be further exploited to build a connection between the proposed subtle noninflammatory microglial changes and the psycho-behavioral manifestations of SCZ. Some metanalytical works on postmortem studies that investigated the extent of microglial morphological and densitometrical alterations in SCZ subjects showed conflicting results [79,80]. Of note, many studies considered in such metanalytical works may have been contaminated by important confounding factors as, for example, the causes of death, co-morbidities, medication, lack of knowledge of disease stage, and/or symptoms predominance [28]. Interestingly, a general decrease in expression of numerous microglia-specific genes (e.g., CX3CR1, CSF1R, TMEM119, TREM2, ITGAM, ITGAX, CD86, IRF8, P2Y12R and OLR1) emerged in SCZ patients as compared to controls in some other experimental [81,82] and metanalytical works [10] (**Table 1**), suggesting a compromise of neuron-microglia communication [28]. However, despite the presence of the abovementioned confounding factors in postmortem studies, a complex alteration of the microglial transcriptome appears consistently detectable [24,83] also in many developmental phases of SCZ animal models [28]. These more subtle alterations, along with other possible finer modification of microglia morphology and dynamics, could indicate the presence of a non-inflammatory basic state of disincorporation of microglia in SCZ. This neurobiological condition can be the first building block for the construction of a connecting line between organic and phenomenological dimensions, passing through the transphenomenal level, offered within the BSs paradigm. Notably, since TMEM119 is a marker of mature and homeostatic/PU.1 expressing microglia [64,84,85], these alterations might involve molecular elements particularly associated with this microglial subset, which has been documented to be partially decreased in multiple conditions of neurodegeneration and brain aging [86].

----------Table 1----------

Regulators of microglial disincorporation in SCZ

Similar to other cell types, microglia could express a certain degree of genetic and epigenetic vulnerability. The concept of microglial "priming" may be generically referred to any informative event (acting on a genetic substrate), capable of permanently conditioning further microglia characteristics and activity. The phenomenon of priming is fundamental for understanding microglial disincorporation. Microglia priming has been typically understood as a form of trained immunity [98], mainly referring to the ability of innate immune cells to develop and display memory for inflammatory and infectious stimuli. However, priming might entail microglia propensity to undergo changes other than those of inflammatory nature. In the following sections, we highlight genetic and environmental factors that are potentially linked to the priming of microglia toward a disincorporation state in SCZ. The model here proposed entails a fundamental genetic substrate, an early priming effect (during the first phase of microglia ontogenetic development) and the subsequent event-process of disincorporation (in presence of further concomitant triggers).

Genetic substrate for dysfunctional priming of microglial disincorporation in SCZ

Genetics seems to significantly contribute to SCZ pathogenesis, considering the estimated 1% of schizophrenia risk in the general population as compared to the 50% risk in individuals with an SCZ twin [99]. The estimated single nucleotide polymorphism (SNP)-based heritability for SCZ is approximately 0.45 [100]. Notably, both pre-genome-wide association studies (GWAS) and GWAS consistently pointed to the presence of SCZ susceptibility loci in the Major Histocompatibility Complex (MHC) region [101,102], one of the most polymorphic and gene dense areas of the human genome [103]. In fact, recent analyses of post-mortem gene expression also verified a correlation between copy number variation (CNV) and mRNA and protein expression of some MHC-linked candidate risk factors [104,105]. HLA molecules are not only fundamental in refining immunity but also involved in neuronal signaling, neuronal/synaptic plasticity, learning memory, behavioral aspects, and CNS anatomical integrity [106-108]. Importantly, MHC loci on chromosome 6 encode information about innate immunity [103], prompting the possibility that MHC-linked genetic variations might contribute to microglial dysfunctional early priming and further disincorporation in SCZ.

A significant part of the association between SCZ and the MHC region concerns some alleles of the complement component 4 (C4). This molecule seems to have the strongest association with SCZ among all loci across the genome [99,102] with documented presence of C4A CNV in SCZ patients [105]. Since C4 activates C3, a complement product required for microglia-mediated synaptic pruning, specific genetic variations regarding C4 gene might trigger a specific disincorporated phenotype of early primed microglia with abnormal features in synaptic elimination in SCZ (e.g., loss of cortical gray matter [109], phagocytosis of stressed neurons, and impairment in neurotrophic factor release [110]. In fact, both higher C4A level [105] and reduced synaptic density [111,112] in the brains of SCZ patients have been observed.

Besides C4, other genetic factors might also be involved in microglial disincorporation in SCZ. For example, the altered microglia-mediated synaptic maturation hypothesis for SCZ seems to involve mutations in CX3CR1/CX3CL1 and CR3/C3 genes [3] or abnormal levels of C1q [113]. Additionally, recent studies of human endogenous retroviruses (HERV) showed some associations between SCZ and the HERV-W type, of which HERV-W envelope protein is predominantly produced by microglia and has been linked to the neurotoxic subtype of these cells [114,115]. Interestingly, the HERV-K type was also associated with the aforementioned alterations of C4A gene [105]. Last but not least, microglial SCZ polygenic score (PGS) [116] was predictive of cognitive functioning to a comparable extent to that of neuronal SCZ-PGS [117], suggesting that dysfunctional priming of disincorporated microglia, promoted by these genetic factors, might have some etiological relevance for the impairment in cognitive function of SCZ patients.

Environmental priming of microglia in SCZ

A growing number of environmental factors could potentially have an epigenetic priming effects on early life microglia and microglial progenitors. In this regard, certain genetic alterations in SCZ, particularly on microglial genes, seem to overlap with a transcriptional signature produced by toxic exposure to traffic-related air pollution (TRAP) [118,119] and other pollutants. Additionally, greater psychosis risk occurred with birth in urban area, which is intrinsically linked to higher exposure to pollution. Despite the difficulties in defining causal links, more recent evidence suggests a possible role of air pollution in altering microglia function and development [120,121]. For example, air pollution has been shown to trigger the production of

immunomodulatory molecules, such as IL-1ß, IL-6, TNF-α in humans and mouse models [122,123]. Genetic associations of various genes encoding these inflammatory cytokines have also been observed in SCZ [17]. Furthermore, pollutants could induce immune and brain development alterations in children and mice, evidenced by activation of microglia TLR4 [124] and increased phagocytic capacity (elevated levels of C5, C5a, and CD68 proteins) [120]. Obviously, urbanicity is not exclusively related to pollution, so the causal link is rather weak. Moreover, the proposed evidence is mainly concerned with inflammatory changes in microglia. However, in line with our hypothesis, these inflammatory alterations could be the result of a more primitive disincorporation condition, possibly fostered (i.e., primed) by the same environmental factors during the early stages of development.

Another possible environmental trigger of microglia disincorporation is the complex alteration in the gut-brain axis and its influence on the immune system [125,126]. More specifically, early alteration of the gut microbiome could lead to increased risk of SCZ, through the mediating role of primitive microglia priming and subsequent disincorporation. In rodents, microbiome-dependent developmental priming of microglia has been documented in a sex-specific manner [127,128], which might provide a potential explanation for slight sex differences in SCZ prevalence.

Furthermore, microbial infection during pregnancy with consequential immune activation and subsequent induction of disincorporated microglia seems to increase the risk of neuropsychiatric disorders, including SCZ [129,130].

