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Research paper

Neuroinflammation and microglial expression in brains of social-isolation rearing model of schizophrenia

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ABSTRACT

Schizophrenia is a psychiatric disorder with a global prevalence of approximately 0.45%. It is considered a mental illness, with negative symptoms, positive symptoms, and cognitive dysfunction. The outcomes of studies on the role of microglia and neuroinflammation have been conflicting. In addition, there is a poor understanding of the sex differences in microglial expression and neuroinflammation markers in the prefrontal cortex, hippocampus, and nucleus accumbens. Understanding the exact roles of neuroinflammation may guide the development of efficient therapeutic drugs that can address the negative, positive, and cognitive symptoms of the disease. We examined the effect of social isolation rearing on schizophrenia-related behaviours in male and female BALB/c mice. The social-isolation rearing protocol started on post-natal day (PND) 21, lasting for 35 days. Animals were assigned to four cohorts, consisting of five animals per group. On PND 56, animals were assessed for behavioural changes. We used enzyme-linked immunosorbent assays to investigate the expression of nuclear factor kappa B (NF-κB), tumour necrosis factor-α (TNF-α), and Interleukin-1β (IL-1β) in the hippocampus, nucleus accumbens, and prefrontal cortex. Immunohistochemistry was used to assess the expression of microglia in the three brain regions. Our study showed that isolation rearing led to increasing locomotion, heightened anxiety, depression, and a reduced percentage of prepulse inhibition. There was a significant increase ($p < 0.05$) in anxiety in the female isolation mice compared to male isolation mice. Furthermore, isolation rearing significantly increased microglia count ($p < 0.05$) in the hippocampus, nucleus accumbens, and prefrontal cortex, only in the male group. There was microglial hyper-activation as evident in the downregulation of CX3CR1 in both male and female social-isolation groups. Male social-isolation mice showed a significant increase ($p < 0.05$) in neuroinflammation markers only in the nucleus accumbens while the female social-isolation mice showed a significant increase ($p < 0.05$) in neuroinflammation markers in both the nucleus accumbens and hippocampus. The study showed that therapeutic interventions aimed at modulating CX3CR1 activity and reducing inflammation may be beneficial for patients with schizophrenia.

1. Introduction

Schizophrenia is a psychiatric disorder with a global prevalence of approximately 0.45% (World Health Organisation, 2022). It is a mental illness with negative symptoms (social withdrawal, avolition), positive symptoms (hallucinations, delusions), and cognitive dysfunction. Despite the availability of antipsychotic drugs for several decades, personal and societal costs remain expensive. It was estimated that the direct costs of schizophrenia range between 7% and 12% of the gross national product in Western countries (Kotzeva et al., 2022). There are many antipsychotic drugs available for the management of schizophrenia; however, they often produce adverse severe side effects and are slow-acting (Boyda et al., 2020).

Studies have demonstrated the presence of neuroinflammation in the focal region after the onset of psychosis in the hippocampi of patients with schizophrenia compared to the healthy control group (Fineberg et al., 2013). A meta-analysis revealed that non-steroidal anti-inflammatory drug adjunctive therapy had a beneficial effect on symptoms of schizophrenic patients, especially in the early stages of the disorder (Cho et al., 2019; Nitta et al., 2013), suggesting the role of inflammation in

Abbreviations: PND, Postnatal Day; NF-κB, Nuclear factor kappa B; TNF-α, Tumour necrosis factor-α; IL-1β, Interleukin-1β; PPI, Prepulse Inhibition; dB, Decibel; ELISA, Enzyme-linked immunosorbent assays.

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schizophrenia. Furthermore, human studies showed increased nuclear factor kappa B (NF-κB), tumour necrosis factor-α (TNF-α), and Interleukin-1β (IL-1β) in the brain tissue of patients with schizophrenia (Murphy et al., 2021; Fillman et al., 2014). However, the cause of the neuroinflammation remains unknown.

Microglia, the resident immune cells of the brain, is known to be associated with most neuropathologies (Bachiller et al., 2018). It exerts its effects partly by secreting cytokines such as TNF-α, IL-1β, and NF-κB (Albensi, 2019). Neurodegeneration observed in the course of schizophrenia was hypothesized to be induced by microglial activation (Laskaris et al., 2016). Positron Emission Transmission (PET) imaging and post-mortem studies on patients with schizophrenia revealed evidence of microglial activation (De Picker et al., 2021; Selvaraj et al., 2018); although, some similar studies found no microglial activation (Di Biase et al., 2017; Hafizi et al., 2017). However, it should be noted that antipsychotic medications used by these patients may also be responsible for microglial activation (Li et al., 2023). Indeed, a PET study by Conen et al. (2020) revealed no significant difference in microglial activation between untreated patients and control group. However, the sample size in the study was limited and the translocator protein marker used is not specific to microglia but is also expressed in astrocytes and endothelial cells. Therefore, a large-sample size human study that assesses unmedicated individuals with schizophrenia and animal developmental studies that do not involve the use of chemical inductions may explain the exact role of microglia in schizophrenia.

CX3CR1 is a chemokine receptor found in microglia and it regulates the attraction of microglia to the sites of neuroinflammation (Hickman et al., 2019). An animal study using a social isolation model in rodents reported increased microglial expression of CX3CR1 in the nucleus accumbens, hippocampus, and medial prefrontal cortex (Zhou et al., 2020). However, their study did not report whether an increase in microglial count accompanied the increased expression. Another animal study reported a decrease in microglial count and microglial hyper-activation in the hippocampus (Al Omam et al., 2022) though other brain regions were not considered. Thus, it is unknown whether this same effect occurs in other essential brain regions implicated in schizophrenia.

Studies have indicated sex disparities in the incidence and symptoms of schizophrenia (Ochoa et al., 2012). Early age of onset was reported in men compared to women (Giordano et al., 2021). Morgan et al. (2008) found a higher prevalence of depressive symptoms in women, while other studies found no significant clinical difference in symptoms of schizophrenia between males and females (Hayashi et al., 2002). Additionally, female patients showed better treatment outcomes than male patients (Li et al., 2016).

The aim of this study is to assess neuroinflammation and microglial profiles in male and female social-isolation mice model of schizophrenia. We hypothesise that neuroinflammation and microglial activation plays no role in social-isolation induced schizophrenia-related behaviours. Social isolation rearing is a known model that has been used to develop schizophrenia-related behaviours such as impaired pre-pulse inhibition function, heightened anxiety state, aggression, and cognitive impairments in laboratory rodents (Sánchez-González et al., 2020). These behaviours, termed 'isolation syndrome' (Jones et al., 2011), resemble some of the symptoms of schizophrenia. The relevance of the social-isolation animal model is heightened by recent studies, which found that social isolation during the Covid-19 lockdown worsened the mental health of all age groups (Grolli et al., 2021; Loades et al., 2020).

1.1. Methods

Eight (8) female mice and two male BALB/c mice were procured from the animal house of the University of Ilorin, Ilorin, Kwara State, Nigeria. They were grouped into two groups with a ratio of 4 female mice to 1 male mouse and were given free access to food and water and allowed to breed. Twenty (20) of the offspring from the female mice were randomly selected for the study. On PND 21, pups were separated from their mothers and reared either in social isolation (i.e. one mouse in a cage) or in groups (five mice in a cage). The Grouped-house has two groups, while the social isolation also has two groups, as detailed below.

