

1 **Microglia and Brain Plasticity in Acute Psychosis and Schizophrenia** 2 **Illness Course: a Meta-review**

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19 **Abstract**

20 **OBJECTIVE:** Schizophrenia poses a tremendous health, social, and economic burden upon patients
21 and society, indicating current treatment options remain inadequate. Recent findings from several
22 lines of evidence have pointed to the importance of immune system involvement in not only
23 premorbid neurodevelopmental, but also subsequent symptom generation and ageing processes of
24 brain change in schizophrenia. In this meta-review, we use the summarized evidence from recent
25 quantitative systematic reviews and meta-analyses of several subspecialties to critically evaluate the
26 hypothesis that immune-related processes shape the symptomatic presentation and illness course of
27 schizophrenia, both directly and indirectly through altered neuroplasticity.

28 **METHODS:** We performed a data search in PubMed for English-language systematic reviews and
29 meta-analyses from 2010 to 2017. The methodological quality of the systematic reviews was assessed
30 with the AMSTAR instrument. In addition, we review in this paper 11 original publications on TSPO
31 PET imaging in schizophrenia.

32 **RESULTS:** We reviewed 26 systematic reviews and meta-analyses. Evidence from clinical
33 observational studies of inflammatory or immunological markers and randomized controlled drug

34 trials of immunomodulatory compounds as add-on in the treatment of schizophrenia suggests
35 psychotic exacerbations are accompanied by immunological changes different from those seen in
36 non-acute states, and that the symptoms of schizophrenia can be modified by compounds such as
37 NSAID and minocycline. Information derived from *post-mortem* brain tissue analysis and PET
38 neuroimaging studies to evaluate microglial activation have added new perspectives to the available
39 evidence, yet these results are very heterogeneous. Each research domain comes with unique
40 opportunities as well as inherent limitations. A better understanding of the (patho-)physiology of
41 microglial cells and their role in neuroplasticity is key to interpreting the immune-related findings in
42 the context of schizophrenia illness exacerbations and progression.

43 CONCLUSIONS: Evidence from clinical studies analyzing patients' blood and cerebrospinal fluid
44 samples, neuroimaging and *post-mortem* brain tissue suggests that aberrant immune responses may
45 define schizophrenia illness' course through altered neuroplasticity representing abnormal ageing
46 processes. Most findings are however prone to bias and confounding, and often nonspecific to
47 schizophrenia, and a multidisciplinary translational approach is needed to consolidate these findings
48 and link them to other schizophrenia hypotheses.

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1. Introduction

Schizophrenia is a devastating and complex central nervous system (CNS) disorder that affects approximately 1% of the world population. Despite antipsychotic drugs being available for several decades, personal and societal costs remain highly expensive: the World Health Organization estimated that the direct costs of schizophrenia range between 7% and 12% of the gross national product in Western countries (1). Current treatment options are inadequate, both due to side-effects that disable patients and threaten their compliance to treatment (2) as well as the drugs' lack of efficacy evidenced by the absence of clinically relevant improvements on negative and cognitive symptoms, and up to one third of schizophrenia patients not responding at all (3-5). Acute psychosis is common and relapse prevention represents an important treatment issue in schizophrenia. An estimated 82% of patients have an illness relapse within 5 years after recovery from first episode psychosis (FEP), and a majority will experience multiple relapses throughout their illness course(6). Furthermore, an high relapse rate is associated with poorer outcomes, including more treatment-resistant symptoms, cognitive deficits and functional disability(7, 8).

These problems emphasize the urgent need for novel therapeutic approaches implicating the identification of new pathophysiological substrates. The illness is syndromic, of heterogeneous presentation and course and while there are well-established criteria in place for making the diagnosis of schizophrenia, the causes are still under debate. This is partly due to the complexity of this human disease that cannot be wholly replicated in animal models (9), as well as the methodological difficulties related to studying brain functionality in living patients. With increased understanding of heritability and ongoing brain changes, a vulnerability-stress model (sometimes described as a two-hit model) has become a prominent hypothesis to explain the pathophysiology of schizophrenia, in which an interaction of genetic, epigenetic and environmental risk factors is assumed to result in brain abnormalities that continue to generate abnormal responses throughout life upon exposure to relevant stressors (10). This presents a modification of the original neurodevelopmental hypothesis which proposed that "a fixed lesion early in life interacts with normal brain maturational events that occur much later" (11). Indeed, it now appears that the neurodevelopmental concept should be extended to encompass altered ongoing neuroplasticity (12-14) creating a background of susceptibility for new insults later in life to catalyze neurodegenerative effects.

In the late 20th century, the investigation of astrogliosis as hallmark for an inflammatory reaction in the schizophrenic brain combined with the expansion of neuropsychiatric genetics shape the debate about the etiology of schizophrenia. The absence of neuroinflammation and the presence of heritable candidate genes was thought to indicate a neurodevelopmental origin and a static rather than a neurodegenerative disease course. Although the earliest study reported the presence of low-grade inflammation (15), subsequent efforts did not replicate this finding. Consequently, the consensus was that reactive astrogliosis is absent in schizophrenia. However, the refinements of immunohistochemistry and modern stereological microscopy have yielded evidence for glial changes and, at the same time, brain imaging studies have increasingly demonstrated that the schizophrenic brain is not static during its clinical course. Over the last decades, immune system involvement has increasingly been implicated in the pathophysiological processes in schizophrenia (16-18). It has been hypothesized to be a driving factor behind both psychotic relapses and the macroscopic brain changes

that occur in schizophrenia, including the characteristic enlarged ventricle size and reductions in grey matter volume, whole-brain volume, and white matter anisotropy (14).

The field has now advanced enough for several major meta-analyses and systematic reviews to have come out in recent years, each compiling the evidence derived from individual studies and large groups of subjects in a variety of sub-disciplines contributing to this work. This meta-review aims to condense the evidence that immunological factors are critically involved in the schizophrenia illness course - modifying symptomatology and prognosis due to progressive brain changes. We critically appraise the current literature, identifying the opportunities and methodological limitations arising from each of the different lines of evidence. We identify areas where crucial information is still lacking and defend the position that opportunities can emerge by adopting a multidisciplinary translational approach to consolidate current findings and link this field of research to other key hypotheses of schizophrenia research. As the schizophrenia psychoneuroimmunology literature is very large, the scope of this review is necessarily limited. The focus will be on specific topics in the schizophrenia literature on neuroinflammation: (1) *in vivo* neuroimaging and (2) neuropathological studies of microglial activation; (3) systemic inflammation and its link to psychosis; (4) clinical trials of immunomodulatory drugs as add-on treatment and (5) neuroplasticity. Other relevant domains such as epidemiological studies, genetic studies, animal studies and clinical studies on changes in related mechanisms such as the kynurenine pathway and oxidative stress markers have their own sub-literature, and will not be dealt with here.