Considering the abundance of steroid receptors expressed by microglial cells [131], early-life stress has also been linked to microglia disincorporation [132]. Prenatal stress is associated with an increased risk of developmental problems in rodents and in nonhumans primates as well as of SCZ in humans [133,134]. Notably, prenatal stress-associated alterations in placental expression of several immune genes was potentially reversible by administration of anti-inflammatory drugs [135]. In rodents, maternal stress could lead to altered microglial morphology and density in the embryonic cortical plate and adult neocortex, which might be related to increased maternal levels of IL-6 [136] as these changes were apparently reversible by IL-6 blockage [137].

Collectively, various environmental factors (pollution, microbiome, and early-life stress) might provide the epigenetic trigger for a subsequent more likely microglial transition toward a disincorporated state. Notably, unlike genetic factors outlined in the section above, this environmentally triggered disincorporated phenotype might be accompanied with maladaptive inflammation as pollutants, microbes, and stress are known to trigger the production of inflammatory mediators in microglia.

Microglial disincorporation and psychopathological progression of SCZ

It is important to considering the level of pathogenetic implication of microglial disincorporation in relation to each specific SCZ progression phase (**Figure 3**). If an adequate incorporation is necessary also to fundamentally optimize primarily online and experience-driven neural computation and activity [69-71], the disincorporated condition should produce a fundamental deficit in basic neural information processing, progressively exposing the subject, on the experiential side, to a primitive state of basic irritation. This condition, borrowed from Basic Symptoms theoretical framework, represents a possible trans-phenomenal link, between neurobiology and the parallel events on the subjective side, where the conscious experience may become more and more complex and progressively enriched with affective tension, due to the discovered unsuitability of the previously acquired cognitive schemas. Thus, strangeness and otherness begin to contaminate the experiential field, starting from its augmented expression at the very cellular and neurobiological level (i.e., the starting event of the disincorporated condition).

As already stated, disincorporation is not just an “event”, but an active “process”, of which the primitive effects can be reinforced by other secondary maladaptive events. All “secondary” neuropathological phenomena (e.g., neurotransmitters alterations, neuronal damage, aberrant synaptic pruning, dysfunctional neuroinflammation etc.) are probably driven by the same necessity to contain the negative drift along the maladaptive spiral. For example, florid psychosis may be seen as the price to pay to gain a temporary stabilization of the initial maladaptive process (via dopamine-dependent immunomodulation of microglial elements [138], and altered synaptic pruning could find its place as a further maladaptive phenomenon in a

more crystallized form of the disease. With disease progression, the consequential phases could be at least partially independent from the initial microglia involvement.

----------Figure 3----------

Limitations

The present work represents a theoretical proposal and, as such, lacks for now an adequate and specific empirical support, beyond what can be deduced through a reinterpretation of the current literature. Moreover, the experimental evidence currently available is often inconsistent and difficult to understand, due to multiple confounding factors, often not adequately managed in the context of the studies conducted. The first limitation of this proposal therefore concerns the unstable foundation on which it was built. Such a limitation is especially referred to postmortem studies results here reported. Moreover, a possible experimental test would require the application of new and sophisticated techniques, perhaps not yet available, in order to capture the subtle alterations that could correspond to the generic condition of disincorporation. Furthermore, the definition of a three-phasic model, which takes into account the genetic starting point, the priming effects and the disincorporation event-process certainly represents a daring speculative attempt. Finally, the attempt to unite the neurobiological level with the phenomenal-experiential one through the trans-phenomenal double-faced element of basic irritation/microglia disincorporation is equally particularly insidious.

Conclusions

This review proposes the idea of disincorporation in susceptible microglial populations as a contributor to SCZ pathogenesis. Through functional coupling with CNS neuro-glial elements, microglia incorporation is maintained, and a homeostatic microglia identity is generically defined. In the anomalous context of SCZ, a primary disincorporated state of microglia was elicited by various genetic and environmental factors, with possible contribution to the development of psychopathology in this disease. Of note, microglial "pro-inflammatory" shift might not necessarily occur with microglial disincorporation, depending on the nature of the triggering factor and/or disease stage. In the context of disincorporation, the pro-inflammatory shift expressed by microglia may be reinterpreted as an identity "regression" to more "immune" (and less incorporated) phenotypes, rather than a primitive change into novel microglial forms. As such, caution must be exerted during therapeutic considerations for SCZ as a disease with possible "neuroinflammatory" background. At the same time, there is a need for more physiologically relevant vitro/in vivo/ex vivo methodology to study microglia in detail, such as such three-dimensional cerebral organoids and chimeric animal models engrafted with human induced pluripotent stem cells (iPSC)-derived microglia [139,140], to reveal other potential contributors to the development of microglial disincorporation. A possible experimental approach to validate this hypothesis could make use of the new methodologies mentioned above. For example, iPSC-derived microglia obtained from schizophrenic patients could be implanted into mouse models to test for the appearance of any deficit in cognitive information processing, since iPSC-derived microglia appear to retain species-specific transcriptomic differences in the expression of genes at risk for neurological disease [140]. Despite ongoing debate concerning these topics, pharmacological targeting of the immune system produces a certain reduction in SCZ symptomatology [141,142], providing the promising preliminary evidence for further therapeutic investigation into the role of microglia and their aberrant disincorporated state in this neuropsychiatric illness. Further studies are needed. However, the hope is that the present theoretical proposal can provide an interpretative framework to better investigate not only the role of microglia in SCZ, but also the connection between neuroinflammatory states and other highly relevant psychopathological conditions [143,144].

References

[1] Prinz M, Jung S, Priller J. Microglia Biology: One Century of Evolving Concepts. *Cell*. 2019;179(2):292-311. doi: 10.1016/j.cell.2019.08.053.

[2] Szepesi Z, Manouchehrian O, Bachiller S, Deierborg T. Bidirectional Microglia–Neuron Communication in Health and Disease. *Front Cell Neurosci*. 2018;12:323. doi: 10.3389/fncel.2018.00323.

[3] Paolicelli RC, Bolasco G, Pagani F, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science*. 2011;333:1456-8. doi: 10.1126/science.1202529.

[4] Garaschuk O, Verkhratsky A. Physiology of Microglia. *Methods Mol Biol*. 2019;2034:27-40. doi: 10.1007/978-1-4939-9658-2_3.

[5] Costanza A, Xekardaki A, Kövari E, Gold G, Bouras C, Giannakopoulos P. Microvascular burden and Alzheimer-type lesions across the age spectrum. *J Alzheimers Dis*. 2012;32:643-52. doi: 10.3233/JAD-2012-120835.

[6] Costanza A, Amerio A, Aguglia A, et al. Hyper/neuroinflammation in COVID-19 and suicide etiopathogenesis: Hypothesis for a nefarious collision? *Neurosci Biobehav Rev*. 2022;136:104606. doi: 10.1016/j.neubiorev.2022.104606.

[7] Serafini G, Costanza A, Aguglia A, et al. The Role of Inflammation in the Pathophysiology of Depression and Suicidal Behavior: Implications for Treatment. *Med Clin North Am*. 2023;107:1-29. doi: 10.1016/j.mcna.2022.09.001.

[8] Brisch R, Wojtylak S, Saniotis A, et al. The role of microglia in neuropsychiatric disorders and suicide. *Eur Arch Psychiatry Clin Neurosci*. 2022;272(6):929-945. doi: 10.1007/s00406-021-01334-z.