Group-housed (Control) Male Group = 5 male mice reared together in a transparent cage. (Male Control Group).

Group-housed (Control) Female Group = 5 female mice reared together in a transparent cage. (Female Control Group).

Social-Isolation Male Group = 5 male mice reared in individual transparent cages (Male Social-Isolation Group).

Social-Isolation Female Group = 5 female mice reared in individual transparent cages (Female Social-Isolation Group) Figs. 1 and 2.

Animals were given free access to water and food and were left undisturbed for 5 weeks (at PND 56) when they were used for behavioural studies. Animals were maintained on a 12 h light–dark cycle (light off: 7 pm), and the animal house was under controlled humidity (45 ± 15%), controlled noise, and room temperature (27 ± 1^0 C). The Guide for the Care and Use of Laboratory Animals was followed. No environmental enrichments were provided for the grouped-house and isolation groups.

1.2. Behavioural procedures

All the behavioural procedures were conducted during light cycle. There was no human interference in the room where behavioural studies were performed. The mice's behaviours in each test were video recorded. Using the video, the behaviours of the animals were scored manually by an investigator who was blind to how the animals were reared.

1.3. Open field test

Open field test was used to assess anxiety-like behaviour and locomotion in the mice. Increased line crossing indicates increased locomotion (Seibenhener & Wooten, 2015). Furthermore, increased time spent at the periphery shows anxiety (Seibenhener & Wooten, 2015) and may be taken to represent the positive symptoms of schizophrenia (Temmingh & Stein, 2015). The open field chamber used has a dimension of 45 × 45 × 45 cm. The floor was segmented with 4 × 4 grid drawn to divide the floor into equal squares and an additional 20 × 20 cm square zone was drawn in the centre (Al Omran et al., 2022). Animals were placed at this centre and were recorded for 6 min.

1.4. Tail suspension test

Depression is a core symptom in patients with schizophrenia (Temmingh & Stein, 2015). Depressive-like behaviour was therefore assessed by measuring the immobility time of mice using the tail suspension test. The mice were suspended by their tails using tail suspension boxes with the dimensions 55 × 15 X 11.5 cm as described by Can et al., (2011). Mice were videotaped for 6 min and the total immobility time was recorded (defined as the time when the limbs were immobile).

1.5. Prepulse inhibition (PPI) test

Impaired prepulse inhibition, though not unique to schizophrenia, is a preattentional sensorimotor gating deficit found in schizophrenia and has been demonstrated in social isolation animal model (Zhou et al., 2020; Sánchez-González et al., 2019). Sensorimotor gating deficits observed in schizophrenia have usually been assessed in laboratory animals using a PPI test (Jones et al., 2011). PPI was assessed using a startle chamber containing a speaker, load cell, small fan, and restrainer holding the test animal. The closed startle chamber, constructed locally, has a dimension of 45 × 40 x 30 cm and is soundproof to isolate the animal from unwanted environmental noise. A 58 dB background noise was presented throughout the test session using a small fan which also

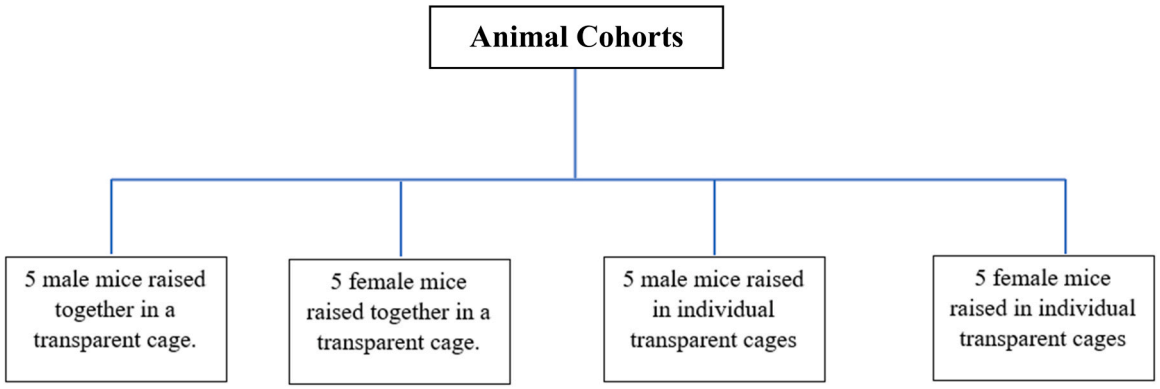

Fig. 1. Animal Cohorts.

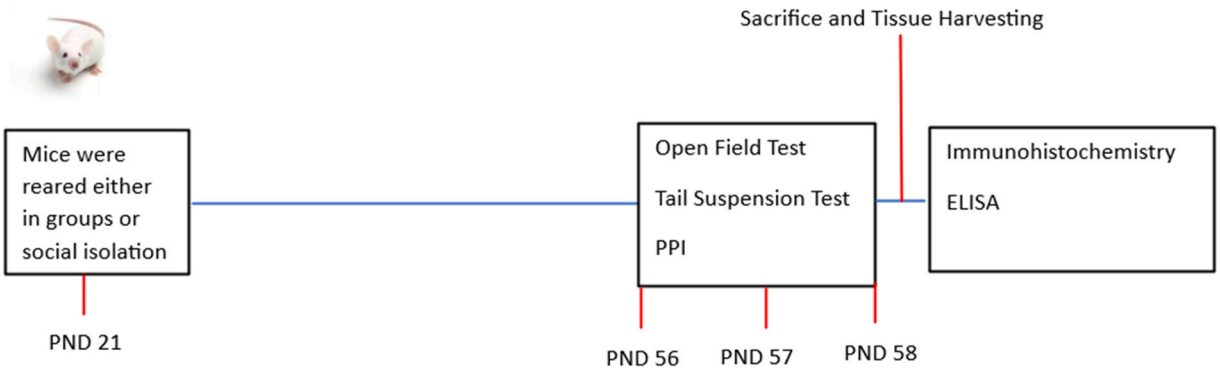

Fig. 2. Schematic representation of the study design.

provided ventilation. The acoustic stimuli presentation contains prepulse trials and startles trials. Prepulse trials consist of 20 msec of 68 dB, 71 dB, and 77 dB, while the pulse trial consists of 40 msec of 120 dB of sound. A complete presentation following the methods described by Curzon et al. (2009) includes;.

1. Four successive trials of 120 dB (40 msec)
2. (a) Pulse alone (120 dB of 40 msec)

(b) No stimulus.
(c) 68 dB of 20 msec followed by 120 dB.
(d) 71 dB of 20 msec followed by 120 dB.
(e) 77 dB of 20 msec followed by 120 dB.

Each trial type was presented 12 times with a variable interval (range: 12–30 s) between each presentation. There was an interval of 100 msec between the prepulse and pulse trial. Acoustic stimuli were designed using FL studio software, while the sound intensity was measured using a digital-level sound metre (Benetech). The startle response was measured using a four-decimal sensitive analytical balance containing a load cell sensor (Precisa, UK).

PPI was calculated as 1 - [(startle response for prepulse + pulse/ startle response to pulse alone)] x 100.