2. Methods

We undertook a qualitative "review of reviews" outlining the summarized evidence for immune system involvement in the illness course of schizophrenia. The PubMed database was searched for English-language publications from January 2010 to August 2017. The different search strings employed per domain are detailed below. For each of these, the PubMed filter 'Article Type' was set to categories 'Systematic Review' and 'Meta-analysis'. Besides the articles found through the PubMed database, a manual review of reference lists of included articles was performed to identify additional papers. A consensus was reached among authors on the studies retained or discarded on the basis of the following inclusion and exclusion criteria. The included articles 1) were meta-analyses and systematic quantitative reviews; 2) were investigating immune-related alterations in the blood, cerebrospinal fluid (CSF) or brain 3) of patients meeting DSM-IV or DSM-V criteria for the schizophrenia spectrum. All included studies are summarized in Table 1. We excluded publications focusing on preclinical research, or premorbid immunological risk factors such as epidemiological or genetic background risks. Articles were excluded if they: 1) were narrative or qualitative review articles 2) were reviews on topics outside the scope of this paper (as indicated in the inclusion criteria); 3) were reviews of *in vitro* or animal studies; 4) were reviews of genome or gene expression studies; 5) were reviews of epidemiological risk factors for schizophrenia; or 6) were not published in English. We assessed the methodological quality of the systematic reviews using the AMSTAR instrument (19); scores are presented in Table 1.

Meta-analyses of immunological or inflammatory changes in the blood, CSF and brain of schizophrenia patients were identified using the following search string: *schizophrenia[Title/Abstract] AND (*inflamm*[Title/Abstract] OR *immune*[Title/Abstract] OR cytokine*[Title/Abstract] OR chemokine*[Title/Abstract] OR *glia*[Title/Abstract])*. 82 articles were retrieved, of which 15 were included. Other studies were excluded because they did not study schizophrenia patients (n=15); concerned genome or gene expression studies (n=11); were narrative or qualitative review papers (n=13); or were outside the scope of this paper (n=24). Two additional articles were added manually after review of the reference list.

To identify meta-analyses pertaining to randomized clinical trials (RCTs) of anti-inflammatory agents in schizophrenia, the following search string was used: *schizophrenia[Title/Abstract] AND (RCT*[Title/Abstract] OR randomized[Title/Abstract]) AND (minocycline[Title/Abstract] OR NSAID[Title/Abstract] OR *inflamm*[Title/Abstract])*. This generated 11 articles, of which 8 were included. 3 studies were excluded for the following reasons: does not concern clinical trials (n=1); is a narrative review (n=1); compound is outside the scope of paper (n=1).

Because no meta-analysis or systematic review could be identified on positron emission tomography (PET) studies of microglial activation in schizophrenia, we chose to add the eleven original studies to this paper, as we believe this is an important sub-discipline in this research field. These papers were identified using a Pubmed and Medline search executed in August 2017 without limitation of time, language or publication type. The search string combined following terms: *(schizophrenia[Title/Abstract] OR psychosis[Title/Abstract]) AND (TSPO*[Title/Abstract] OR Translocator[Title/Abstract] OR *PK11195[Title/Abstract] OR *PBR*[Title/Abstract])*.

3. Microglial activation influencing the course of schizophrenia

The hallmark of neuroinflammation is the activation of microglia, the resident immune cells in the CNS (20), which are central to the current immune-related hypothesis in schizophrenia. Microglia are critically involved in the organization of the neuronal network during brain development (21) by primarily pruning excess synapses (22). Experimental models of schizophrenia showed that maternal infection during embryogenesis contributes to the presence of activated microglia in the offspring (23). This suggests that neonatal infections might induce perinatal microglia priming (24), a process in which highly sensitized microglia lead to an exaggerated response by example during the maturation processes occurring at the adolescent age and which can result in behavioral changes (25-27). This has led to the so called “microglia hypothesis” of schizophrenia, positioning exaggerated microglial activation as a key factor in the etiology of schizophrenia (17). In this hypothesis, exposure to inflammatory responses and/or genetically rooted excessive synapse pruning in the perinatal period (28) triggers an activated or primed state of microglia in adulthood (17), leading to ongoing systemic and central abnormal immune responses which drive schizophrenia symptoms such as psychotic episodes, and cause an unfavorable illness course, with progressive loss of function.

The presence of such ongoing microglial activation in schizophrenia patients has been supported both by some *post-mortem* studies as well as a few *in vivo* PET imaging studies using specific ligands (29, 30) that visualize and quantify microglial activation.

3.1. Neuroimaging studies on microglial activation

PET radioligands for microglial activation have recently been developed allowing for the first time functional imaging of neuroinflammation *in vivo*. These radiotracers selectively bind to the 18-kDa translocator protein (TSPO; previously called peripheral benzodiazepine receptor), which is expressed on the outer mitochondrial membrane of microglia in their activated states. Interestingly, although the (patho-)physiological function of the TSPO, which is related to cholesterol transport for steroid production in microglia, is not well understood (31), these tracers have been proven very useful and are now increasingly being used for the prospective *in vivo* study of human microglial activation in several CNS conditions. This means the evolution of early and late inflammatory changes can be followed over months and correlated with symptom severity in clinical longitudinal studies. The technique also allows to monitor the effects of treatment and to evaluate new anti-inflammatory or neuroprotective strategies. The oldest and most frequently used tracer is PK11195 radiolabeled with carbon-11, but its high lipophilicity and non-specific binding are thought to impede accurate quantification of tracer uptake. Since then, several newer second-generation tracers have emerged for use with either carbon-11 or fluor-18 labelling, boasting an improved pharmacokinetic profile and an up to 80-fold higher specific binding as demonstrated by animal blocking studies(32). Second generation tracers radiolabeled with 18-F, which has a longer half-life, have made this technique available for nuclear imaging centers without cyclotron. although their use is complicated by the existence of a polymorphism (*rs6971*) in humans that occurs in 30% of the European population. Therefore, genotyping of study subjects at this locus is essential to allow interpretation of TSPO PET studies, by stratifying between genetic groups and exclusion of low-affinity subjects.

