Scientific Correspondence

Microglial contribution to synaptic uptake in the prefrontal cortex in schizophrenia

Efficient synaptic communication is crucial to maintain healthy behavioural and cognitive processes. In neurodevelopmental diseases, like schizophrenia, affected individuals can exhibit behavioural symptoms like psychosis, hallucinations and alterations in decision-making. A reduction in cortical grey matter volume and enlarged ventricles in the brains of schizophrenia cases has been consistently reported [1,2]. This reduction in cortical volume is likely to be an outcome of neuronal and synaptic loss, which has also been reported in schizophrenia but the results have varied between brain area and synaptic markers examined [3–7]. A meta-analysis of the expression of synaptic markers in the disease has shown reduced levels of presynaptic markers in the frontal cortex which are heavily implicated in schizophrenia, but not in unaffected areas like the temporal and occipital lobes [8]. Synapses are crucial mediators of brain communication [9], and so, such synaptic alterations can have an impact on brain network connectivity, a process known to be affected in schizophrenia [10]. There are several factors during brain development that influence brain connectivity, with nonneuronal contributors playing an important role in synaptic formation and network maturation [11,12]. One of these nonneuronal contributors are microglia, the resident brain immune cells and primary phagocytes of the brain [12,13]. Gliosis is commonly observed during loss of brain homeostasis. Microglia have also been shown to facilitate neural network shaping in development by phagocytosing synapses using the complement system [14]. However, microglia can be aberrantly involved in synaptic elimination in nonphysiological contexts, as observed in animal models of Alzheimer’s disease [15]. Here, we performed a human post mortem study to investigate the role of microglia in synaptic engulfment in schizophrenia. We examined microglial burden using Iba1 which labels the microglial cytoplasm and reflects microglial motility and homeostasis. Iba1 is considered as a pan-microglial marker and has been observed to be increased in a subset of neurodegenerative diseases [16]. Our other microglial marker, CD68, labels the lysosomal compartment of microglia [17].

We studied post mortem brains from 10 control and 10 schizophrenia cases from the dorsolateral prefrontal cortex which is affected in schizophrenia [1]. Cortical sections were stained with Iba1 and CD68 to label microglia (Figure 1A–D). We observed that there was no difference in either Iba1 (P = 0.315) or CD68 (P = 0.794) area coverage of the cortex (burden) between the schizophrenia and control cohorts (Figure 1E,F) (full statistical results found in Table S1). Furthermore, there was no difference in the co-localization between CD68 and Iba1 in controls and schizophrenia brains (P = 0.639), suggesting the co-expression of the two markers per single cell is unchanged (Figure 1G).

Figure 1. Microgliosis burdens unchanged in control and schizophrenia tissue. Representative confocal images of immunohistochemistry stained sections for the microglial markers Iba1 (cyan) and CD68 (magenta) in control (A,B) and schizophrenia (C,D) tissue. Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Scale bars in large images represent 20 and 10 μm in the expanded inserts (denoted by dotted white lines). The insert images of A and B are represented as 3D-reconstructions in B and D, respectively (scale bar, 10 μm). 3-D reconstructions made on ParaView. Quantification of Iba1 burdens (% area), CD68 burdens (% area) and Iba1 + CD68 co-expression (% area) are shown in panels E, F, and G, respectively. Representative confocal images of the pre-synaptic marker synapsin I (green) engulfed by CD68 (magenta) and Iba1 (cyan) in control (H–J) and schizophrenia tissue (K–M). H and K show individual panels of each stain and lastly the merged image, with white arrowheads pointing to sites of co-localisation between CD68 and synapsin I. I and L are expanded images of H and K, with orthogonal views indicating where CD68 and synapsin I co-localise inside Iba1 positive cells. J and M represent 3D reconstructions from H and K, generated on ParaView. Synapsin I burdens are shown in panel N. In O, the co-localisation index of CD68 and synapsin I is quantified for control and schizophrenia cases, where similar levels of synaptic engulfment by microglia are observed. By normalizing each image to their respective CD68 burden or Iba1 burden there is still no statistical change in the engulfment of synapsin I by CD68 (P and Q, respectively). Each data point represents a mean average of 20 images taken per case, where n = 10 per group. Linear mixed-effects model assessed statistical significance, considering P ≤ 0.05 for significance. All scale bars in H–M represent 10 μm.

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Though no difference in microglial burdens between the two cohorts was observed, we aimed to assess whether microglia were involved in synaptic engulfment in schizophrenia. To do this, we quantified the amount of co-localization between synapsin I and CD68 (% area), as a measure of engulfed synaptic material in the microglial phago-lysosomal compartment (Figure 1H–M). Firstly, in our cohort we did not find a significant difference in the cortical area occupied by synapsin I staining between the schizophrenia and control groups ($P = 0.956$) (Figure 1N). Furthermore, we found no difference in synaptic engulfment by microglia between the schizophrenia and control cases ($P = 0.413$) (Figure 1O). Additionally, when we normalized this co-localization to their respective CD68 or Iba1 burdens, there was still no statistical difference between schizophrenia and control tissue ($P = 0.167$ and $P = 0.964$ respectively) (Figure 1P, Q). Of note, we have also shown microglia are capable of ingesting other presynaptic proteins like synaptophysin, as well as the post-synaptic protein PSD-95 (Figures S1 and S2). Our data therefore suggest that at the time of death, microglia do not appear to be involved in aberrant synaptic internalization in patients with schizophrenia.