1.6. Preparation of brain tissues for enzyme-linked immunosorbent assays (ELISA)

Brain tissue was collected after the mice were sacrificed using cervical dislocation. Hippocampus, nucleus accumbens, and prefrontal cortex were excised, rinsed, and stored in ice-cold phosphate buffer solution. The brain tissues were homogenised in ice-cold phosphate buffer saline. The homogenates were in turn centrifuged at 5000 revolutions per minute for 10 min at 4 °C. The supernatants were used for the biochemical analysis.

Interleukin-1β, NF-κB, and TNF-α concentration in the homogenates were assessed using Sandwich-ELISA method (Elabscience Biotech. Texas, USA). The methods described by the manufacturer were followed. Briefly, the microplate wells are pre-coated with specific antibodies. Samples from the homogenates were added to the wells to combine with the specific antibodies. Then biotinylated detection antibody and Avidin-Horseradish Peroxidase conjugate were added to the wells. After washing the free components, only the wells that contain the biochemicals of interest (IL-1β, NF-κB, and TNF-α) appeared blue in colour. A stop solution was added to terminate the enzyme-substrate reaction and the colour turned yellow. The intensity of the colour developed was measured using a Spectrophotometer at 450 nm. For each of the biochemicals of interest, three replicates of ELISA were done.

1.7. Harvesting of brain tissue for immunohistochemistry

The mice for the immunohistochemical study were transcardially perfused with a phosphate-buffered solution, and then with 10% formal saline which served as a pre-fixative. Thereafter, the whole brain was harvested and the three tissues of interest were separated and post-fixed in 10% formal saline.

1.8. Immunohistochemistry

The tissues were trimmed, fixed in 10% neutral buffered formalin for routine histological processing and paraffin embedding. Thin Section (4 μm) of the tissue were then micro-sectioned using microtome, floated and mounted on charged glass slides. The slides were labelled, arranged in racks and placed in oven at 50–60^0 C for 20–30 min to melt excess paraffin. The slides containing the tissue were further deparaffinised and prepared for heat-induced antigen retrieval (in citrate buffer solution

(10 mM citric acid, pH 6.0). The immunohistochemical staining was performed using the Thermo Scientific Pierce Peroxidase IHC Detection Kit (36000, Thermo Scientific, USA) with slight modification of the procedure. Endogenous peroxidase activity was quenched by incubating tissue for 30 min in Peroxidase Suppressor, washed three times in Wash Buffer. A blocking buffer was added to the slides and incubated for 30 min. Excess buffer was blotted from the tissue sections, before the addition of primary antibody, CX3CR1 (Cat #PA1–28839) at a dilution of 1:100, and left overnight in a humidified chamber at 4 °C. Afterward, slides were washed two times for 3 min with Wash Buffer. The tissue sections were treated with Biotinylated Secondary Antibody and incubated for 30 min. The slides were washed three times for 3 min each with Wash Buffer, treated and incubated with the Avidin/Streptavidin-HRP for another 30 min, and washed three times for 3 min each with Wash Buffer.

1.9. Staining

The tissues were incubated with Metal Enhanced DAB (3,3′-diaminobenzidine) Substrate Working Solution for 5 min for desired staining to be achieved. The slides were rinsed with distilled water and drained. The entire tissue sections were subsequently counterstained with Mayer's haematoxylin stain, and incubated for 1–2 min at room temperature. The slides were washed several times with distilled water. DAB stained the glial cells with blue colour while haematoxylin stain provided a background staining that aids visualization of the tissue sections.

1.10. Scoring

The slides were mounted with coverslips and DPX mountant. Photomicrographs were taken with digital camera (Amscope MU900) attached to the microscope (Olympus C2X). The images were quantified for staining intensity using open-source Fiji while the quantification of microglial cells was done using cell counter plug-in of ImageJ.

1.11. Statistical analysis

The results obtained were expressed as mean ± SEM. The effects of social isolation on the different parameters were analysed using a one-way ANOVA followed by Turkey's Multiple Comparison Tests. Two-way ANOVA analysis was performed to assess the interaction of gender and social isolation. Graphs were plotted using Graph pad 8.02.263 (GraphPad Software Inc., CA, U.S.A). The significant level was set at $p < 0.05$. The observed power for the sample size was 91%.

2. Data

2.1. Social isolation increased locomotive activity in the open field test

To determine the impact of social isolation on the locomotive activity of the mice, the number of lines crossed in the open field was assessed. Locomotor hyperactivity is an indication of psychotic agitation in schizophrenia and other positive symptoms of the disease (Lotter et al., 2020). Fig. 3 shows the effect of social isolation on locomotor activity in the open field test. One-way ANOVA analysis revealed that social isolation caused increased locomotion in isolation groups compared to the control groups ($F_{(3,16)} = 12.56$, $p = 0.001$). Two-way ANOVA analysis revealed no statistically significant interaction between gender and social isolation ($F_{(1,16),} = 0.07$, $p = 0.935$).

2.2. Social isolation increased thigmotaxis in the open field test

The role of social isolation on anxiety was assessed by measuring the total duration spent at the periphery in the open-field test, a phenomenon known as thigmotaxis (Seibenhener and Wooten, 2015). Fig. 4 illustrates the effect of social isolation on thigmotaxis in the open-field test. One-way ANOVA revealed that social isolation led to anxiety in isolation groups compared to the control groups ($F_{(3,16)} = 12.55$, $p = 0.001$). Two-way ANOVA showed a significant gender effect on thigmotaxis with female mice spending more time at the periphery regardless of the rearing condition ($F_{(1,16)} = 38.56$, $p = 0.00005$). Also,

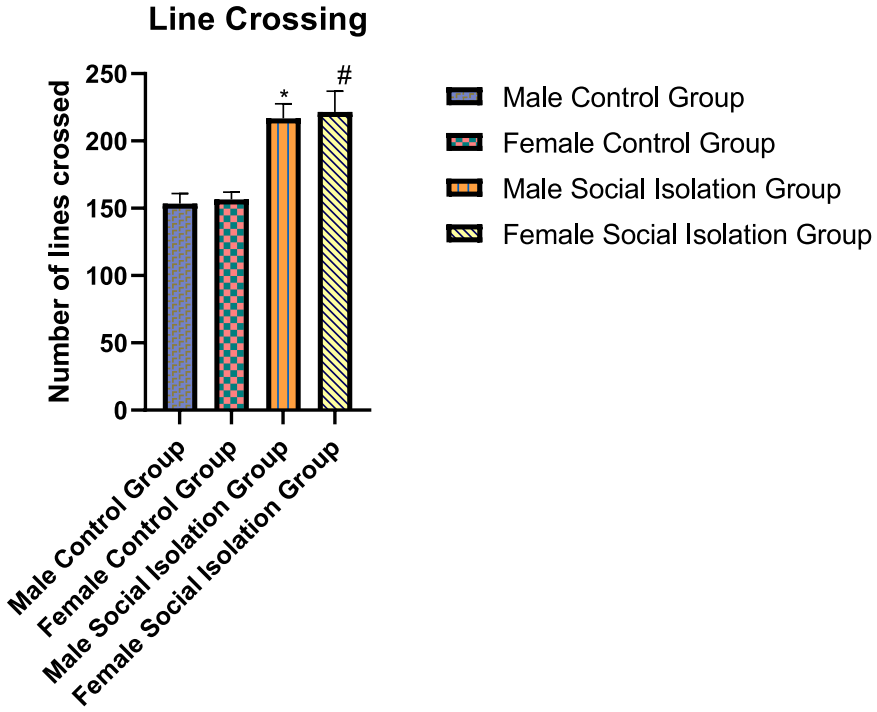

Fig. 3. Effect of social isolation on locomotor activity in open field test. *= significant difference from the male control group at $p < 0.05$. # = significant difference from the female control group at $p < 0.05$. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