[9] Rahimian R, Wakid M, O'Leary LA, Mechawar N. The emerging tale of microglia in psychiatric disorders. *Neurosci Biobehav Rev*. 2021;131:1-29. doi: 10.1016/j.neubiorev.2021.09.023.

[10] Snijders GJLJ, van Zuiden W, Sneeboer MAM, et al. A loss of mature microglial markers without immune activation in schizophrenia. *Glia*. 2021;69(5):1251-1267. doi: 10.1002/glia.23962.

[11] North HF, Weissleder C, Fullerton JM, Sager R, Webster MJ, Weickert CS. A schizophrenia subgroup with elevated inflammation displays reduced microglia, increased peripheral immune cell and altered neurogenesis marker gene expression in the subependymal zone. *Transl Psychiatry.* 2021;11(1):635. doi: 10.1038/s41398-021-01742-8.

[12] Mondelli V, Vernon AC, Turkheimer F, Dazzan P, Pariante CM. Brain microglia in psychiatric disorders. *Lancet Psychiatry*. 2017;4(7):563-572. doi: 10.1016/S2215-0366(17)30101-3.

[13] Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R, Jones PB. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *Lancet Psychiatry*. 2015;2:258-70. doi: 10.1016/S2215-0366(14)00122-9.

[14] Frydecka D, Krzystek-Korpacka M, Lubeiro A, et al. Profiling inflammatory signatures of schizophrenia: A cross-sectional and meta-analysis study. *Brain Behav Immun.* 2018;71:28-36. doi: 10.1016/j.bbi.2018.05.002.

[15] Upthegrove R, Manzanares-Teson N, Barnes NM. Cytokine function in medication-naive first episode psychosis: A systematic review and meta-analysis. *Schizophr Res*. 2014;155:101-8. doi: 10.1016/j.schres.2014.03.005.

[16] Wang AK, Miller BJ. Meta-analysis of Cerebrospinal Fluid Cytokine and Tryptophan Catabolite Alterations in Psychiatric Patients: Comparisons Between Schizophrenia, Bipolar Disorder, and Depression. *Schizophr Bull*. 2018; 44:75-83. doi: 10.1093/schbul/sbx035.

[17] Rodrigues-Amorim D, Rivera-Baltanás T, Spuch C, et al. Cytokines dysregulation in schizophrenia: A systematic review of psychoneuroimmune relationship. *Schizophr Res*. 2018;197:19-33. doi: 10.1016/j.schres.2017.11.023.

[18] Yeo IJ, Yun J, Son DJ, et al. Overexpression of transmembrane TNFα in brain endothelial cells induces schizophrenia-relevant behaviors. *Mol Psychiatry*. 2023 Feb;28(2):843-855. doi: 10.1038/s41380-022-01846-7.

[19] Kroken RA, Sommer IE, Steen VM, et al. Constructing the immune signature of schizophrenia for clinical use and research; an integrative review translating descriptives into diagnostics. *Front Psychiatry.* 2019;10:753. doi: 10.3389/fpsyt.2018.00753.

[20] Orhan F, Schwieler L, Fatouros-Bergman H, et al. Increased number of monocytes and plasma levels of MCP-1 and YKL-40 in first-episode psychosis. *Acta Psychiatr Scand*. 2018;138:432-40. doi: 10.1111/acps.12944

[21] De Picker LJ, Victoriano GM, Richards R, et al. Immune environment of the brain in schizophrenia and during the psychotic episode: A human post-mortem study. *Brain Behav Immun*. 2021;97:319-327. doi: 10.1016/j.bbi.2021.07.017.

[22] Nasib LG, Gangadin SS, Rossum IW van, et al. The effect of prednisolone on symptom severity in schizophrenia: A placebo-controlled, randomized controlled trial. *Schizophr Res*. 2021;230:79-86. doi: 10.1016/j.schres.2021.01.024.

[23] Warrington TP, Bostwick JM. Psychiatric Adverse Effects of Corticosteroids. *Mayo Clin Proc*. 2006;81:1361-7. doi: 10.4065/81.10.1361.

[24] Gandal MJ, Zhang P, Hadjimichael E, et al. Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. *Science*. 2018; 362:eaat8127. doi: 10.1126/science.aat8127.

[25] Drzyzga Ł, Obuchowicz E, Marcinowska A, Herman ZS. Cytokines in schizophrenia and the effects of antipsychotic drugs. *Brain Behav Immun*. 2006;20:532-45. doi: 10.1016/j.bbi.2006.02.002.

[26] Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-Analysis of Cytokine Alterations in Schizophrenia: Clinical Status and Antipsychotic Effects. *Biol Psychiatry.* 2011;70:663-71. doi: 10.1016/j.biopsych.2011.04.013.

[27] Mantere O, Trontti K, García-González J, et al. Immunomodulatory effects of antipsychotic treatment on gene expression in first-episode psychosis. *J Psychiatr Res*. 2019;109:18-26. doi: 10.1016/j.jpsychires.2018.11.008.

[28] Rodrigues-Neves AC, Ambrósio AF, Gomes CA. Microglia sequelae: brain signature of innate immunity in schizophrenia. *Transl Psychiatry*. 2022;12(1):493. doi: 10.1038/s41398-022-02197-1.

[29] Sierra A, de Castro F, Del Río-Hortega J, Rafael Iglesias-Rozas J, Garrosa M, Kettenmann H. The "Big-Bang" for modern glial biology: Translation and comments on Pío del Río-Hortega 1919 series of papers on microglia. *Glia*. 2016;64:1801-40. doi: 10.1002/glia.23046.

[30] Węgiel J, Wiśniewski HM, Dziewiątkowski J, et al. Reduced number and altered morphology of microglial cells in colony stimulating factor-1-deficient osteopetrotic op/op mice. *Brain Res*. 1998;804:135-9. doi: 10.1016/s0006-8993(98)00618-0.

[31] Wang Y, Szretter KJ, Vermi W, et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat Immunol*. 2012;13:753-60. doi: 10.1038/ni.2360.

[32] Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FMV. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nature Neuroscience*. 2007;10:1538-43. doi: 10.1038/nn2014.

[33] Gomez Perdiguero E, Klapproth K, Schulz C, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*. 2014; 518:547-51. doi: 10.1038/nature13989.

[34] Ginhoux F, Greter M, Leboeuf M, et al. Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages. *Science*. 2010;330:841-5. doi: 10.1126/science.1194637.

[35] Zhan L, Krabbe G, Du F, et al. Proximal recolonization by self-renewing microglia re-establishes microglial homeostasis in the adult mouse brain. *PLoS Biol*. 2019;17:e3000134. doi: 10.1371/journal.pbio.3000134.

[36] Réu P, Khosravi A, Bernard S, et al. The Lifespan and Turnover of Microglia in the Human Brain. *Cell Rep.* 2017;20:779-84. doi: 10.1016/j.celrep.2017.07.004.

[37] Askew K, Li K, Olmos-Alonso A, et al. Coupled Proliferation and Apoptosis Maintain the Rapid Turnover of Microglia in the Adult Brain. *Cell Rep*. 2017;18:391-405. doi: 10.1016/j.celrep.2016.12.041.