As a meta-analysis is lacking for this specific research domain, the results of the existing studies are summarized in Table 2. Interestingly, whereas some PET studies have revealed that activated microglia are present in frontal and temporal lobes and total grey matter in: participants at ultra-high risk of psychosis (33), patients within the first 5 years of disease onset (34) or during a psychotic state (35); other PET studies have shown no difference in microglial activation between healthy controls and patients in various clinical states (ultra-high risk, first-episode psychosis, after a recent diagnosis of schizophrenia or in chronic phases of the illness) (36-41) or even decreased uptake in first-episode medication-naïve patients (42). With four new negative papers published in the last year (39-41, 43), it seems this field is experiencing an episode of catharsis after the initial enthusiasm that accompanied the first positive exploratory results almost ten years earlier, and it is becoming increasingly unclear whether there is indeed enhanced TSPO uptake in schizophrenia.

One major drawback of this new technology is that it comes with considerable technical requirements. First, the kinetic properties of the TSPO tracers currently require complex dynamic scanning protocols (60-125 minutes PET scan, often with an arterial line) which are considered a challenging and relatively invasive procedure towards patients. Secondly, because of the low resolution yielded by PET, co-localization with additional MRI images is essential to assess regional specific uptake. Taken together with the restriction for patients not to use benzodiazepines prior to the scan, these technical requirements arguably compromise the generalizability of PET study samples due to significant non-response bias in patient groups with the highest symptomatic burden and limit the future clinical use of the procedure.

For studies to be conducted effectively, adequate statistical power is necessary, yet because of the high technical and financial burden of these studies, sample sizes are typically rather small. Even after stratifying for genotype, high inter-subject variability is seen in imaging with TSPO tracers. This variability may be due to technical variables such as (i) the degree of tracer plasma protein binding, which is estimated to introduce up to 30% of variability in primary outcome measure V_T (33), (ii) a relatively low signal-to-noise ratio (particularly with first-generation tracers), or (iii) other unidentified technical or clinical confounders. Intra-subject variability, measured as test-retest reliability, depends upon a number of factors including scanner noise, input function noise, tracer kinetics and any fluctuating biological variability of the target within the inter scan period, with decreased reliability causing a loss of statistical power that is not accounted for in typical sample size calculations. For second-generation TSPO tracers, intraclass correlation coefficients have been reported around 0.76-0.92 for grey matter and 0.32-0.64 for white matter. (42) (Ottoy *et al*, submitted).

Furthermore, a complicated kinetic modelling procedure is involved, in which the kinetic properties of the tracer together with the chosen mathematical model and outcome measure have a large impact on the results, as evidenced by the inconsistent results found across the TSPO PET imaging studies. The dominant methodology has been to adopt a regular two tissue compartmental model (2TCM). However, a model which also accounts for endothelial and vascular TSPO binding (2TCM-1K) was recently shown to have improved performance in second generation TSPO tracers, probably because in these tracers a higher ligand specific TSPO affinity leads to considerable endothelial binding in the blood-brain barrier. Additionally, a less invasive reference tissue model was developed to study TSPO binding using first generation tracer PK11195 without arterial input function (39). A second important consideration is that the outcome measure is normally either BP (binding potential, i.e. a combined measure of the density of available receptors and the affinity of the radioligand to that receptor) or V_T (total volume of distribution; i.e. the ratio of the radioligand concentration in the region of interest to that in plasma at equilibrium). This means the increased uptake observed in a certain region of interest could reflect either increased non-specific tracer binding as well as a true biological signal(44). Strikingly in one of the studies, no difference was found between patients and controls when using the regular outcome measure V_T ; yet when the authors accounted for inter-subject variability in the input function by using DVR (distribution volume ratio, i.e. the ratio of the V_T in the region of interest to V_T in the whole brain) as their outcome measure, large effect sizes (Cohen's $d > 1.7$) were found (33). This alternative region-based approach has been subject to the criticism that it does not clarify whether the higher distribution volume ratio values in schizophrenia subjects are a result of greater microglial activation in the region of interest or of lesser microglial activation in other regions included in the denominator of the outcome measure, such as the cerebellum or white matter(45). The authors of the original study thereupon defended their choice by explaining DVR is superior to V_t when plasma input function measures are unreliable (such as is the case with TSPO tracers, which generally have $< 5\%$ free fraction in plasma) or subject to systematic group differences, for instance caused by elevated acute phase plasma proteins and cytokines in schizophrenia.(46)

Also remarkably, when one of the pioneering author groups in this field replicated their study in a larger patient sample and using a reference region instead of arterial input function, they no longer found a difference between patients and controls as they did in the original study (34, 39). Finally, the direct biological relationship between microglia and TSPO binding in vivo is still not fully understood. The exact cellular source of the upregulated TSPO binding in CNS pathology remains subject to discrepancy, caused by the extrapolation of in vitro data to the in vivo situation and differences in the models used (for instance lesions with or without blood-brain barrier damage) with literature reporting TSPO expression not only on microglial cells but also on astrocytes and some neuronal subtypes, as well as endothelial cells. In non-human primates endotoxin-induced systemic inflammation caused marked increases in the signal of a second generation TSPO tracer, confirmed post-mortem to be largely due to microglial binding (47). Although this animal model is not suitable to study the brain changes of schizophrenia patients - which are much more subtle than those in many “typical” inflammatory disorders and autoimmune disorders - the source of TSPO radioligand binding has not yet been studied in more relevant models such as the (maternal) Poly I:C viral mimetic challenge. Given the subtlety of these changes as well as the different cell types involved, it is possible to imagine a scenario in which small increases and decreases in different cell types could cancel each other out causing no diagnosis-related differences regarding TSPO- binding to be found despite biological changes being present.

Finally, even if increased TSPO binding truly reflects increased microglial activation, PET images do not provide the detailed morphological and functional data required to appreciate the function of microglia in pathophysiological states. Interpretation of such findings is therefore complex as they may reflect both a protective response, or a neuroinflammatory process which causes psychotic symptoms and/or neurodegenerative effects.

3.2. Neuropathological studies on microglia

A recent paper of two mega-analyses of combined micro-array data in brain and blood tissues re-emphasizes the raised expression of innate immune system genes in the brain but also in the blood of people with schizophrenia (48). However, anatomopathological investigation has been the gold standard for thorough characterization and investigation of human tissue. It offers the best possible resolution to evaluate changes at the cell level as well as interactions between microglial cells and other brain functional components such as neurons. Although data derived from *post-mortem* brain tissue of schizophrenia patients is scarce, an association between schizophrenia and microglial activation, particularly in white matter regions, has been observed (26). Yet a definitive statement cannot be made on whether neuroinflammation is present in schizophrenic *post-mortem* brain samples due to the significant number of negative studies and conflicting results that have been published by different groups with sometimes the use of the same microglial marker (37).

