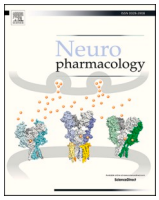
Neuropharmacology 278 (2025) 110566

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



Targeting P2X7 mitigates neurobehavioural alterations in a mouse model of post-acute sequelae of SARS-CoV-2 infection

Dorottya Szabó [a,b,1], Pál Tod [a,1], Fruzsina Maácz [a,b], Flóra Gölöncsér [a], Bernadett Iring-Varga [a,b], Bibiána Török [a], Gyula Zsidei [c], Bernadett Pályi [c], Zoltán Kis [c], Beáta Sperlágh [a,b,*]

[a] Laboratory of Molecular Pharmacology, HUN-REN Institute of Experimental Medicine, Budapest, 1083, Hungary
[b] János Szentágothai School of Neurosciences, Semmelweis University School of PhD Studies, Budapest, 1083, Hungary
[c] National Biosafety Laboratory, National Center for Public Health and Pharmacy, Budapest, Hungary

ARTICLE INFO

Handling Editor: Dr J Young

Keywords:
Mouse-adapted SARS-CoV-2
Post-COVID
Depression
Anxiety
P2X7
Neuroinflammation
JNJ 47965567

ABSTRACT

The COVID-19 pandemic has imposed a significant global health burden, leading to various long-term consequences, including persistent neuropsychiatric symptoms in a substantial proportion of infected individuals. This study investigates the role of the purinergic receptor P2X7 in mediating behaviour changes in a mouse model of post-acute sequelae of SARS-CoV-2 infection (PASC). We show that infection with a mouse-adapted SARS-CoV-2 strain induces anxiety- and depression-like behaviours in male mice, associated with elevated P2X7 receptor expression in the prefrontal cortex and hippocampus, as well as increased IFN-γ levels in the striatum. To assess the therapeutic potential of P2X7 antagonism, we administered the selective P2X7 antagonist JNJ 47965567 *in vivo*. Pretreatment with JNJ 47965567 mitigated the behavioural changes and reduced IFN-γ levels, suggesting a potential therapeutic role for P2X7 antagonists in the management of post-COVID neuropsychiatric symptoms. Our findings support the involvement of neuroinflammation in the symptoms of PASC and highlight the P2X7 pathway as a potential innovative therapeutic target for alleviating anxiety and depression in affected individuals and in other sequelae of post-viral neuropsychiatric conditions.

1. Introduction

Although the COVID-19 pandemic has been officially declared over, its impact on global health remains profound. Excess mortality, which accounts for additional deaths from all pandemic-related causes, is estimated to range between 19.1 and 36 million worldwide (Edouard Mathieu et al., 2024). While the acute phase of the pandemic has subsided, the virus continues to circulate globally, and many individuals recovering from SARS-CoV-2-induced viral infection experience lingering physical, psychological, and cognitive symptoms, even after biochemical evidence indicating the viral replication has ceased. Collectively, these symptoms are referred to as 'long COVID' (Chippa et al., 2025). Among these cases, post-acute sequelae of SARS-CoV-2 infection (PASC) encompass new, recurring, or persisting symptoms that officially arise after the acute phase of the COVID-19 infection has resolved (Jiao et al., 2024). Despite extensive research in progress worldwide, there are currently no clear diagnostic methodologies or treatments for PASC (Takao and Ohira, 2023). The signs and symptoms involved include fatigue, dyspnea, palpitation, dysosmia, subclinical fever, hypertension, alopecia, sleep problems, loss of concentration, amnesia, numbness, pain, gastrointestinal symptoms, depression, anxiety, memory and cognitive deficits (Takao and Ohira, 2023; Zeng et al., 2023). Some individuals with PASC continue to experience long-term symptoms, classified as post-COVID Condition (PCC), which typically emerge within three months of the initial infection, persist for at least two months, sometimes affecting multiple organs without an alternative explanation (Organization, 2025). Approximately 6 in every 100 people who contract COVID-19 go on to develop post-COVID-19 conditions (Organization, 2025).

The mental health impact of COVID-19 is particularly concerning.

This article is part of a special issue entitled: Tribute to Francesco Di Virgilio published in Neuropharmacology.