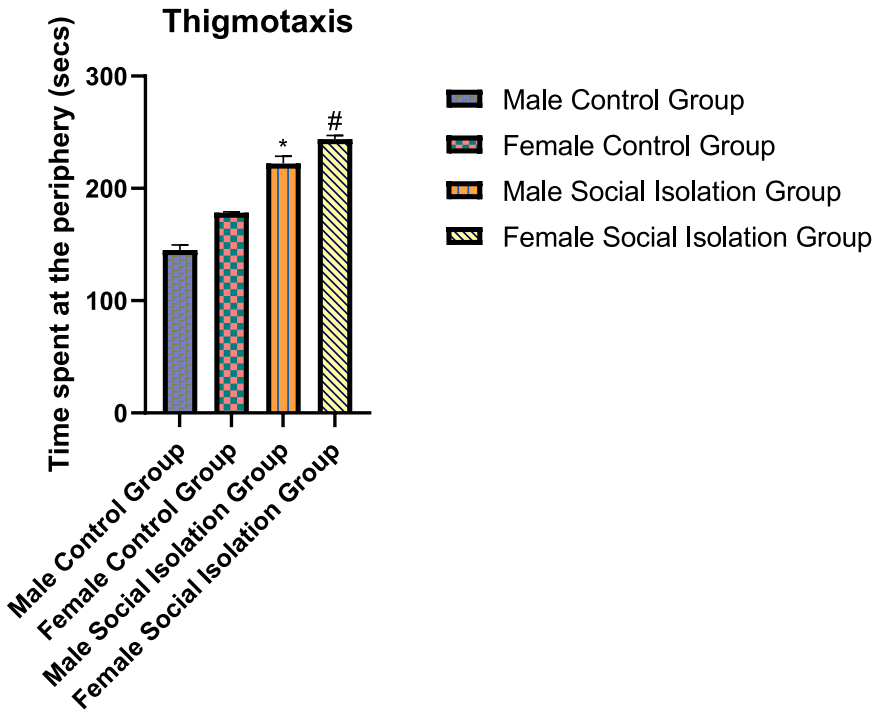

Fig. 4. Effect of social isolation on thigmotaxis in open field test. *= significant difference from the male control group at $p < 0.05$. # = significant difference from the female control group at $p < 0.05$. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

one-way ANOVA showed that a significant amount of time was spent at the periphery in the female social isolation group compared to the male social isolation group ($F_{(3,16)} = 12.55$, $p = 0.021$).

2.3. Social isolation increased immobility time in the tail suspension test

The impact of social isolation on depression was assessed using a tail suspension test. As shown in Fig. 5, one-way ANOVA revealed that social isolation produced a significant increase in immobility time in the isolation group compared to the control group ($F_{(3,16)} = 17.99$, $p = 0.00003$). However, two-way ANOVA analysis revealed no significant interaction between gender and social isolation rearing ($F_{(1,16)} = 0.331$, $p = 0.574$).

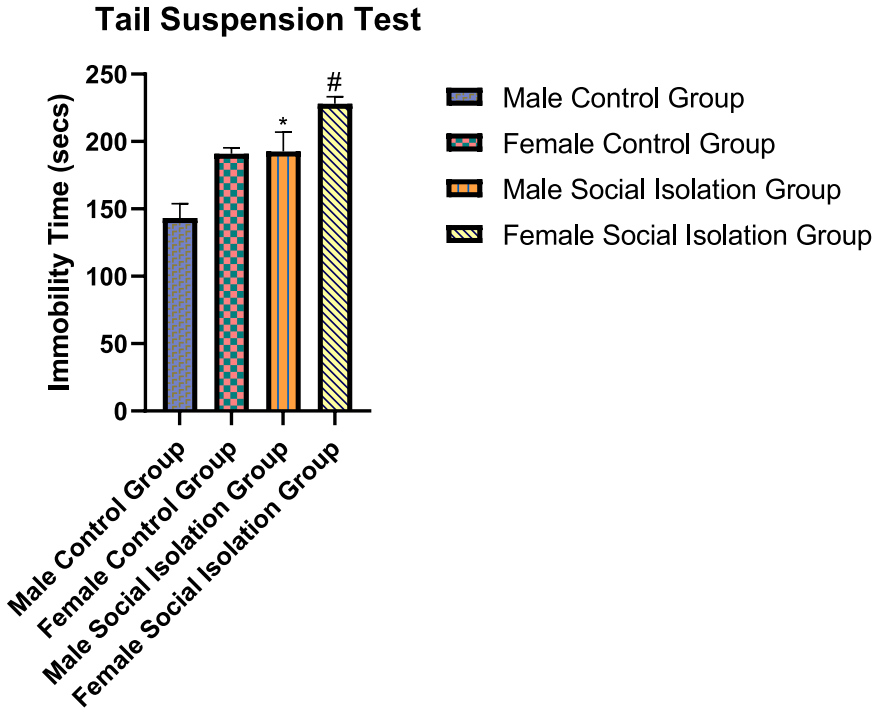

Fig. 5. Effect of social isolation on immobility time in tail suspension test. *= significant difference from the male control group at $p < 0.05$. # = significant difference from the female control group at $p < 0.05$. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

2.4. Social isolation caused impairments in PPI

Fig. 6 shows the effect of social isolation on percentage pre-pulse inhibition. One-way ANOVA analysis revealed that social isolation led to significantly decreased PPI in the isolation group compared to the control group ($F_{(3,16)} = 20.63$, $p = 0.001$). A two-way ANOVA analysis revealed that there was no statistically significant interaction between social isolation and gender ($F_{(1,16)} = 1.112$, $p = 0.37$).

2.5. Effect of social isolation rearing on neuroinflammation in the prefrontal cortex

Fig. 7 A, 7B, and 7C show the effect of isolation rearing on the IL-1β, NF-κB, and TNF-α respectively in the prefrontal cortex. One-way ANOVA analysis revealed that social isolation has no significant effect on the IL-1β ($F_{(3,16)} = 1.049$, $p = 0.407$, NF-κB ($F_{(3,16)} = 0.668$, $p = 0.58$), and TNF-α ($F_{(3,16)} = 3.918$, $p = 0.07$) concentrations in the isolation groups compared to control groups. Likewise, two-way ANOVA showed no significant interaction between social isolation and gender on IL-1β ($F_{(1,16)} = 0.099$, $p = 0.758$), TNF-α ($F_{(1,16)} = 1.75$, $p = 0.211$), and NF-κB ($F_{(1,16)} = 1.256$, $p = 0.284$).