As the largest meta-analysis in this field, Trepanier *et al* reviewed a total of 119 articles on neuroinflammation in *post-mortem* schizophrenic brains, 22 of which looked at a range of microglial markers. Of these, 11 studies reported an increase in microglial markers in *post-mortem* brains, whereas 8 studies found no effect and 3 studies found a decrease in microglial markers. HLA-DR is the strongest risk factor for schizophrenia and a component of the major histocompatibility class II involved in “non-self” recognition, antigen presentation and the most used marker of microglial

activation in schizophrenia. The systematic review by Trepanier *et al* reported differential expression of HLA-DR by immunohistochemistry in 11 out of 13 *post-mortem* studies (9 studies reporting increased and 2 decreased expression).. The same review reported unchanged expression in 3 out of 3 studies using CD68, a marker of microglial lysosomes indicative of phagocytic microglia and involved in clearance of neuronal debris in neurodegeneration, and 2 out of 2 studies with Iba1 (ionised calcium-binding adapter molecule 1), a protein involved in membrane ruffling (49) and thus a marker associated with microglial motility, a function essential to brain surveillance (50).

More recently, Van Kesteren *et al* reviewed 41 studies on immune involvement in *post-mortem* schizophrenic brains, with 11 studies investigating microglia on 181 patients and 159 controls. A significant increase in microglia was observed (SMD = 0.69**) over all studies, yet again with significant heterogeneity. The authors also reported a significant increase in the overall expression of pro-inflammatory molecular components (14 studies on 330 patients and 323 controls; SMD = 0.37**).

It is worth noting that these two main meta-analyses included among their results a paper which demonstrated increased microglial staining and increases in IL1 β and TNF expression in the frontal cortex, but was retracted afterwards (51).

The most evident problem for this kind of study is the limited availability of patients' brain tissue. Since *post-mortem* research is by definition at the end of life, many clinical confounders related to the life and illness history of both patients and controls may complicate the interpretation of data. Known confounders include gender, age at death, use of medication etc. The cause of death should also be considered, as the presence of systemic inflammation, hematological malignancies or neurological illness may confound the results, as can suicide, which is common in schizophrenia and has been linked to the presence of elevated pro-inflammatory cytokines regardless of diagnosis (52). It is therefore essential that all tissue samples are accompanied by detailed clinical information and that the control group has been mindfully selected. Heterogeneity may also be explained in part by the qualitative vs. quantitative analysis, the brain region and cortical layer in which the markers are measured (37). Furthermore, there may be differences in the diagnosis methods, inclusion and exclusion criteria, storage conditions, and demographics variables among the brain banks which provide the brain samples.

Besides the microglial cells, structural and functional abnormalities in the other two glial cell types have also been studied in relation to inflammation in schizophrenia. For instance, evidence related to reduced numbers and impaired cell maturation of oligodendrocytes has been linked to white matter abnormalities and disturbed inter- and intra-hemispheric connectivity. Astrocyte dysfunction, as suggested by some studies demonstrating abnormal expression of a variety of astrocyte-related genes as well as S100B protein, may contribute to certain aspects of disturbed neurotransmission in schizophrenia.(53) However, these studies fall outside the scope of this paper and are not reviewed here.

4. Systemic inflammation and its association with psychosis in schizophrenia

327 Clinical research has focused to a great extent on peripheral immune system alterations in adult
328 schizophrenia patients. Clinical observational studies in adult patients are relatively easy to organize
329 and considered non-invasive, as blood sampling is often part of routine medical practice. The
330 resulting large sample sizes and a lower selection bias, imply study samples are generally more
331 representative of clinical populations. Furthermore, the identification of potential peripheral
332 biomarkers will be useful to confirm diagnostic or prognostic questions in clinical practice.

333 To assess the role of immunity in illness symptomatology, it is important to carefully differentiate
334 patient groups according to their clinical characteristics. This was done for the first time by Miller *et al*
335 *al*, who reviewed published data of levels of cytokines (or cytokine receptors or antagonists), and
336 later also of blood monocytes or lymphocytes, *toxoplasma gondii* and auto-antibody titers in patients
337 with schizophrenia or related psychotic disorder and healthy control subjects in a series of meta-
338 analyses. Their analyses included both cross-sectional studies and longitudinal studies in patients with
339 an acute exacerbation of psychosis at baseline and following a period of antipsychotic treatment. All
340 studies included in this paper met strict criteria for defining patients' clinical status (as either acutely
341 relapsed inpatients (AR), first-episode psychosis (FEP), stable medicated outpatients (SO) or
342 treatment resistant psychosis (TR)) and the meta-analyses perform quite well on the AMSTAR scale.
343 Yet together, the meta-analyses published by this group of authors make up 6 out of the 12 meta-
344 analyses on blood inflammatory/immunological markers in schizophrenia (54-58).

345 Based on these meta-analyses, alterations in cytokines, chemokines, lymphocytes and oxidative stress
346 markers have been demonstrated in the blood of patients with schizophrenia during an acute psychotic
347 event which normalize with antipsychotic treatment ("state" markers IL-1 β , IL-6, TGF- β and
348 increased CD4/CD8 ratio), as opposed to other "trait" markers that remain elevated throughout the
349 stages of the illness (55, 59). In general, effect sizes for FEP are similar in direction and magnitude
350 to those in AR, indicating prior exposure to antipsychotic medication does not change the "acute"
351 profile. Interestingly, a similar finding was reported in patients with chronic schizophrenia infected
352 by *toxoplasma gondii*, with positive IgM antibody titers (a marker of acute/recent infection, or also
353 potentially persistent infection or reinfection, possibly with a different genotype) linked to acute
354 psychotic exacerbations (57). Thus it seems that differential patterns of immunological activation
355 exist in different "states" of the schizophrenia illness course, in which patients who are experiencing
356 an acute psychotic episode can be distinguished from those who are in symptomatic remission.