In human post mortem tissue from both patients with schizophrenia and age-matched controls, we found presynaptic proteins inside microglial cells in the frontal cortex of the brain, but no difference in the levels of synaptic internalization between the two groups. A limitation of our post mortem tissue is that it provides a snapshot of the disease many years after onset, which does not address the mechanism involved in synaptic internalization by microglia. A greater sample size in an independent cohort will be useful to assess the reproducibility of these results and to stratify by confounding variables like sex and age. This would also allow us to assess whether confounding factors like depression, psychosis, systemic inflammation and use of antipsychotic drugs affect these microglial and synaptic interactions. Furthermore, we have looked through all six cortical layers in a nonbiased manner, but we cannot exclude layer specific differences in gliosis, synapse loss or synaptic engulfment by microglia. However, this study is unique by the type of assessment performed on schizophrenia tissue is scarce.

With gliosis being reported in multiple brain disorders, we assessed microgliosis in schizophrenia. As described above, we found no differences in microglial burden between disease and control groups. This suggests that microglial activation is not a sustained event in chronic schizophrenia, and if any changes do occur in these cells it would instead likely involve functional alterations. Previous literature looking at CD68 expression in control and schizophrenia cases has also reported a similar outcome [18]. It is possible that if any changes in glial dynamics were to occur, they may be seen closer to disease onset, and that by the time the brains were donated approximately 35 years later, any changes would have subsided. This would be consistent with the observations published to visualize and quantify microglial activation in vivo with positron emission computed tomography (PET) using specific ligands of the translocator protein TSPO [19]. The PET studies have revealed that activated microglia are present in patients within the first 5 years of disease onset or during a psychotic state, whereas other PET studies in chronic schizophrenia have shown no difference in microglial activation between healthy controls and these patients. Nevertheless, TSPO signals are not a perfect read-out of microglia-mediated inflammation as they are influenced by age and are not microglia-specific [20, 21].

Although developmental synaptic alterations, like synapse loss, have been characterized in individuals with schizophrenia [3, 8], there are key unanswered questions that remain. For instance, it is not clear how the synapse elimination is mediated, the extent to which it drives behavioural symptoms, or whether it is the outcome of other disease-specific pathologies. Right now, a prominent mechanism for synaptic elimination in development is the use of the classical complement cascade (CCC), where it has been shown to sculpt neural circuits by tagging less electrically active synapses [14]. Recent research has now implicated complement as a signal for aberrant synapse elimination in disease [12, 22]. Specifically, variants of C4 of the CCC are associated with a greater risk of developing schizophrenia [23], as well as poorer brain connectivity and schizophrenia-like behavioural deficits in mice [24].

Currently, a suggested mechanism by which complement-tagged synapses are cleared is by microglial recruitment for synaptic removal. In co-cultured neuron and microglia-like cells from human induced pluripotent stem cells from control and schizophrenia lines, increased levels of the excitatory post-synaptic protein PSD-95 was reported phagocytosed in the schizophrenia...
Interestingly, this increased phagocytic activity was mainly driven by the presence of schizophrenia-derived microglia. Indeed, when schizophrenia neurons were co-cultured with microglia from control patients, the phagocytic index was reduced, indicating that in schizophrenia microglia have intrinsic differences in their phagocytic response. It is worth noting that induced stem cells are a good model for understanding human disease but represent a developmentally earlier phenotype, and not that of the age of the donor. Therefore, this supports a role for phagocytic microglia in early stages of the illness and may explain why we did not see any changes in phagocytic ability of microglia towards synapses in chronic schizophrenia, as we are not studying the developmental time-frame.

In conclusion, we report that microglia in human post mortem tissue internalize presynaptic proteins physiologically, and that this does not appear to be altered in the chronic form of schizophrenia, in contrast with our observation in AD. Nevertheless, given the typically early onset of schizophrenia and that synapse loss is likely to have occurred years before brain collection, we cannot make assumptions on the role of microglia in synaptic clearance at the start of the disease. Looking forward, it would be interesting to study the difference between young vs. older cases in terms of synaptic uptake by microglia, and phenotype these changes in several brain areas to investigate any region-specific differences. Lastly, longitudinal PET imaging of the presynaptic marker SV2A [26] and TSPO microglial marker would enable exploration of any microglia-synapse association during the course of the illnesses.

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Peer Review

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

M. Tzioras*†
A. J. Stevenson*†
D. Boche‡,1
T. L. Spires-Jones*†,1
*UK Dementia Research Institute, †Centre for Brain Discovery Sciences, The University of Edinburgh, Edinburgh, and ‡Clinical Neurosciences, Clinical and Experimental Sciences Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK
†Equal contribution.

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Supporting information
Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1. Microglia can engulf synaptophysin in control and schizophrenia human post mortem tissue. Representative confocal images of the presynaptic marker synaptophysin, Sy38 (green), CD68 (magenta) and Iba1 (cyan) in control (A–C) and schizophrenia tissue (D–F). Nuclei are counterstained with DAPI. A and D show individual panels of each stain and lastly the merged image, with white arrowheads pointing to sites...
of co-localization between CD68 and synaptophysin. B and E are expanded images of A and D, with orthogonal views indicating where CD68 and synaptophysin co-localize. C and F represent 3D reconstructions from A and D, generated on ParaView. All scale bars represent 10 μm.

**Figure S2.** Microglia can engulf PSD-95 in control and schizophrenia human post mortem tissue. Representative confocal images of the presynaptic marker PSD-95 (green), CD68 (magenta) and Iba1 (cyan) in control (A–C) and schizophrenia tissue (D–F). Nuclei are counterstained with DAPI. A and D show individual panels of each stain and lastly the merged image, with white arrowheads pointing to sites of co-localization between CD68 and PSD-95. B and E are expanded images of A and D, with orthogonal views indicating where CD68 and PSD-95 co-localize. C and F represent 3D reconstructions from A and D, generated on ParaView. All scale bars represent 5 μm.

**Table S1.** Linear mixed-effects model outcomes from R Studio.

**Appendix S1.** Methods.

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