* Corresponding author. Laboratory of Molecular Pharmacology, HUN-REN Institute of Experimental Medicine, Budapest, 1083, Hungary.
E-mail address: sperlagh@koki.hu (B. Sperlágh).

[1] These authors contributed equally to the study.

https://doi.org/10.1016/j.neuropharm.2025.110566
Received 28 April 2025; Received in revised form 3 June 2025; Accepted 15 June 2025
Available online 16 June 2025

Studies have shown that 90 % of individuals hospitalized with COVID-19 and 25 % of non-hospitalized adults experienced at least one brain or mind-related symptom, such as fatigue, headache, sleep disturbances, depression, or anxiety-six months post-illness (Frontera and Simon, 2022). D'Hondt et al. found that individuals with PCC had a 50 % probability of both anxiety and depression three months post-infection, significantly higher than those without PCC or a matched uninfected control group (D'Hondt et al., 2024). Symptoms of depression can include a loss of interest in activities, feeling down or hopeless, difficulty in sleeping, low energy, and trouble concentrating, while anxiety manifests as excessive worry, nervousness, irritability, and difficulty relaxing (Frontera and Simon, 2022). D'Hondt et al. also found that factors such as acute COVID-19 symptoms, financial difficulties following infection, and specific post-COVID-19 symptoms were associated with a worsening of anxiety and depression over time (D'Hondt et al., 2024). While these symptoms may diminish, their persistence and associated risks can greatly impact quality of life.

The underlying mechanisms driving these neuropsychiatric symptoms are complex and potentially involve SARS-CoV-2-induced neuroinflammation (Iqbal et al., 2023; Monje and Iwasaki, 2022). Neuroinflammation could affect cognition and behaviour by disrupting the integrity of the brain vasculature, neurotransmission and neurogenesis, acute effects that may persist in COVID-19 survivors with long-term COVID symptoms (Klein et al., 2021). This neuroinflammation may be triggered by pathways including the activation of microglia and astrocytes, leading to the release of pro-inflammatory cytokines further contributing to neuronal dysfunction observed in long COVID (Iqbal et al., 2023; Monje and Iwasaki, 2022).

Purinergic P2X7 receptors are ligand-gated non-selective cation-channels expressed by immune and CNS cells (Burnstock, 2016). Abundant in neurons and glia, they play a key role in inflammation, primarily via NLRP3 inflammasome activation and IL-1β release (Sperlágh and Illes, 2014). IL-1β, besides inducing fever, influences cell proliferation, differentiation, apoptosis, and behavioural changes (Yin et al., 2024). In the adult brain, P2X7 contributes to neuronal functions, such as axonal growth, branching and neurotransmitter release (Miras-Portugal et al., 2017), regulates neural progenitor cells (Oliveira et al., 2016), and modulates hippocampal neurogenesis including cell death and neurotrophic factor signalling (Csölle et al., 2013; Leeson et al., 2019). P2X7 receptor (P2X7) activation by increased extracellular ATP can lead to cell lysis, necrosis, or apoptosis, depending on the stimulus type, duration, and cell (Bidula et al., 2019). Thus, in CNS-related inflammatory conditions with high ATP release, P2X7 receptors may hinder regeneration and cell renewal.

The P2X7 and its downstream signalling pathway, known for its role in neuroinflammation, may also contribute to immune dysregulation in SARS-CoV-2 infection (García-Villalba et al., 2022; Lécuyer et al., 2023). P2X7 modulates SARS-CoV-2 replication via NLRP3 inflammasome activation (Lécuyer et al., 2023), and elevated soluble P2X7 (sP2X7) levels in COVID-19 patients correlate with disease severity and C-reactive protein (CRP) levels (García-Villalba et al., 2022). Similarly, high serum P2X7 in early COVID-19 predict adverse clinical outcomes, suggesting sP2X7 serves not only as a potential disease marker (Vultaggio-Poma et al., 2023), but also as a peripheral indicator of systemic inflammation, similarly to CRP. Immunocytochemistry analysis of microglial cultures shows that the spike protein - a surface glycoprotein of SARS-CoV-2 responsible for host cell entry and a key pathogen-associated molecular pattern (PAMP) - increases P2X7 expression, while hippocampal tissue from spike-infused animals exhibits elevated P2X7 mRNA and receptor expression in CA3/DG (dentate gyrus) microglia (Alves et al., 2023). P2X7 receptors have a critical regulatory role in long-term neuroinflammation through their influence on cytokine release, microglial activation, BBB disruption, chemotaxis of peripheral immune cells to the CNS, differentiation of T lymphocytes, monocytes and dendritic cells, metalloproteinase secretion and activation, and modulation of inflammatory signalling pathways (Oliveira-Giacomelli et al., 2021). SARS-CoV-2-induced cell destruction elevates extracellular ATP, triggering P2X7 hyperactivation, which stimulates NLRP3 inflammasome-mediated neuroinvasion and inflammation, a process linked to psychiatric disorders (Ribeiro et al., 2021). In this context, P2X7 receptor antagonism may be a promising strategy to prevent or treat neurological complications of SARS-CoV-2 infection (Ribeiro et al., 2021). Notably, brain P2X7 is an emerging therapeutic target, with its antagonism already showing promise in treatment-resistant depression (Bhattacharya et al., 2016).