2.6. Effect of social isolation rearing on neuroinflammation in the hippocampus

Fig. 8 A, 8B, and 8C show the effect of isolation rearing on IL-1β, NF-κB, and TNF-α respectively in the hippocampus. One-way ANOVA analysis revealed social isolation led to a significant increase in the IL-1β concentration ($F_{(3,16)} = 8.334$, $p = 0.003$) and TNF-α ($F_{(3,16)} = 16.49$, $p = 0.001$) in the female social isolation group compared to the female control group. One-way ANOVA analysis showed a significant decrease was observed in the male isolation group compared to the male control group ($F_{(3,16)} = 16.49$, $p = 0.027$). Two-way ANOVA showed a significant interaction between rearing condition and gender on TNF-α ($F_{(1,16)} = 44.51$, $p = 0.00002$). Hence, social isolation produced neuroinflammation in the hippocampus via IL-1β and TNF-α in the female mice.

2.7. Effect of social isolation rearing on neuroinflammation in the nucleus accumbens

Fig. 9 A, 9B, and 9 C show the effect of isolation rearing on IL-1β, NF-κB, and TNF-α respectively in the nucleus accumbens. One-way ANOVA analysis showed that social isolation rearing caused a significant increase in IL-1β ($F_{(3,16)} = 14.26$, $p = 0.0003$) and TNF-α ($F_{(3,16)} = 20.01$, $p = 0.0005$) in the isolation groups compared to the control groups. Furthermore, One-way ANOVA analysis showed a significant increase in NF-κB in the male social isolation group compared to the control group ($F_{(3,16)} = 12.43$, $p = 0.001$). Two-way ANOVA showed there is no gender and isolation rearing interaction in IL-1β concentration ($F_{(1,16)} = 3.83$, $p = 0.074$) and TNF-α ($F_{(1,16)} = 2.09$, $p = 0.178$) while gender and social isolation rearing interaction was found in the NF-κB concentration ($F_{(1,16)} = 8.22$, $p = 0.014$).

2.8. Effect of social isolation rearing on microglial count

Figs. 10A, 10B, and 10C show the effect of social isolation rearing on microglia in the prefrontal cortex, hippocampus, and nucleus accumbens respectively. One-way ANOVA analysis showed a significant increase in the microglia count of the male social isolation group compared to the males in the control group in the prefrontal cortex ($F_{(3,16)} = 34.56$, $p = 0.007$), hippocampus ($F_{(3,16)} = 112.11$, $p = 0.0004$), and nucleus accumbens ($F(3,16) = 87.53$ $p = 0.004$). On the other hand, one-way ANOVA analysis showed a significant decrease in microglia count in the female social isolation group compared to the females in the control group in the prefrontal cortex ($F_{(3,16)} = 34.56$, $p = 0.002$) and hippocampus ($F_{(3,16)} = 112.11$, $p = 0.0002$). Two-way ANOVA showed a significant interaction between social isolation rearing and gender in the prefrontal cortex ($F_{(1,16)} = 54.29$, $p = 0.0007$), hippocampus ($F_{(1,16)} = 151.66$, $p = 0.00002$), and nucleus accumbens ($F_{(1,16)} = 121.88$, $p = 0.00004$).

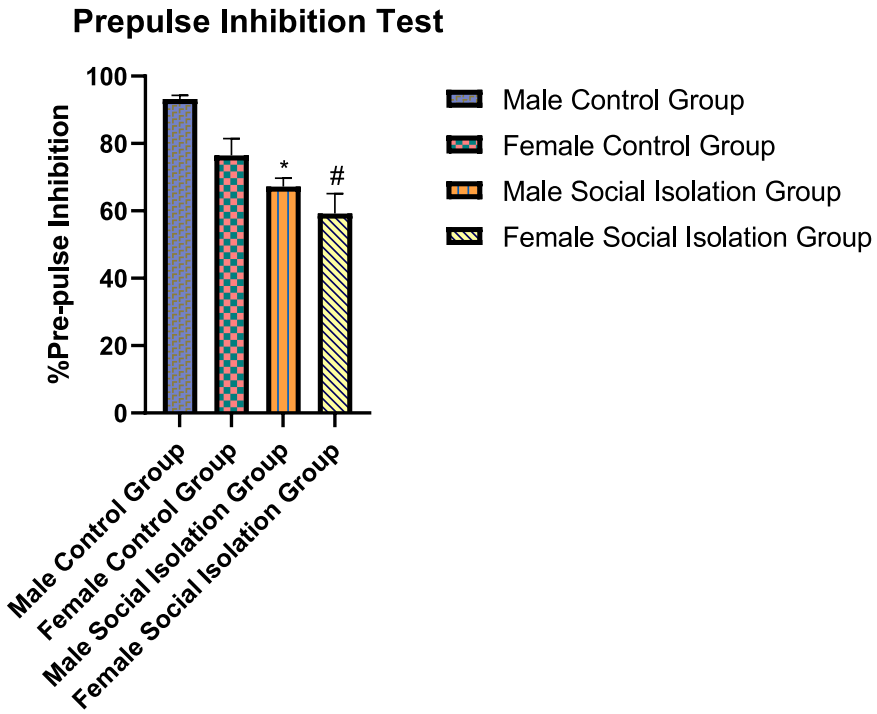

Fig. 6. Effect of social isolation in PPI test. *= significant difference from the male control group at $p < 0.05$. # = significant difference from the female control group at $p < 0.05$. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

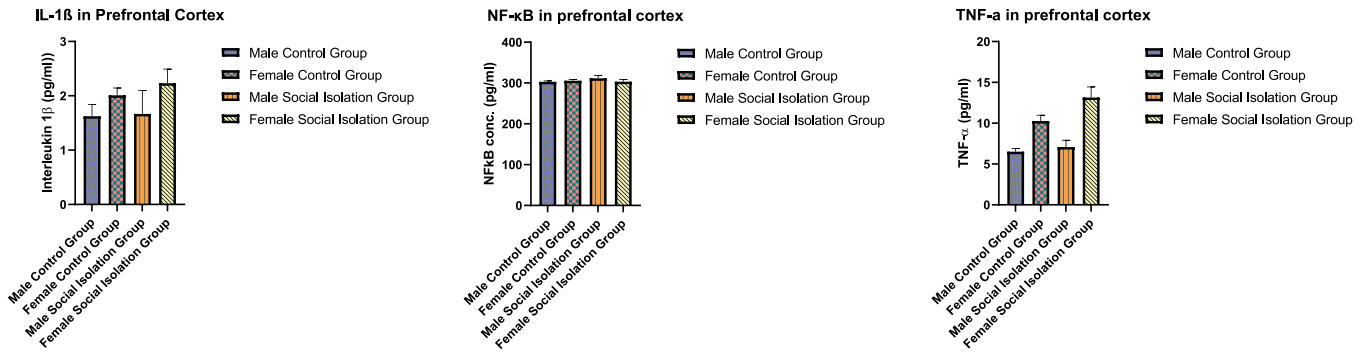

Fig. 7. Effect of social isolation on neuroinflammation in the prefrontal cortex. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