[38] Nimmerjahn A. Optical window preparation for Two-photon imaging of microglia in mice. *Cold Spring Harb Protoc*. 2012; 7:587-93. doi: 10.1101/pdb.prot069286.

[39] Davalos D, Grutzendler J, Yang G, et al. ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci*. 2005; 8:752-8. doi: 10.1038/nn1472.

[40] Nimmerjahn A, Kirchhoff F, Helmchen F. Neuroscience: Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science*. 2005; 308:1314-8. doi: 10.1126/science.1110647.

[41] Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci*. 2009; 29:3974-80. doi: 10.1523/JNEUROSCI.4363-08.2009.

[42] Hanamsagar R, Alter MD, Block CS, Sullivan H, Bolton JL, Bilbo SD. Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia*. 2018;66:460. doi: 10.1002/glia.23277.

[43] Geirsdottir L, David E, Keren-Shaul H, et al. Cross-Species Single-Cell Analysis Reveals Divergence of the Primate Microglia Program. *Cell*. 2020;181:746. doi: 10.1016/j.cell.2020.04.002.

[44] Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. *Physiol Rev*. 2011;91:461-553. doi: 10.1152/physrev.00011.2010.

[45] Matcovitch-Natan O, Winter DR, Giladi A, et al. Microglia development follows a stepwise program to regulate brain homeostasis. *Science*. 2016;353:aad8670. doi: 10.1126/science.aad8670.

[46] Bohlen CJ, Friedman BA, Dejanovic B, Sheng M. Microglia in Brain Development, Homeostasis, and Neurodegeneration. *Annu Rev Genet*. 2019; 53:263-88. doi: 10.1146/annurev-genet-112618-043515.

[47] Parkhurst CN, Yang G, Ninan I, et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell*. 2013; 155:1596-609. doi: 10.1016/j.cell.2013.11.030.

[48] Sharma K, Bisht K, Eyo UB. A Comparative Biology of Microglia Across Species. *Front Cell Dev Biol.* 2021;9:718. doi: 10.3389/fcell.2021.652748.

[49] Gosselin D, Skola D, Coufal NG, et al. An environment-dependent transcriptional network specifies human microglia identity. *Science.* 2017; 356:1248–59. doi: 10.1126/science.aal3222.

[50] Gomez-Nicola D, Perry VH. Microglial dynamics and role in the healthy and diseased brain: A paradigm of functional plasticity. *Neuroscientist.* 2015;21:169-84. doi: 10.1177/1073858414530512.

[51] Böttcher C, Schlickeiser S, Sneeboer MAM, et al. Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. *Nat Neurosci*. 2019;22:78-90. doi: 10.1038/s41593-018-0290-2.

[52] Grabert K, Michoel T, Karavolos MH, et al. Micro-glial brain region−dependent diversity and selective regional sensitivities to aging. *Nat Neurosci*. 2016;19:504–16. doi: 10.1038/nn.4222.

[53] Safaiyan S, Besson-Girard S, Kaya T, et al. White matter aging drives microglial diversity. *Neuron*. 2021;109:1100-1117.e10. doi: 10.1016/j.neuron.2021.01.027.

[54] Hammond TR, Dufort C, Dissing-Olesen L, et al. Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity.* 2019; 50:253-271.e6. doi: 10.1016/j.immuni.2018.11.004.

[55] Liu YU, Ying Y, Li Y, et al. Neuronal network activity controls microglial process surveillance in awake mice via norepinephrine signaling. *Nat Neurosci.* 2019; 22:1771-81. doi: 10.1038/s41593-019-0511-3.

[56] Koning N, Swaab DF, Hoek RM, Huitinga I. Distribution of the Immune Inhibitory Molecules CD200 and CD200R in the Normal Central Nervous System and Multiple Sclerosis Lesions Suggests Neuron-Glia and Glia-Glia Interactions. *J Neuropathol Exp Neurol.* 2009;68:159-67. doi: 10.1097/NEN.0b013e3181964113.

[57] Paolicelli RC, Bisht K, Tremblay MÈ. Fractalkine regulation of microglial physiology and consequences on the brain and behavior. *Front Cell Neurosci.* 2014;8:129. doi: 10.3389/fncel.2014.00129.

[58] van Beek EM, Cochrane F, Barclay AN, Berg TK van den. Signal Regulatory Proteins in the Immune System. *J Immunol*. 2005;175:7781-7. doi: 10.4049/jimmunol.175.12.7781.

[59] Hanisch UK. Microglia as a source and target of cytokines. *Glia*. 2002 Nov;40(2):140-155. doi: 10.1002/glia.10161

[60] Réaux-Le Goazigo A, Van Steenwinckel J, Rostène W, Mélik Parsadaniantz S. Current status of chemokines in the adult CNS. *Prog Neurobiol*. 2013;104:67-92. doi: 10.1016/j.pneurobio.2013.02.001.

[61] Inoue K. Microglial activation by purines and pyrimidines. *Glia*. 2002 Nov;40(2):156-163. doi: 10.1002/glia.10150.

[62] Spittau B, Dokalis N, Prinz M. The Role of TGF β Signaling in Microglia Maturation and Activation. *Trends Immunol.* 2020; 41:836-48. doi: 10.1016/j.it.2020.07.003.

[63] Fazio F, Ulivieri M, Volpi C, Gargaro M, Fallarino F. Targeting metabotropic glutamate receptors for the treatment of neuroinflammation. *Curr Opin Pharmacol*. 2018;38:16-23. doi: 10.1016/j.coph.2018.01.010.

[64] Kierdorf K, Erny D, Goldmann T, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nature Neuroscience.* 2013;16:273-80. doi: 10.1038/nn.3318.

[65] McCluskey LP, Lampson LA. Local immune regulation in the central nervous system by substance P vs. glutamate. *J Neuroimmunol*. 2001;116:136-46. doi: 10.1016/s0165-5728(01)00295-8.

[66] Kuhn SA, Van Landeghem FKH, Zacharias R, et al. Mi-croglia express GABAB receptors to modulate interleukin release. *Molecular and Cellular Neuroscience*. 2004;25:312-22. doi: 10.1016/j.mcn.2003.10.023.

[67] Basso M, Bonetto V. Extracellular vesicles and a novel form of communication in the brain. Front *Neurosci*. 2016;10:127. doi: 10.3389/fnins.2016.00127.

[68] Rufino-Ramos D, Albuquerque PR, Carmona V, Perfeito R, Nobre RJ, Pereira de Almeida L. Extracellular vesicles: Novel promising delivery systems for therapy of brain diseases. *Journal of Controlled Release*. 2017;262:247-58. doi: 10.1016/j.jconrel.2017.07.001.

[69] Akiyoshi R, Wake H, Kato D, Horiuchi H, et al. Microglia Enhance Synapse Activity to Promote Local Network Synchronization. *eNeuro.* 2018;5(5):ENEURO.0088-18.2018. doi: 10.1523/ENEURO.0088-18.2018.

[70] Li Y, Du XF, Liu CS, Wen ZL, Du JL. Reciprocal Regulation between Resting Microglial Dynamics and Neuronal Activity In Vivo. *Dev Cell*. 2012;23:1189-202. doi: 10.1016/j.devcel.2012.10.027.

[71] Tremblay MĚ, Lowery RL, Majewska AK. Microglial interactions with synapses are modulated by visual experience. *PLoS Biol*. 2010;8: e1000527. doi: 10.1371/journal.pbio.1000527.