357 Yet the correlation between peripheral and brain inflammation is far from certain. Data from CSF
358 samples might be better at representing brain immunity changes than the peripheral blood samples;
359 however such CSF samples are much less readily available (58). In their meta-analysis, Wang *et al*
360 identified 16 studies of cytokines in CSF, but did not stratify according to patients' clinical state (58).
361 Although the authors cautioned that their findings needed to be interpreted with caution due to the
362 small numbers of studies and subjects, they reported that in schizophrenia many CSF alterations were
363 also concordant with those reported in the peripheral blood. Communication between the systemic
364 immune system and the brain and its consequence on microglia is a critical poorly understood
365 component of the inflammatory response to systemic disease (60). Systemic infections activate
366 neural and humoral pathways that communicate with the brain and initiate a coordinated set of
367 metabolic and behavioral changes (61). However, these adaptive responses may become maladaptive

when microglia have been “primed” by an ongoing pathology and respond to a systemic inflammatory challenge by switching their phenotype to an aggressive pro-inflammatory state (24), adversely affecting neuronal function and potentially leading to a psychotic decompensation through modulating effects of pro-inflammatory cytokines on neurotransmitter function (24).

Another recurring problem in the abovementioned clinical observational studies seems to be that although the effect sizes found in meta-analyses are moderate to large, for a majority of cytokines (or cytokine receptors or antagonists) assessed, there was significant heterogeneity in effect size estimates (55). Indeed it appears that due to the heterogeneity encountered, no individual assay seems apt to reliably differentiate between different patient groups in clinical practice, and large sample sizes are needed to detect these immunological disturbances which may reflect that they are either very low-grade and subtle differences, or they only occur in a subgroup of patients. The result is that both selection bias and methodological factors such as the choice of assay or analysis method and controlling for various confounding variables have a large impact on the results. In the meta-analysis on blood cytokine levels by Miller *et al*, 97% of studies controlled for age and gender as confounding factors, whereas the potential effects of race (41%), body mass index (35%), and smoking (24%) were often not considered (55). Furthermore, similarities have been found in the pattern of cytokine alterations during acute and chronic phases of illness in schizophrenia, bipolar disorder and major depressive disorder, pointing out the possibility of common underlying pathways that are not specific to schizophrenia (62).

5. Clinical trials of immunomodulatory drugs as add-on treatment for schizophrenia

Following the concept that neuroinflammation may play a central role in the symptomatology and prognosis of schizophrenia, novel therapeutic prospects have arisen which aim to modulate immune effects to influence schizophrenia disease course. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and celecoxib have been investigated for therapeutic intervention, as well as other compounds with anti-inflammatory properties which readily cross the blood-brain barrier such as davunetide (derived from activity-dependent neurotrophic protein (ANAP), a growth factor released by glial cells), fatty acids such as eicosapentaenoic acids and docosahexaenoic acids, estrogens, minocycline (a broad-spectrum tetracycline antibiotic with inhibitory effects on microglia), N-acetylcysteine (NAC), and even potent immunosuppressant drugs such as methotrexate. These drugs are being investigated as augmentation to antipsychotics in patients with a diagnosis of a schizophrenia spectrum disorders, and effects measured as change in symptom severity on the Positive and Negative Syndrome Scale (PANSS) or Brief Psychiatric Rating Scale (BPRS). The results of the most well-studied compounds (i.e. minocycline and NSAID) are summarized in Table 3. Well-designed clinical trials offer the clinical relevance the healthcare field and community are waiting for. Since these trials are run with immunomodulatory drugs that are already available on the market, they come with the additional advantage of immediate usability and well-known clinical profiles in the case of positive outcome.

A first meta-analysis on the effect of NSAIDs as add-on to antipsychotics was published by Sommer *et al* in 2012 and included 5 double-blind, randomized, placebo-controlled trials (Four studies on celecoxib, and 1 on acetylsalicylic acid), reporting on 264 patients. The authors reported a mean effect

size of 0.43, which was significant at $P = 0.02$ in favor of NSAIDs on total symptom severity (63). This paper was subsequently criticized by Nitta *et al*, who replicated the meta-analysis, adding 3 more unpublished studies to the analysis demonstrating an overall less convincing case (64). Nitta *et al* conducted subanalyses based on treatment setting and disease phase, demonstrating a significant improvement in PANSS total scores with NSAIDs in studies of inpatients (Hedges' $g = 0.44$, $P = 0.029$) and first-episode patients (Hedges' $g = 0.39$, $P = 0.048$), but not in outpatients and chronic patients (64).

In 2014, Sommer *et al* reviewed double-blind randomized placebo-controlled trials of a broad range of immunomodulatory compounds. Weak to moderate beneficial effects were reported with the use of aspirin (Hedges' $g = 0.3$, $P = 0.001$), NAC (Hedges' $g = 0.45$, $P = 0.009$), and estrogens (Hedges' $g = 0.9$, $P = 0.001$); while addition of celecoxib, EPA/DHA fatty acids, davunetide, and minocycline did not show efficacy (64, 65). That same year, a second meta-analysis by Oya *et al* was published reviewing RCTs with minocycline (66). Compared to Sommer *et al*, this meta-analysis included two more original studies and excluded one study which was only published as congress proceeding. In this meta-analysis, minocycline was superior to placebo for decreasing PANSS total scores (66). Finally, during the course of 2017, two more meta-analyses on RCTs of minocycline in schizophrenia were published around the same time. Both Solmi *et al* and Xiang *et al* demonstrated minocycline's superiority versus placebo for reducing PANSS total scores, negative symptom scores and general psychopathology scores. While standard mean deviations (SMDs) for the different outcome measures are quite similar across the last three meta-analyses, only in Xiang *et al* the effect on positive symptom scores reached significance level –probably due to the higher number of RCTs and study subjects included in this meta-analysis (67, 68). A third meta-analysis published in 2017 evaluated celecoxib as add-on for schizophrenia once more, this time demonstrating a significant improvement of total and positive symptoms scores (69).

Clearly the major limitation of these studies is that for each compound, the number of individual studies is relatively low. As a result, even though they have been well executed and gain the highest scores on the AMSTAR assessment, the outcomes within and between the different meta-analyses remain relatively heterogeneous. It seems therefore too early to make conclusions on the efficacy on symptom severity of schizophrenia of augmentation with anti-inflammatory agents. Stratifying results per symptom domain makes sense, as celecoxib seems to be more effective against positive symptoms, and minocycline against negative symptoms. Overall, we would argue that too little information is currently available about the clinical determinants of groups that may benefit from these treatments to optimize study cohort selection, as well as the nature of the immune alterations – causing the choice of study drug to be virtually unguided and ranging a complete spectrum from food supplements to potent immunosuppressant drugs.