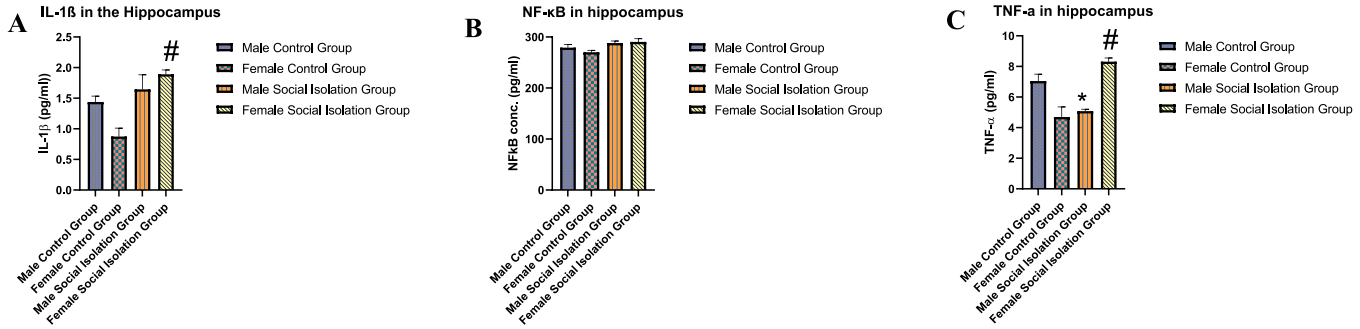

Fig. 8. Effect of social isolation on neuroinflammation in the hippocampus. *= significant difference from the male control group at $p < 0.05$. # = significant difference from the female control group at $p < 0.05$. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

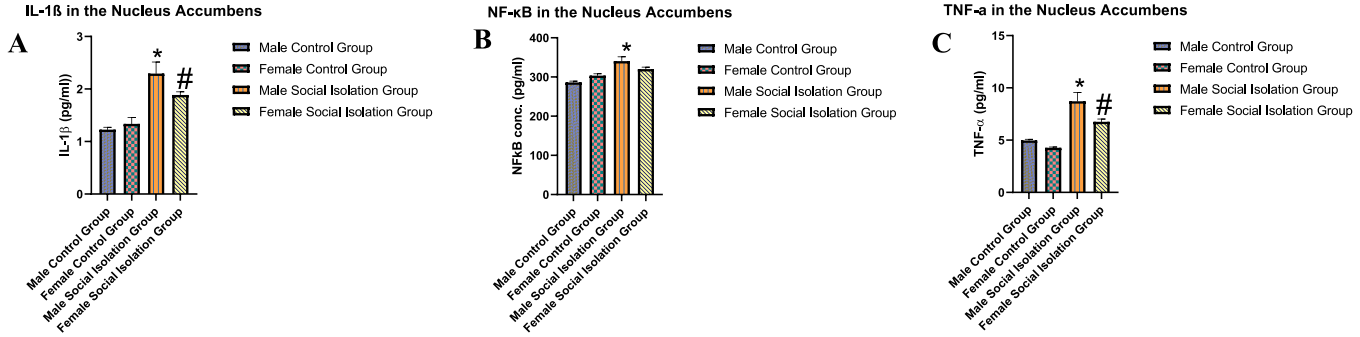

Fig. 9. Effect of social isolation on neuroinflammation in the nucleus accumbens. * = significant difference from the male control group at $p < 0.05$. # = significant difference from the female control group at $p < 0.05$. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

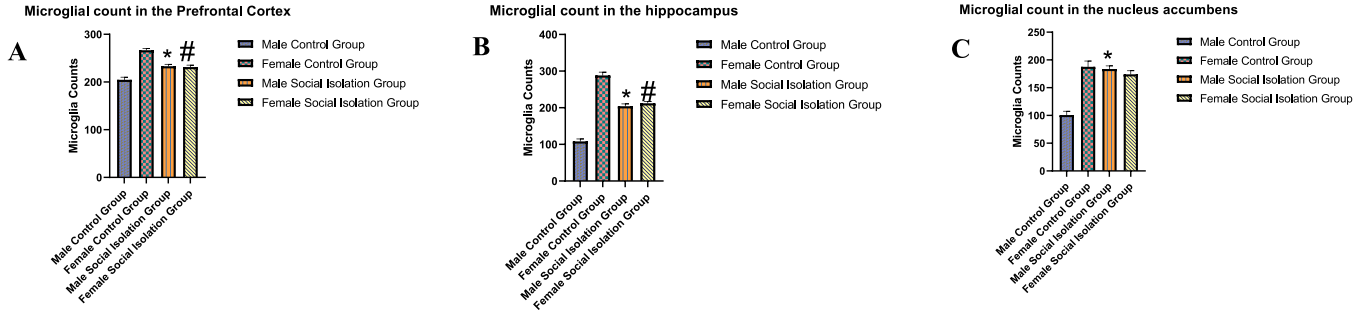

Fig. 10. Effect of social isolation on microglial count in the **A**. prefrontal cortex, **B**. hippocampus, **C**. nucleus accumbens. *= significant difference from the male control group at $p < 0.05$. # = significant difference from the female control group at $p < 0.05$. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

2.9. Effect of social isolation on CX3CR1 expression

Fig. 11 and 12 shows the CX3CR1 immunopositive cells. In the prefrontal cortex, one-way ANOVA shows that isolation rearing led to significant up-regulation of CX3CR1 in male mice compared to the male control group ($F_{(3,16)} = 38.68$, $p = 0.0004$) while in the hippocampus, isolation rearing led to significant downregulation of CX3CR1 in female mice compared to the female control group ($F_{(3,16)} = 48.26$, $p = 0.00002$). In the nucleus accumbens, one-way ANOVA showed that isolation rearing caused significant downregulation of CX3CR1 in both male and female isolation groups compared to male ($F_{(3,16)} = 85.93$, $p = 0.002$) and female control groups ($F_{(3,16)} = 85.93$, $p = 0.00003$) respectively. Two-way ANOVA showed a significant interaction between social isolation and gender in the CX3CR1 expression in the prefrontal cortex ($F_{(1,16)} = 48.19$, $p = 0.0001$), hippocampus ($F_{(1,16)} = 49.14$, $p = 0.0001$), and nucleus accumbens ($F_{(1,16)} = 32.51$, $p = 0.0005$).

3. Discussion

The current study seeks to understand the behavioural, neuroinflammatory, microglial counts, and expression of CX3CR1 in social-isolation rearing. Summarily, the male social isolation group showed a significant increase in NF-κB, TNF-α, and IL-1β only in the nucleus accumbens. In contrast, the female social isolation group showed a significant increase in TNF-α and IL-1β only in the hippocampus. Also, this study found no significant difference in neuroinflammation markers in the prefrontal cortex in the isolation groups compared to the control groups (Fig. 7).

The behavioural studies revealed an increase in locomotion (Fig. 3), heightened anxiety (Fig. 4), depression (Fig. 5), and impaired PPI (Fig. 6) in social isolation groups. Furthermore, isolation rearing led to a significant increase in microglial count only in the male group. However, there was downregulation of CX3CR1 in the hippocampus (Fig. 12a) and nucleus accumbens (Fig. 12c) of isolation-reared groups while upregulation of CX3CR1 was found in the prefrontal cortex of male social isolation group (Fig. 12b).

This study revealed that social isolation led to increased locomotor activity, depression, anxiety, and impaired prepulse inhibition function. Previous studies have shown these impairments (Al Oman et al., 2022; Lander et al., 2017). Hence, social isolation used in this study was able to induce schizophrenia-related behaviours in both male and female mice.