[72] Basilico B, Ferrucci L, Ratano P, et al. Microglia control glutamatergic synapses in the adult mouse hippocampus. *Glia*. 2022;70:173-95. doi: 10.1002/glia.24101.

[73] Basilico B, Ferrucci L, Khan A, di Angelantonio S, Ragozzino D, Reverte I. What microglia depletion approaches tell us about the role of microglia on synaptic function and behavior. *Front Cell Neurosci*. 2022;16:1022431. doi: 10.3389/fncel.2022.1022431.

[74] Maggini C, Raballo A. Self-Centrality, Basic Symptoms Model and Psychopathology in Schizophrenia. *Psychopathology*. 2004;37:69-75. doi: 10.1159/000077581.

[75] Gross G. The 'basic' symptoms of schizophrenia. *Br J Psychiatry Suppl*. 1989;(7):21–5; discussion 37-40.

[76] Huber G, Gross G. The concept of basic symptoms in schizophrenic and schizoaffective psychoses. *Recenti Prog Med.* 1989;80:646-52.

[77] Schultze-Lutter F, Michel C, Schmidt SJ, et al. EPA guidance on the early detection of clinical high risk states of psychoses. *Eur Psychiatry*. 2015;30:405-16. doi: 10.1016/j.eurpsy.2015.01.010.

[78] Klosterkötter J. The Meaning of Basic Symptoms for the Genesis of the Schizophrenic Nuclear Syndrome. *Psychiatry Clin Neurosci*. 1992;46:609-30. doi: 10.1111/j.1440-1819.1992.tb00535.x.

[79] Van Kesteren CFMG, Gremmels H, De Witte LD, et al. Immune in-volvement in the pathogenesis of schizophrenia: a meta-analysis on postmortem brain studies. *Translational Psychiatry.* 2017;7:e1075-e1075. doi: 10.1038/tp.2017.4.

[80] Trépanier MO, Hopperton KE, Mizrahi R, Mechawar N, Bazinet RP. Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. *Molecular Psychiatry.* 2016;21:1009-26. doi: 10.1038/mp.2016.90.

[81] Hill SL, Shao L, Beasley CL. Diminished levels of the chemokine fractalkine in post-mortem prefrontal cortex in schizophrenia but not bipolar disorder. *World J Biol Psychiatry*. 2021 Feb;22(2):94-103. doi: 10.1080/15622975.2020.1755451.

[82] Bergon A, Belzeaux R, Comte M, et al. CX3CR1 is dysregulated in blood and brain from schizophrenia patients. *Schizophr Res*. 2015 Oct;168(1-2):434-43. doi: 10.1016/j.schres.2015.08.010.

[83] Gandal MJ, Haney JR, Parikshak NN, et al. Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap. *Science*. 2018; 359:693-7. doi: 10.1126/science.aad6469.

[84] Satoh J ichi, Kino Y, Asahina N, et al. TMEM119 marks a subset of microglia in the human brain. *Neuropathology.* 2016;36(1):39-49. doi: 10.1111/neup.12235.

[85] Bennett ML, Bennett FC, Liddelow SA, et al. New tools for studying microglia in the mouse and human CNS. *Proc Natl Acad Sci U S A*. 2016;113:E1738-46. doi: 10.1073/pnas.1525528113.

[86] Krasemann S, Madore C, Cialic R, et al. The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity.* 2017;47:566-581.e9. doi: 10.1016/j.immuni.2017.08.008.

[87] Gehrmann J, Banati RB, Kreutzberg GW. Microglia in the immune surveillance of the brain: human microglia constitutively express HLA-DR molecules. *J Neuroimmunol*. 1993 Nov-Dec;48(2):189-98. doi: 10.1016/0165-5728(93)90191-z.

[88] Fahrenhold M, Rakic S, Classey J, et al. TREM2 expression in the human brain: a marker of monocyte recruitment? Brain Pathology. 2018;28:595-602. doi: 10.1111/bpa.12564.

[89] Butovsky O, Weiner HL. Microglial signatures and their role in health and disease. Nat Rev Neurosci. 2018 Oct;19(10):622-635.CD86 ref. doi: 10.1038/s41583-018-0057-5.

[90] Wlodarczyk A, Holtman IR, Krueger M, et al. A novel microglial subset plays a key role in myelinogenesis in developing brain. *EMBO J.* 2017 Nov 15;36(22):3292-3308. doi: 10.15252/embj.201696056.

[91] Keren-Shaul H, Spinrad A, Weiner A, et al. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell*. 2017;169(7):1276-1290.e17. doi: 10.1016/j.cell.2017.05.018.

[92] Kamphuis W, Kooijman L, Schetters S, Orre M, Hol EM. Transcriptional profiling of CD11c-positive microglia accumulating around amyloid plaques in a mouse model for Alzheimer's disease. *Biochim Biophys Acta*. 2016;1862(10):1847-60. doi: 10.1016/j.bbadis.2016.07.007.

[93] Zrzavy T, Hametner S, Wimmer I, Butovsky O, Weiner HL, Lassmann H. Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain*. 2017;140(7):1900-1913. doi: 10.1093/brain/awx113.

[94] Shippy DC, Watters JJ, Ulland TK. Transcriptional response of murine microglia in Alzheimer's disease and inflammation. *BMC Genomics*. 2022;23(1):183. doi: 10.1186/s12864-022-08417-8.

[95] Wang ZT, Zhong XL, Tan MS, et al. Association of lectin-like oxidized low density lipoprotein receptor 1 (OLR1) polymorphisms with late-onset Alzheimer disease in Han Chinese. *Ann Transl Med*. 2018;6(10):172. doi: 10.21037/atm.2018.04.31.

[96] Masuda T, Tsuda M, Yoshinaga R, Tozaki-Saitoh H, Ozato K, Tamura T, Inoue K. IRF8 is a critical transcription factor for transforming microglia into a reactive phenotype. *Cell Rep*. 2012;1:334-40. doi: 10.1016/j.celrep.2012.02.014.

[97] Amadio S, Parisi C, Montilli C, Carrubba AS, Apolloni S, Volonté C. P2Yreceptor on the verge of a neuroinflammatory breakdown. *Mediators Inflamm*. 2014; 2014:975849. doi: 10.1155/2014/975849.

[98] Haley MJ, Brough D, Quintin J, Allan SM. Microglial Priming as Trained Immunity in the Brain. *Neuroscience*. 2019;405:47–54. doi: 10.1016/j.neuroscience.2017.12.039.

[99] Stefansson H, Ophoff RA, Steinberg S, et al. Common variants conferring risk of schizophrenia. *Nature.* 2009;460:744-7. doi: 10.1038/nature08186.

[100] Zheng J, Erzurumluoglu AM, Elsworth BL, et al. LD Hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. *Bioinformatics*. 2017;33:272. doi: 10.1093/bioinformatics/btw613.

[101] Dennison CA, Legge SE, Pardiñas AF, Walters JTR. Genome-wide association studies in schizophrenia: Recent advances, challenges and future perspective. *Schizophr Res*. 2020;217:4-12. doi: 10.1016/j.schres.2019.10.048.

[102] Farrell MS, Werge T, Sklar P, et al. Evaluating historical candidate genes for schizophrenia. *Molecular Psychiatry.* 2015;20:555-62. doi: 10.1038/mp.2015.16.