6. The microglial phenotype

Besides the specific limitations of the studies mentioned above, one of the main problems underlying their limited clinical usefulness is our very limited basic knowledge about one of its key players in

human brain, the microglial cell. Our understanding about the physiology of microglia is derived mainly from studies of tissue macrophages (20). However, in contrast to the latter, microglia are derived from myeloid precursors migrating from the yolk sac to the CNS early in embryonic development (E8.5) (70), and throughout life reside behind the blood-brain barrier where they are difficult to study *in vivo*. They account for 0.5 to 16.5% of the total number of cells in the human brain depending of the region explored (71). Microglia are highly plastic cells (50) that can adapt their behavior and morphology to changes in their environment and adopt different profiles (20). This defines microglial activation as a spectrum, in which transient microglial activation can include adaptive and beneficial physiological and behavioral responses, whereas maladaptive immune responses can lead to neuronal dysfunction and tissue damage. Microglia are critically involved in the organization of the neuronal network (21). They have an important role in synaptic pruning (28, 72) during brain development as well as in adult life, optimizing synaptic communication (73). Microglia can develop a range of functional phenotypes beyond the classic M1 (classical activation; an activation state in which microglia would adopt a deleterious function) versus M2 (alternative activation; in which immune cells adopt a regulatory or tolerance-inducing profile) paradigm (20). However, it is the specific manner in which microglia are activated and the phenotype that they adopt that is important in determining the influence of microglia in neurodegeneration (60). Morphology does not indicate the function of microglia, which means that even if the presence of enhanced microglial activation in certain patient groups or disease states is proven unequivocally, we still do not know what this means in terms of microglial activity and whether to interpret this as beneficial or harmful. The activation may be pathological or part of an endogenous compensatory response to some other aspect of the disease process.

Neuropathological studies remain the gold standard to gain answers to these questions. Therefore, the use of a single microglial marker, often different between the *post-mortem* studies (Iba1, CD68 or HLA-DR), is not sufficient to identify the phenotype expressed by microglia. Furthermore, if their phenotype is related to a certain clinical state or illness phase, knowledge of the time course of the microglial activation is relevant. Adding to the complexity of microglia, some authors have argued that the microglial activation status is most probably a reflection of the history of life events (defined as innate immune memory (74)), including prenatal and *perimortem* influences, as well as an individual's genetic background (75), emphasizing the need for detailed clinical information in *post-mortem* or PET investigations of microglia.

7. Neuroplasticity and microglia

Neuroplasticity refers to the ability of the brain to develop and finetune its neural connections, including adjustments in response to changes in the environment, by alteration in the neurons or glial cells via cell division/apoptosis and synaptic/neurite remodeling. Microglia are key facilitators for neuronal plasticity during brain development (21, 22) and in adult life (76). Constant surveillance of the microenvironment by microglial processes (50) and their attraction to active rather than non-stimulated synapses imply that microglia might monitor the functional state of synapses leading to plastic changes in healthy adult brain (77). This could occur through remodeling of the extracellular spaces and elimination of synaptic elements, regulation of the neurotransmitters present in the

487 synaptic cleft such as glutamate, or by direct contact with synaptic elements (77, 78) via the
488 complement components (28).

489 Research, summarized in this paper, has identified alterations in the key actors and mechanisms
490 involved in neuroplasticity. A quantitative meta-analytic summary of studies focused on neuron
491 density provides support for the finding of altered neuron density in schizophrenia, with variation
492 dependent on age (14). The vertical micro-circuits within the cortex, known as minicolumns,
493 normally become thinner with age in controls (79), indicating a reduction in neuropil, but not in
494 schizophrenia (80). The role that microglia may play in this process is strongly suggested by the
495 finding that microglia are involved in synaptic pruning during healthy brain development (58). Given
496 that neuropil expansion is a correlate of synapse number, an abnormality of microglia may be
497 understood to be a basis for reduced synaptic and neuropil modulation.

498 The quantitative neuropathological changes in the cerebral cortex of schizophrenia, suggest that the
499 “reduced neuropil hypothesis” (81) applies in early life in schizophrenia, but subsequently it is
500 “reduced neuroplasticity” which leaves the cells and minicolumns relatively widely spaced in later
501 life, compared to the typical pattern of healthy control neuropil thinning. This is consistent with
502 neuroimaging data and offers a microanatomical explanation to account for many of the larger scale
503 functional–anatomical changes observed in structural neuroimaging studies (14), which have shown
504 that over time schizophrenia is associated with progressive decreases of whole brain volume and
505 whole brain grey matter (82), frontal grey and white matter, parietal white matter and temporal white
506 matter (83).

507 Consequently, an altered ageing trajectory is implicated as a factor in the pathogenesis of
508 schizophrenia. This hypothesis is supported by the finding that the decrease in brain-derived
509 neurotrophic factor (BDNF) which occurs in older age is amplified in schizophrenia. BDNF is a
510 regulator of microglial-mediated synaptic plasticity (84), suggesting a greater reduction in
511 neuroplasticity with advancing age in schizophrenia (85). The progressive anatomical abnormalities
512 of brain structure recorded in longitudinal studies indicating greater severity following the first
513 episode of illness, may be the cumulative effect of reduced neuroplasticity over time. One of the
514 consequences at the level of symptoms may be that a shift from positive symptoms to more negative
515 symptoms, which has been reported during the disease course, is consistent with the implications of
516 reduced plasticity – ie. negative symptoms represent a relative loss of functions, that may become
517 more dominant due to insufficient plasticity to keep up with ongoing cognitive demand, rather than
518 the presence of additional cognitive phenomena described as positive symptoms.

519 **8. Conclusion and future directions: multimodality as key to the future of** 520 **neuroinflammation research in schizophrenia**

521 The evidence from the psychoneuroimmunology research field converges on a progressive illness
522 model of schizophrenia in which primed microglia contribute to altered neuroplasticity, leading to
523 structural and chemical abnormalities that accumulate as patients age. This model integrates both
524 neurodevelopmental and neurodegenerative hypotheses in schizophrenia, as the combination of a pre-
525 or perinatal immunological vulnerability serves as a background for ongoing abnormal peripheral and

526 brain immune responses which throughout life constitute a basis for illness exacerbation and
527 progressive defects. Yet although the psychoneuroimmunology field provides a compelling
528 pathophysiological model, at this moment it has not attained a clinically significant level of relevance
529 to comprehensively explain the etiology, diagnosis or potential treatment of the disorder. In addition
530 many of the findings appear non-specific to schizophrenia. To enhance the clinical relevance of this
531 field, further links are needed to connect the immune-related hypotheses to other major research fields
532 and hypotheses of schizophrenia psychopathology.