Understanding the mechanisms behind the gender differences in psychosis may provide insight into opportunities to deliver gender-specific treatments for patients with psychosis. According to Palanza and Parmigiani (2017), it is often impossible to attribute findings from male-exclusive studies to female behaviour. However, among all the behavioural parameters examined, no significant sex differences were found between the two isolation groups except for anxiety. The female isolation-reared mice showed higher anxiety levels than the male mice. Animal behavioural studies have also proved that female rodents are more susceptible to anxiety than males (Zilkha et al., 2021). Also, human studies that examined isolation due to the Covid-19 pandemic reported that the female gender is more likely to report anxiety (Sykes et al., 2021). Hence, more attention should be paid to anxiety in the female gender in mental disorders such as schizophrenia.

There is evidence that NF-κB, TNF-α, and IL-1β play essential roles in stress-induced behavioural deficits, such as in the social-isolation model (Al Oman et al., 2022). However, there have been conflicting reports on the role of neuroinflammation in schizophrenia and other mental disorders. In human studies, neuroinflammation was reported in some studies but found not to be significant in others (Fineberg and Ellman, 2013). Nevertheless, recent social isolation animal studies found a significant increase in cytokines in the hippocampus (Al Oman et al., 2022; Alshammari et al., 2020) although Du Preez et al. (2020) did not observe changes in neuroinflammation. In contrast, this study observed an interesting pattern. There was no significant difference in the NF-κB, TNF-α, and IL-1β concentrations in the prefrontal cortex of both male and female social isolation groups compared to the control groups (Fig. 7). However, there was an increase in NF-κB, TNF-α, and IL-1β concentrations in the hippocampus of the female social isolation group but not in the male social isolation group. In the nucleus accumbens however, male social isolation mice showed increased NF-κB, TNF-α, and IL-1β (Fig. 9) while the female social isolation group showed increased TNF-α and IL-1β (Fig. 9). Hence, there is evidence of neuroinflammation in the hippocampus and nucleus accumbens of female isolation mice while neuroinflammation is seen only in the nucleus accumbens of male mice. These results show that neuroinflammation is essential in the pathogenesis of social stress-induced schizophrenia-related behavioural deficits. The hippocampus is believed to play a central role in the development and maintenance of anxiety disorders (Baksh et al., 2021). The increased NF-κB, TNF-α, and IL-1β concentrations in the hippocampus of female isolation mice (Fig. 8) may therefore explain the reason for heightened anxiety in female compared to male mice. Indeed, different animal studies found that increased NF-κB, TNF-α, and IL-1β in different brain regions produce schizophrenia-related behavioural deficits (Richardson et al., 2022; Gomes et al., 2020; Hsu et al., 2014). Therefore, female and male patients with such symptoms may benefit from anti-inflammatory drugs as suggested in the literature (Cho et al., 2019; Nitta et al., 2013).

Microglia plays a critical role in initiating and mediating neuroinflammation in the brain (DiSabato et al., 2016). However, the role of microglia in schizophrenia and other mental disorders is still in contention. In human studies, Hafizi et al. (2017) found no difference in microglial activation, while Bloomfield et al. (2016) found an increase in activated microglia. This study found down regulation of microglial CX3CR1 in the hippocampus (Fig. 11b) and nucleus accumbens (Fig. 11c) of both male and female social-isolation groups; an indication of microglial hyper-activation (Pawelec et al., 2020; Wolf et al., 2013). In addition to promoting the activation of microglia, the deficiency of

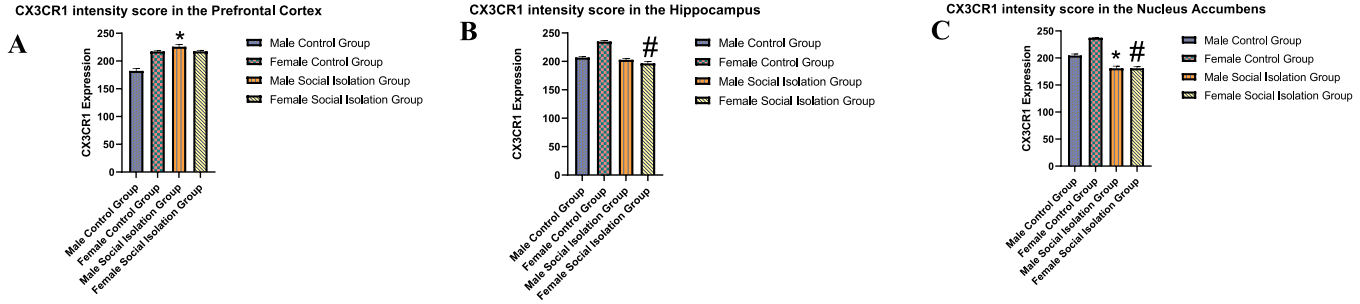

Fig. 11. Effect of social isolation on CX3CR1 expression in the **A**. prefrontal cortex, **B**. hippocampus, **C**. nucleus accumbens. * = significant difference from the male control group at $p < 0.05$. # = significant difference from the female control group at $p < 0.05$. Results are expressed as the mean ± SEM [n = 5, one-way ANOVA and two-way ANOVA].