[103] Trowsdale J, Knight JC. Major histocompatibility complex genomics and human disease. *Annu Rev Genomics Hum Genet.* 2013;14:301-23. doi: 10.1146/annurev-genom-091212-153455.

[104] Yang Y, Chung EK, Zhou B, et al. Diversity in intrinsic strengths of the human complement system: serum C4 protein concentrations correlate with C4 gene size and polygenic variations, hemolytic activities, and body mass index. *J Immunol*. 2003;171:2734-45. doi: 10.4049/jimmunol.171.5.2734.
[105] Sekar A, Bialas AR, de Rivera H, et al. Schizophrenia Working Group of the Psychiatric Genomics Consortium, Daly MJ, Carroll MC, Stevens B, McCarroll SA. Schizophrenia risk from complex variation of complement component 4. *Nature*. 2016;530:177-83. doi: 10.1038/nature16549.
[106] Boulanger LM. Immune Proteins in Brain Development and Synaptic Plasticity. *Neuron*. 2009;64:93-109. doi: 10.1016/j.neuron.2009.09.001.
[107] Goddard CA, Butts DA, Shatz CJ. Regulation of CNS synapses by neuronal MHC class I. *Proc Natl Acad Sci U S A*. 2007;104:6828-33. doi: 10.1073/pnas.0702023104.
[108] Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ. Functional Requirement for Class I MHC in CNS Development and Plasticity. *Science.* 2000;290:2155-9. doi: 10.1126/science.290.5499.2155.
[109] Rački V, Petrić D, Kučić N, Gržeta N, Jurdana K, Rončević-Gržeta I. Cortical gray matter loss in schizophrenia: Could microglia be the culprit? *Med Hypotheses*. 2016;88: 18-21. doi: 10.1016/j.mehy.2015.12.021.
[110] Kamitaki N, Sekar A, Handsaker RE, et al. Complement genes contribute sex-biased vulnerability in diverse disorders. *Nature*. 2020;582:577-81. doi: 10.1038/s41586-020-2277-x.
[111] Lewis S. Microglia go pruning. *Nat Rev Neurosci*. 2011;12:492-3. doi: 10.1038/nrn3095.
[112] Osimo EF, Beck K, Reis Marques T, Howes OD. Synaptic loss in schizophrenia: a meta-analysis and systematic review of synaptic protein and mRNA measures. *Mol Psychiatry*. 2019;24:549-61. doi: 10.1038/s41380-018-0041-5.
[113] Han M, Zhang J chun, Hashimoto K. Increased Levels of C1q in the Prefrontal Cortex of Adult Offspring after Maternal Immune Activation: Prevention by 7,8-Dihydroxyflavone. *Clinical Psychopharmacology and Neuroscience.* 2017;15:64-7. doi: 10.9758/cpn.2017.15.1.64.
[114] Perron H, Hamdani N, Faucard R, et al. Molecular characteristics of Hu-man Endogenous Retrovirus type-W in schizophrenia and bipolar disorder. *Transl Psychiatry*. 2012;2:e201. doi: 10.1038/tp.2012.125.
[115] Gruchot J, Kremer D, Küry P. Human endogenous retroviruses: ammunition for myeloid cells in neurodegenerative diseases? *Neural Regen Res.* 2020;15:1043-4. doi: 10.4103/1673-5374.270311.
[116] Pouget JG, Gonçalves VF, Spain SL, et al. Genome-Wide Association Studies Suggest Limited Immune Gene Enrichment in Schizophrenia Compared to 5 Autoimmune Diseases. *Schizophr Bull*. 2016;42:1176-84. doi: 10.1093/schbul/sbw059.
[117] Dudbridge F. Power and Predictive Accuracy of Polygenic Risk Scores. *PLoS Genet*. 2013;9:e1003348. doi: 10.1371/annotation/b91ba224-10be-409d-93f4-7423d502cba0.
[118] Horsdal HT, Agerbo E, McGrath JJ, et al. Association of Childhood Exposure to Nitrogen Dioxide and Polygenic Risk Score for Schizophrenia With the Risk of Developing Schizophrenia. *JAMA Netw Open*. 2019; 2:e1914401-e1914401. doi: 10.1001/jamanetworkopen.2019.14401.
[119] Genc S, Zadeoglulari Z, Fuss SH, Genc K. The adverse effects of air pollution on the nervous system. *J Toxicol*. 2012;2012:782462. doi: 10.1155/2012/782462.
[120] Babadjouni R, Patel A, Liu Q, et al. Nanoparticulate matter exposure results in neuroinflammatory changes in the corpus callosum. *PLoS One.* 2018;13:e0206934. doi: 10.1371/journal.pone.0206934.
[121] Cheng H, Saffari A, Sioutas C, Forman HJ, Morgan TE, Finch CE. Nanoscale particulate matter from urban traffic rapidly induces oxidative stress and inflammation in olfactory epithelium with concomitant effects on brain. *Environ Health Perspect*. 2016;124:1537-46. doi: 10.1289/EHP134.
[122] Gruzieva O, Merid SK, Gref A, et al. Exposure to Traffic-Related Air Pollution and Serum Inflammatory Cytokines in Children. *Environ Health Perspect*. 2017;125:067007. doi: 10.1289/EHP460.
[123] Bos I, De Boever P, Emmerechts J, et al. Changed gene expression in brains of mice exposed to traffic in a highway tunnel. *Inhal Toxicol*. 2012;24:676-86. doi: 10.3109/08958378.2012.714004.
[124] Woodward NC, Levine MC, Haghani A, et al. Toll-like receptor 4 in glial inflammatory responses to air pollution in vitro and in vivo. *J Neuroinflammation.* 2017;14(1):84. doi: 10.1186/s12974-017-0858-x.

[125] Schwarz E, Maukonen J, Hyytiäinen T, et al. Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment re-sponse. *Schizophr Res*. 2018;192:398-403. doi: 10.1016/j.schres.2017.04.017.

[126] Zheng P, Zeng B, Liu M, et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Sci Adv.* 2019;5:eaau8317. doi: 10.1126/sciadv.aau8317. Erratum in: Sci Adv. 2019 Jun 21;5(6):eaay2759.

[127] Erny D, Hrabě de Angelis AL, Jaitin D, et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci*. 2015;18(7):965-77. doi: 10.1038/nn.4030.

[128] Thion MS, Low D, Silvin A, et al. Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. *Cell.* 2018;172(3):500-516.e16. doi: 10.1016/j.cell.2017.11.042.

[129] Pearce BD. Schizophrenia and viral infection during neurodevelopment: a focus on mechanisms. *Molecular Psychiatry.* 2001;6:634-46. doi: 10.1038/sj.mp.4000956.

[130] Brown AS, Derkits EJ. Prenatal infection and schizophrenia: A review of epidemiologic and translational studies. *AM J Psychiatry*. 2010;167:261-80. doi: 10.1176/appi.ajp.2009.09030361.

[131] Sierra A, Gottfried-Blackmore A, Milner TA, McEwen BS, Bulloch K. Steroid hormone receptor expression and function in microglia. *Glia*. 2008;56:659-74. doi: 10.1002/glia.20644.