533 Such an interesting new link between immune alterations and neuroplasticity, in which aberrant
534 microglial activation could cause reduced synaptic and neuropil modulation, has recently emerged,
535 but still needs to be comprehensively investigated in the human brain. Furthermore, knowledge on
536 the physiology and functional phenotypes of microglia, as well as their link with systemic
537 inflammation, is crucially insufficient to interpret current findings. We would therefore argue that
538 studies acting as a bridge between the preclinical and clinical studies are crucially missing. We
539 therefore advocate multimodal research studies that explore neuroinflammatory mechanisms both in
540 experimental models and schizophrenia patients; using *in vivo* multimodal imaging and analysis of
541 immunological markers and microarchitectural changes in *post-mortem* brain tissue as translational
542 means. In particular the underutilized human brain approach has a powerful translational nature in
543 complex neuropsychiatric conditions (9). Overcoming individual limitations, this combined approach
544 evidently generates added value and has a high feasibility, as it is supported by literature, preliminary
545 data and active involvement of highly skilled research teams situated within the field that already
546 possess the necessary know-how. The advent of a greater recognition of the role of the immune
547 system and its effects on neuroplasticity in schizophrenia will allow identification of potential novel
548 therapeutic avenues, thereby finally bringing the psychoneuroimmunology research field to its full
549 potential.

550

551 9. Tables

552 **Table 1: Summarized evidence of immune processes influencing the course of schizophrenia**

	Research methods	Meta-analysis (MA)/systematic review (SR)	N studies	N patients	AMSTAR score (out of 11)	Outcomes
Clinical studies on peripheral expression of biomarkers and cells	Case-control study on blood and CSF inflammatory markers and immune cells (patients versus controls OR comparison of patients in different states)	MILLER 2011 (55): MA blood cytokine studies	40	1324 SZ; 1154 C	9	Trait and state increases of inflammatory cytokines; - in schizophrenia patients blood <i>trait</i> markers are IL-12, IFN- γ , TNF α , sIL-2R, CRP, CD56 cells; <i>state</i> markers are IL-1b, IL6, TGF- β , CD4 cells; - in unmedicated FEP blood trait markers are TNF- α , IL-17, and IFN- γ state markers are IL-6 and IL-2, and possibly IL-1 β - in CSF increased IL-1 β , IL-6, IL-8.
		MILLER 2013 (56): MA blood lymphocyte studies	16	488 SZ; 525 C	9	
		TOURJMAN 2013 (86): MA blood cytokine studies and AP	23	762 SZ	6	
		MILLER 2014 (87): MA blood CRP studies	8	N/A	N/A	
		UPTHEGROVE 2014 (88): MA blood cytokine studies in drug-naïve FEP	23	570 SZ; 683 C	9	
		GUO 2015(89): MA blood cytokine studies	21	N/A	N/A	
		GOLDSMITH 2016 (62): MA blood cytokine studies acute – chronically ill	40	N/A	N/A	
		FERNANDES 2016 (90): MA blood CRP studies	71	85000 SZ+C	N/A	
		WANG & MILLER 2017 (58): MA CSF cytokine studies	16	N/A	N/A	
		CAPUZZI 2017 (91): MA blood cytokine studies on effect of AP in FEP drug-naïve patients	8	505 SZ	6	
	Case-control study on	EZEOKI 2013 (54): MA antibody titer studies	81	N/A	8	Increased prevalence of positive titers for 20 different autoantibodies

	auto-antibody titers	MONROE 2015 (57): MA T. gondii titer studies	16	2535 SZ; 1707 C	9	Increase in T gondii IgM antibodies in acute psychosis
Post-mortem studies	Case-control study on histological or molecular findings in <i>post-mortem</i> brain tissue	BAKHSI 2015 (14): SR of neuronal density studies	30	360 SZ; 390 C	4	Age-dependent increases in neuronal density Approximately half of <i>post-mortem</i> studies reported increased microglial markers Similar patterns of heightened innate immune gene expression in both brain and blood in schizophrenia.
		NAJJAR & PEARLMAN 2015 (30): SR white matter pathology and neuroinflammation studies	15	350 SZ; 346 C	5	
		TREPANIER 2016 (92): SR neuroinflammation markers	119	N/A	4*	
		HESS 2016 (48): 2 mega-analyses of microarray data from <i>post-mortem</i> prefrontal cortices and <i>ex-vivo</i> blood tissues	19 (brain) 25 (blood)	315 (brain) 578 (blood)	N/A	
		VAN KESTEREN 2017 (93): MA histological / molecular immunological parameter studies	41	783 SZ; 762 C	9*	
Neuro-imaging	Case-control study on PET imaging for microglial activation	Data summarized in Table 2	11	176 SZ; 175 C	N/A	Increased uptake of tracer with certain tracers and kinetic models in certain patient populations; results vary
Clinical trials	RCT with adjunctive anti-inflammatory drugs.	SOMMER 2012 (63): MA RCT NSAID	5	264 SZ	N/A	Results vary, significant effects have been found for aspirin, celecoxib, estrogens, NAC and minocycline
		NITTA 2013 (64): MA RCT NSAID	8	774 SZ	10	
		SOMMER 2014 (65): MA RCT anti-inflammatory agents	24	1938 SZ	8	
		OYA 2014 (66): MA RCT minocycline	4	330 SZ	10	
		SOLMI 2017 (68): MA RCT minocycline	6	413 SZ	11	

		XIANG 2017 (67): MA RCT minocycline	8	548 SZ	9	
		ZHENG 2017 (69): MA RCT celecoxib	8	626 SZ	9	

553 **Legend:** “SZ”=schizophrenia patient group; “C”=control group; “n”=number of subjects;
554 “FEP”=first episode psychosis patients; “MA”=meta-analysis; “SR”=systematic review; “N/A” =
555 not applicable/not available; “ES”=effect size; “RCT”=randomized clinical trial; “RR”=relative
556 risk; “CSF”=cerebrospinal fluid;
557 * Included results from a paper which was later retracted.
558
559

560 **Table 2: PET studies with TSPO tracer evaluating microglial activation in schizophrenia**
 561 **patients *versus* controls**