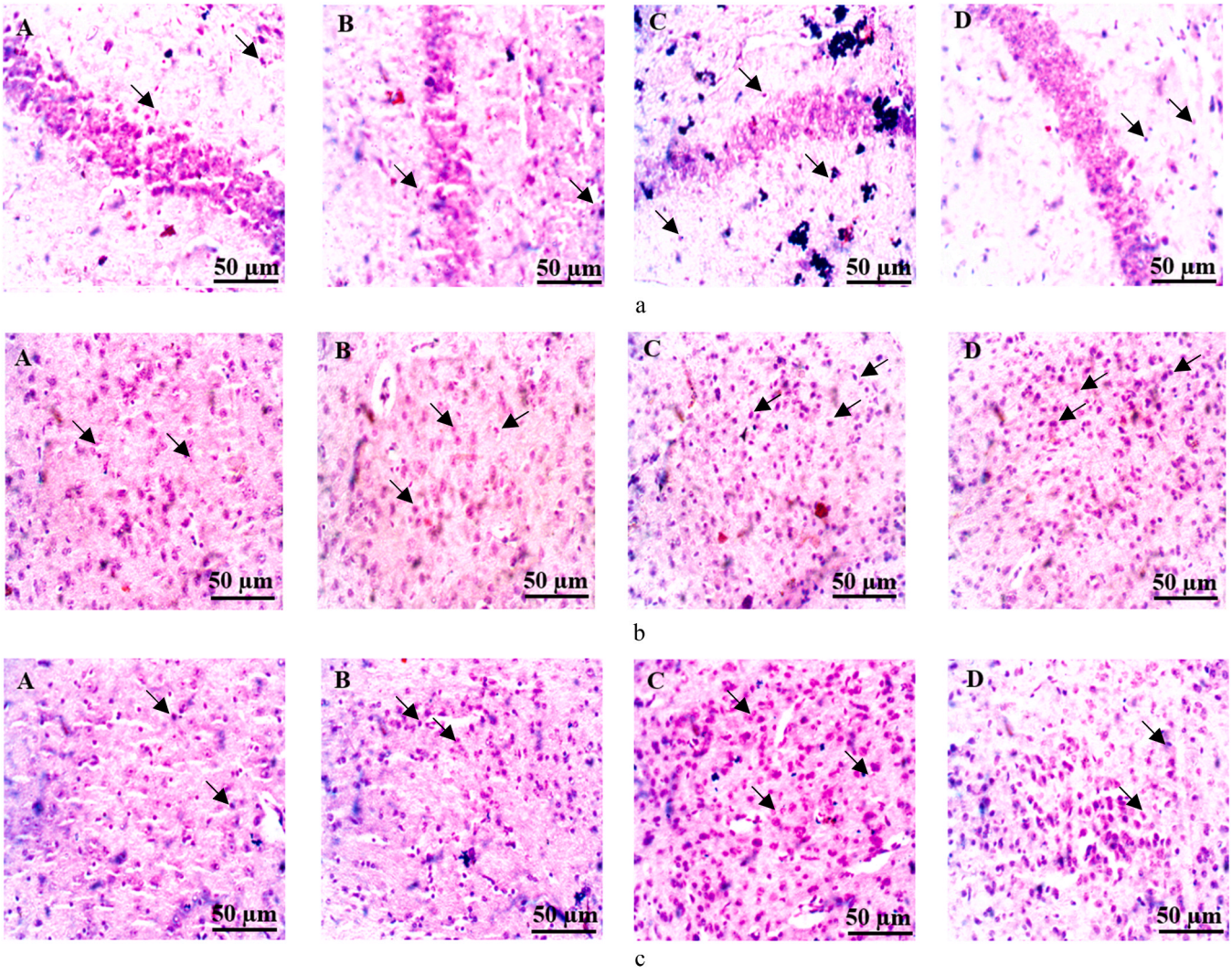

Fig. 12. a: Representative photomicrographs of the effect of social isolation rearing on the expressions of CX3CR1 immunopositive cells in the hippocampus (A), Male Control Group (B) Female Control Group (C) Male Social Isolation Group (D) Female Social Isolation Group. CX3CR1 Immunopositive cells, stained blue, are labelled with black arrowheads. Magnification: x400. b:Representative photomicrographs of the effect of social isolation rearing on the expressions of CX3CR1 immunopositive cells in the prefrontal cortex (A), Male Control Group (B) Female Control Group (C) Male Social Isolation Group (D) Female Social Isolation Group. CX3CR1 Immunopositive cells, stained blue, are labelled with black arrowheads. Magnification: x400. c: Representative photomicrographs of the effect of social isolation rearing on the expressions of CX3CR1 immunopositive cells in the nucleus accumbens (A), Male Control Group (B) Female Control Group (C) Male Social Isolation Group (D) Female Social Isolation Group. CX3CR1 Immunopositive cells, stained blue, are labelled with black arrowheads. Magnification: x400.

microglial CX3CR1 stimulates the release of inflammatory factors such as IL-1β (Briones et al., 2014) and leads to impaired cognitive function in animals (Wolf et al., 2013). Human studies such as Zhang et al. (2020), Gandal et al. (2018), and Bergon et al. (2015) consistently reported reduced expression of CX3CR1 in patients with schizophrenia.

Exaggerated neuroinflammation, microglial activation, and down-regulation of CX3CR1 were linked with protracted depressive-like behaviour in animal studies (Wynne et al., 2010) while microglial activation was found also in clinically depressed patients (Setiawan et al., 2018). Findings from this study, therefore, revealed that regulation of microglial activation may be a therapeutic approach in managing depression in male and female patients with schizophrenia.

In contrast to Al-Omam et al. (2022), this study observed increased microglial count in the three brain regions of isolated male mice. Our finding is supported by a meta-analysis study that found an increase in microglia count in postmortem brains of patients with schizophrenia (van Kesteren et al., 2017). Indeed, PET scans have shown that microglia are increased in unmedicated depressed subjects (Richards et al., 2018), anxiety (Frick et al., 2013), and patients with schizophrenia (Steiner et al., 2008).

Furthermore, recent animal studies that used the social isolation model to induce schizophrenia-related behavioural deficits have found increased microglial activation in the hippocampus, prefrontal cortex, and nucleus accumbens (Lee et al., 2021; Du Preez et al., 2021). These studies, unlike ours, did not assess the microglial count. Hence, it could be concluded that schizophrenia is mediated by increased microglial activation, increased microglia count (in the male gender), and neuro-inflammation. It is not clear why female isolation mice in this study showed decreased microglia counts compared to male isolation mice. However, the decreased microglial count may explain the heightened anxiety reported in female isolation mice. Rosin et al. (2018) showed that depletion of microglia caused anxiolytic-like behaviour that is more pronounced in female mice than in male mice.

Despite the evidence of microglial hyper-activation found in this study, there still exists controversy surrounding the use of minocycline, a known microglial inhibitor, in the management of schizophrenia. For example, while Deakin et al. (2018) noted that clinical trials suggested that minocycline is not beneficial in managing the symptoms of the

disease, Kishimoto et al. (2018) recommended its use, especially in patients with an active inflammatory process. Furthermore, Weiler & Dittmar (2019) found that minocycline is able to reduce TNF-α by suppressing NF-κB transcriptional activity and subsequently ameliorate cognitive deficits in patients with schizophrenia (Zhang et al., 2019). Additionally, Shelton et al. (2021) found two novel objects that alleviate sensorimotor gating deficits by reducing microglial activation and modulating TNF-α in the rodent model of schizophrenia. Despite the controversies on the use of minocycline in managing schizophrenia, findings from this study suggest that agents that modulate the CX3CR1 activity, microglial activities, and neuroinflammation may be beneficial in managing schizophrenia in both male and female patients.

A limitation of this study is the non-consideration of the sexual cycle of the female mice. Furthermore, dopamine in the brain tissues was not assessed. Assessment of dopamine could give insight into its modulatory effect on microglial activities.

4. Conclusion

The outcome of our study provided insight into the role of neuroinflammation and microglia in social isolation-induced schizophrenia-related behaviours. We found that neuroinflammation plays a role in the pathogenesis of schizophrenia-related behaviours with some sex differences. Female mice were more susceptible to anxiety, but no sex differences in other behavioural parameters such as depression, and locomotion were observed. Neuroinflammation was observed in the hippocampus and nucleus accumbens of female isolation mice while male isolation mice showed neuroinflammation only in the nucleus accumbens. The down-regulation of CX3CR1 found in the hippocampus and nucleus accumbens of male and female mice, indicating the hyperactivation of microglia, suggested the role of microglia activation in schizophrenia.

Consent for Publication

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Ethical Approval

The study was approved by the Ethical review committee, University of Ilorin. There is no human subject involved in this study and informed consent is not applicable.

Authors' Contributions

Gideon Opeyemi Ayilara designed and performed the experiments. Bamidele Victor Owoyele provided mentorship throughout all the stages of research.

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Nil.

CRediT authorship contribution statement

Ayilara G. O conceived and designed the study, under the supervision of Owoyele B. V. Ayilara G. O reviewed the literature, collected the data, and draughted the manuscript. Owoyele B. V reviewed the manuscript. The two authors attest to approving the final manuscript.

Declaration of Competing Interest

Nil.

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