[132] Catale C, Gironda S, Iacono L Lo, Carola V. Microglial Function in the Effects of Early-Life Stress on Brain and Behavioral Development. *Journal of Clinical Medicine.* 2020;9:468. doi: 10.3390/jcm9020468.

[133] Weinstock M. The long-term behavioural consequences of prenatal stress. *Neurosci Biobehav Rev*. 2008;32:1073-86. doi: 10.1016/j.neubiorev.2008.03.002.

[134] Pugliese V, Bruni A, Carbone EA, et al. Maternal stress, prenatal medical illnesses and obstetric complications: Risk factors for schizophrenia spectrum disorder, bipolar disorder and major depressive disorder. *Psychiatry Res*. 2019;271:23-30. doi: 10.1016/j.psychres.2018.11.023.

[135] Bronson SL, Bale TL. Prenatal Stress-Induced Increases in Placental Inflammation and Offspring Hyperactivity Are Male-Specific and Ameliorated by Maternal Antiinflammatory Treatment. *Endocrinology*. 2014;155:2635-46. doi: 10.1210/en.2014-1040.

[136] Dahlgren J, Samuelsson AM, Jansson T, Holmäng A. Interleukin-6 in the Maternal Circulation Reaches the Rat Fetus in Mid-gestation. *Pediatric Research.* 2006;60:147-51. doi: 10.1203/01.pdr.0000230026.74139.18.

[137] Gumusoglu SB, Fine RS, Murray SJ, Bittle JL, Stevens HE. The role of IL-6 in neurodevelopment after prenatal stress. *Brain Behav Im*mun. 2017;65:274-83. doi: 10.1016/j.bbi.2017.05.015.

[138] Färber K, Pannasch U, Kettenmann H. Dopamine and noradrenaline control distinct functions in rodent microglial cells. *Molecular and Cellular Neuroscience*. 2005;29:128-38. doi: 10.1016/j.mcn.2005.01.003.

[139] Zhang W, Jiang J, Xu Z, et al. Microglia-containing human brain organoids for the study of brain development and pathology. *Mol Psychiatry*. 2024; 8:96-107. doi: 10.1038/s41380-022-01892-1.

[140] Xu R, Li X, Boreland AJ, et al. Human iPSC-derived mature microglia retain their identity and functionally integrate in the chimeric mouse brain. *Nature Commun.* 2020;11:1-16. doi: 10.1038/s41467-020-15411-9.

[141] Müller N. COX-2 Inhibitors, Aspirin, and Other Potential Anti-Inflammatory Treatments for Psychiatric Disorders. *Front Psychiatry*. 2019; 10:375. doi: 10.3389/fpsyt.2019.00375.

[142] Çakici N, Van Beveren NJM, Judge-Hundal G, Koola MM, Sommer IEC. An update on the efficacy of anti-inflammatory agents for patients with schizophrenia: a meta-analysis. *Psychol Med*. 2019;49:2307-19. doi: 10.1017/S0033291719001995.

[143] A Costanza, M Baertschi, K Weber, A Canuto. Neurological diseases and suicide: from neurobiology to hopelessness. *Rev Med Suisse*. 2015;11:402-5.

[144] Costanza A, Amerio A, Aguglia A, et al. When Sick Brain and Hopelessness Meet: Some Aspects of Suicidality in the Neurological Patient. *CNS Neurol Disord Drug Targets*. 2020;19:257-263. doi: 10.2174/1871527319666200611130804.

Table 1. Possible markers of disincorporated microglia in SCZ.

HLA-DR	MHC class II element constitutively expressed by microglia with regional heterogeneity [87]
CX3CR1	Regulation of microglia numbers/activation, synaptic pruning, and functional brain connectivity [57]
CSF1R	Regulation of microglia cell division, development, maturation, and survival. Classically considered an M3 microglia marker [31]
TMEM119	A stable and highly specific microglia marker, maintained even in the presence of CNS injury [84,85]
TREM2	Marker of myeloid cell recruitment during immune activation [88]
ITGAM	Adhesion and uptake of complement-coated molecules [89]
ITGAX/ CD11c	Marker of a homeostatic subset of microglia with a key role in myelinogenesis and IGF1 production in the developing brain [90]. Particularly expressed also by DAM and Alzheimer-associated microglia [91,92].
CD86/macrosialin	Co-stimulatory receptor for immune cell proliferation. Classically considered an M1 microglia marker [93]
OLR1	An inflammatory and immune system response gene [94], whose variations have been proposed as risk factors for AD [95].
IRF8	A critical transcription factor for the development of mature microglia [64] and for transforming microglia into a "reactive" phenotype [96].
P2Y12R	Regulation of microglial chemotaxis and neuro-glial junctions. A highly specific microglia marker [97].