Author	n SZ	n C	Tracer	Model; Outcome measure	Clinical state <i>Total (T) and positive symptom scale (P) score (PANSS mean \pm SD unless otherwise specified)</i>	DOI (y)	Medication <i>% of patients on antipsychotics (AP); (mean CPZ)</i> <i>Benzodiazepines (BZD) excluded (duration)?</i>	Out- come
VAN BERCKEL 2008 (34)	10	10	[11C]PK11195	2TCM; BP	Undefined Symptom scores unavailable	3.1 ± 1.7	AP 100% BZD ?	SZ>C
DOORDUIN 2009 (35)	7	8	[11C]PK11195	2TCM; BP	Psychosis T 73.6 ± 13.3 P 19.7 ± 3.0	5 ± 6	AP 100% BZD excluded (3x t _{1/2})	SZ>C
BANATI & HICKIE 2009 (94)	16	8	[11C]PK11195	2TCM; BP	Undefined Symptom scores unavailable	Range 0.3-30	Information unavailable	SZ>C
TAKANO 2010 (36)	14	14	[11C]DAA1106	2TCM; BP	Chronic T 77.9 ± 20.1 P 19.1 ± 5.3	19 ± 12	AP 100% BZD excluded (<1m)	SZ=C
KENK 2015 (37)	16	27	[18F]FEPPA	2TCM; V _T	Psychosis T 70.2 ± 9.7 P 19.3 ± 2.2	15 ± 9	AP 100%; (300 CPZ) BZD excluded (duration?)	SZ=C
BLOOMFIELD 2015 (33)	14	14	[11C]PBR28	2TCM-1K; DVR	Undefined T 63.7 ± 18.1 P 17.0 ± 6.1	Undefined	AP ?% BZD excluded (duration?)	SZ>C
COUGHLIN 2016 (38)	12	14	[11C]DPA713	Undefined; V _T	Undefined T unavailable P (SAPS) 3.8 ± 2.5	2.2 ± 1.4	AP ?%; (474.5 CPZ) BZD excluded (6m)	SZ=C
VAN DER DOEF 2016 (39)	19	17	[11C]PK11195	Reference tissue; BP	Undefined T 53 ± 10 P 12 ± 4	1.3 ± 1.1	AP 79% BZD excluded (4w)	SZ=C

COLLSTE 2017 (42)	16	16	[11C]PBR28	2TCM; V _T	FEP drug naïve T 77.4 ± 18.3 P 20.3 ± 4.9	0.7 ± 0.8	AP 0% BZD not excluded	SZ<C
HAFIZI 2017 (43)	19	20	[18F]FEPPA	2TCM; V _T	FEP unmedicated T 68.6 ± 13.0 P 19.2 ± 3.8	2.8 ± 3.3	AP 0% BZD ?	SZ=C
DI BIASE 2017 (40)	33	27	[11C]PK11195	Reference tissue; BP	Recent-onset (n=18) T 68.5* P (BPRS) 12.6 ± 4.6 Chronic (n=15) T 86.5* P (BPRS) 19.5 ± 7.8	1.5 ± 1.0 13.6 ± 8.8	AP 78% BZD ? AP 100% BZD ?	SZ=C SZ=C

Legend: “SZ”= schizophrenia patient group; “C”= control group; “n”= number of subjects; “FEP” = first episode psychosis patients, “DOI”=duration of illness, “2TCM” = two-tissue compartment model, “BP” = binding potential; “V_t”=; “DVR”= distribution volume ratio; “CPZ” = chlorpromazine equivalent;
“SZ>C” = increased uptake of tracer in schizophrenia patients compared to controls ;
“SZ=C” = no difference in tracer uptake between schizophrenia patients and controls;
“SZ<C”= decreased uptake of tracer in schizophrenia patients compared to controls.
* mean BPRS total scores were converted to corresponding PANSS total scores using the equipercentile linking method (95)

572 **Table 3: Outcomes of meta-analyses of randomized controlled trials (RCTs) on minocycline**
 573 **(Table 3a) and NSAID (Table 3b).**

Table 3a: Minocycline	N RCTs	Subjects	Treatment duration	Total symptoms (PANSS total score or BPRS)	Positive symptoms (PANSS positive subscale or BPRS)	Negative symptoms (PANSS negative subscale or SANS)	General symptoms (PANSS general subscale)
Sommer 2014 (minocycline)(65)	4	182 drug-166 placebo	36±18.8 weeks	SMD 0.22			
Oya 2014 (minocycline)(66)	4	173 drug - 157 placebo	25±19.1 weeks	SMD 0.70*	SMD 0.26	SMD 0.86**	SMD 0.50*
Solmi 2017 (minocycline)(68)	6	215 drug - 198 placebo	19.7±17.0 weeks	SMD 0.59*	SMD 0.22	SMD 0.76** (PANSS); SMD 0.60** (SANS)	SMD 0.44*
Xiang 2017 (minocycline)(67)	8	286 drug - 262 placebo	18.5±13.4 weeks	SMD 0.64**	SMD 0.22*	SMD 0.69**	SMD 0.45*

574

Table 3b: NSAID	N RCTs	Subjects	Treatment duration	Total symptoms (PANSS total score or BPRS)	Positive symptoms (PANSS positive subscale or BPRS)	Negative symptoms (PANSS negative subscale or SANS)	General symptoms (PANSS general subscale)
Sommer 2012 (NSAID)(63)	5	N=264					
Nitta 2013 (aspirin)(64)	2	133 drug - 137 placebo	14 ± 2.8 weeks	Hedges <i>g</i> 0.29*			
Nitta 2013 (celecoxib)(64)	6	255 drug - 245 placebo	7.7 ± 2.1 weeks	Hedges <i>g</i> 0.21			
Sommer 2014 (aspirin)(65)	2	133 drug - 137 placebo	14 ± 2.8 weeks	Hedges <i>g</i> 0.30**			
Sommer 2014 (celecoxib)(65)	5	236 drug - 226 placebo	7.2 ± 2.4 weeks	Hedges <i>g</i> 0.15			
Zheng 2017 (celecoxib)(69)	8	316 drug - 310 placebo	8.3 ± 2.3 weeks	SMD 0.47**	SMD: 0.50**	SMD 0.32	SMD 0.35*

575 **Legend: “SMD” = standardized mean difference; * sig at p=0.05; ** sig at p=0.01**

576

577 **Abbreviations**

BPRS	Brief Psychiatric Rating Scale
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
FEP	First episode psychosis
GWAS	Genome-Wide Association Study
IL	Interleukin
MIA	Maternal Immune Activation
MA	Meta-analysis
MRI	Magnetic Resonance Imaging
PANSS	Positive and Negative Syndrome Scale
NAC	N-Acetylcysteine
PET	Positron Emission Tomography
SAPS	Scale for the Assessment of Positive Symptoms
SR	Systematic review
TSPO	Translocator Protein

578

579 **10. Registration**

580 Not Applicable

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587 All authors met ICMJE criteria and all those who fulfilled those criteria were listed as authors. All
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