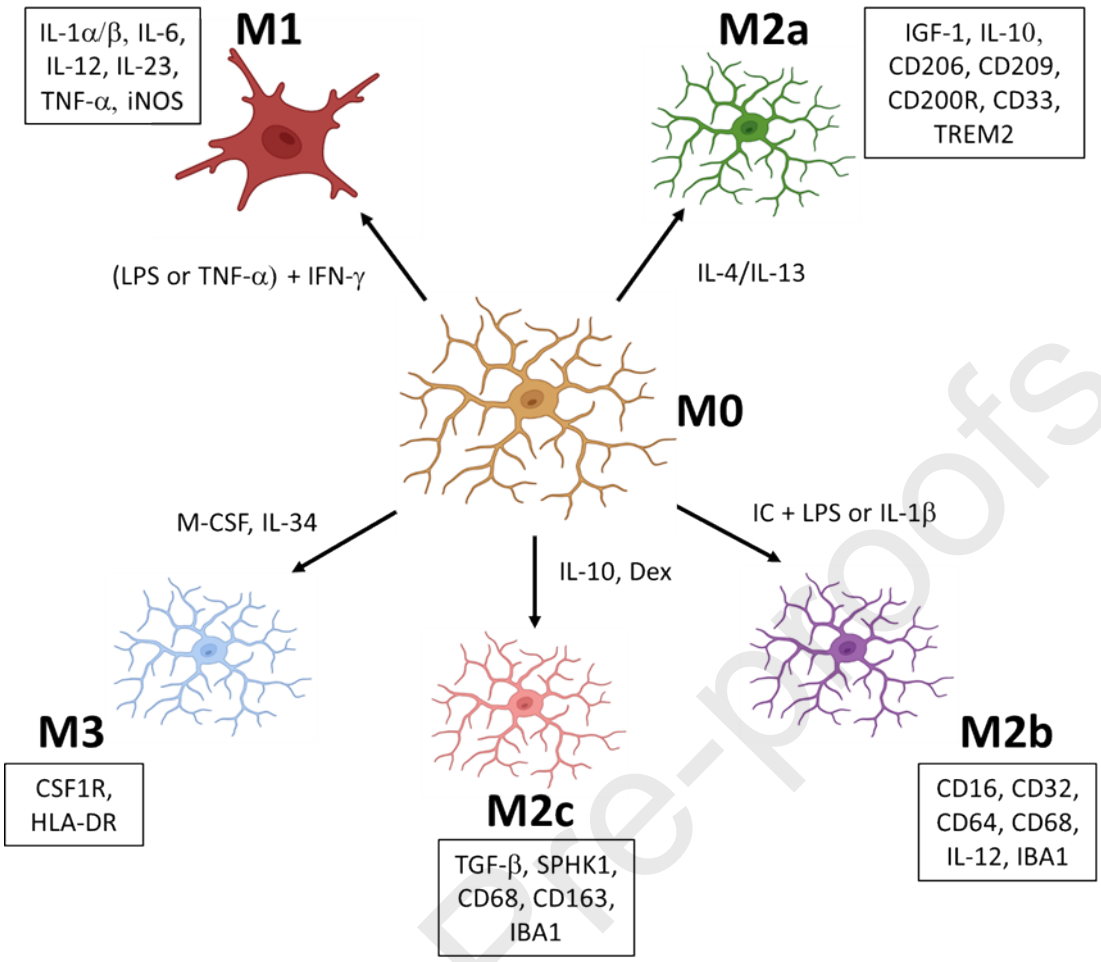

Figure 1 - Discrete forms of microglia molecular and functional polarization, according to M-polarization paradigm. IL. Although this framework represents an advancement in the attempt to capture the complexity of microglial morpho-physiology and pathology, it still presents overly discrete solutions that are disconnected from each other due to the lack of a concept that grounds continuity between different microglial entities. Interleukin; TNF-α:tumor necrosis factor-α; iNOS: inducible nitric oxide synthase; LPS: lipopolysaccharides; INF-γ: interferon-γ; IGF-1: insuline-like growth factor 1; TREM2: triggering receptor expressed on myeloid cells 2; CSF1R: colony-stimulating factor-1; HLA-DR: human leukocyte antigen DR; TGF-β: Transforming Growth Factor-β; SPHK1: Sphingosine Kinase 1; IBA1: ionized calcium binding adaptor molecule 1.

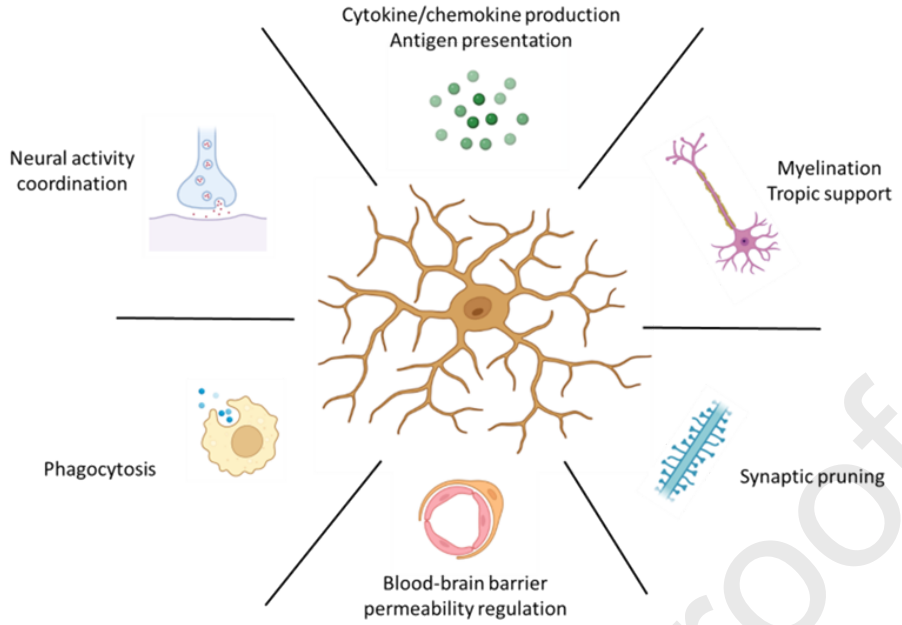

Figure 2 - Different possible expressions of functional coupling between microglia and neuroglial environment with associated aspects of microglia activity. All types of homeostatic coupling are collectively resumed under the continuous concept of incorporation.

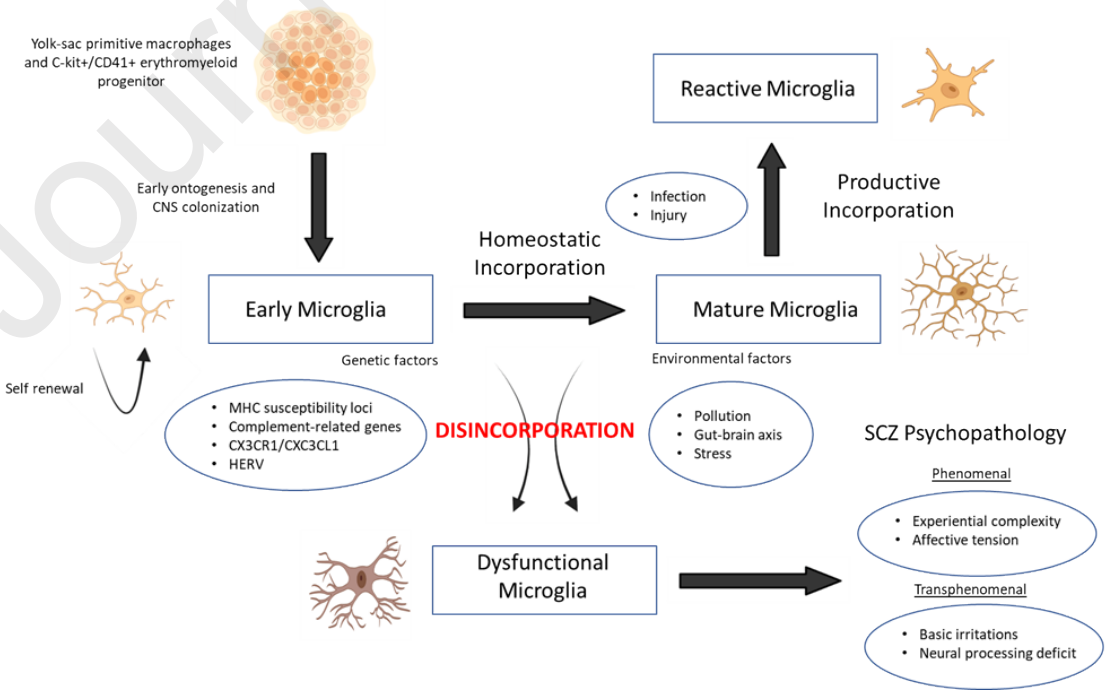

Figure 3 - Concise diagram, echoing the present theoretical proposal on the possible role of disincorporated microglia in the pathogenesis of SCZ. From top left to bottom right: due to exclusive local self-renewal, colonization of the CSN by microglial progenitors quite sharply defines the basic characters of microglial subpopulations at each further individual life stage. Primitive genetic vulnerability and early priming effects affecting microglial progenitors or immature microglia may promote a subsequent event-process of disincorporation later in life, associated with further environmental perturbations (i.e., double/multiple-hit model). If adequate incorporation is also necessary to optimize online, experience-driven neurocomputation, the disincorporated condition should produce a fundamental deficit in basic information processing, progressively exposing the subject, on the experience side, to a primitive state of basic irritation. Basic irritation and neural processing deficits represent the two sides of the same transphenomenal fact, grounded neurobiologically on primitive noninflammatory alterations and on which further phenomenal experiences may arise, during the early stages of SCZ development.

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Fundamental disincorporation and early non-inflammatory microglia alterations: possible bridging phenomena between neurobiology and psychopathology in schizophrenia

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