This research aims to evaluate whether prolonged activation of the P2X7 receptor could also play a key role in developing the persistent neuroinflammation observed in COVID-associated depression and anxiety. First, we induced mouse-adapted SARS-CoV-2 virus infection intranasally to evaluate the sickness behaviour and body-weight changes in mice during COVID-19 infection. Aiming to explore the neuropsychiatric consequences of COVID-19 infection, we performed behaviour tests and detected post-COVID depressive and anxiety symptoms. Next, we administered pharmacological treatments to interfere with P2X7-downstream signalling pathways to evaluate the potential effect of P2X7 in maintaining neurological symptoms related to COVID-19 infection. Finally, infection-induced changes of inflammatory cytokines and P2X7 receptor in the plasma, lung and brain samples were explored in search of additional potential mediators that may play a role in COVID-19-induced long-term impact on brain functioning.

2. Materials and methods

2.1. Animals

In this study, n = 79 male, 8-week-old BALB/cAnNHsd mice, weighing 14–26 g, were used. Mice were purchased from Animalab Hungary Kft. (Vác, Hungary). All procedures were conducted in BSL-3 conditions at the National Biosafety Laboratory of the National Centre for Public Health and Pharmacy (Budapest, Hungary). 5-6 adult littermates were housed together in individually ventilated cages (ISOcage N; Techniplast S.p.A., Buguggiate, Italy) with corncob bedding, using the following environmental settings: 22 °C ± 1 °C; humidity: 50–70 %, natural light conditions. Cardboard bedding materials were placed in all cages for environmental enrichment. All procedures were conducted from 9 a.m. to 2 p.m. The animals were treated in accordance with ethical guidelines; all efforts were made to minimise animal suffering and reduce the number of experimental animals. All procedures and treatments were performed in accordance with the Hungarian Act of Animal Care and Experimentation guidelines (40/2013, II.14), which in line with the Directive 2010/63/EU. Animal experiments were reported in accordance with ARRIVE 2.0 guidelines and were approved by the local Animal Care Committee of the IEM HAS (PE/EA/00161-4/2024).

2.2. SARS-CoV-2 MA10 virus propagation and titre determination

The mouse adapted, MA-10 variant SARS-CoV-2 virus stock (NR-55329) was received from the BEI Resources, USA under a Materials Transfer Agreement. Until use, virus stock was kept at −80 °C. In vitro colonization of the virus stock was strictly followed the slight modification of the procedures described earlier (Leist et al., 2020) and propagated in Vero cells (Nuvonis, Austria) maintained in Virus Production Serum-Free Medium (VP-SFM, Gibco, LifeTechnologies, Germany) supplemented with 2X GlutaMAX-I (Gibco, LifeTechnologies). The 70 % confluent cells were infected with MA-10 at 0.001 multiplicity of infection and were incubated for 5 days at 37 °C with 5 % CO_2. The virus-containing cell culture supernatant was centrifuged at 4,500g for 10 min. A working virus stock was generated from the second passage by concentrating into a 100-fold suspension using a tangential flow filter (TFF-Easy, HansaBioMed).

The 50 % tissue culture infectious dose ($TCID_{50}$) per mL of the

concentrated virus stock was determined using endpoint dilution assay. Vero cells were seeded in VP-SFM into 96-well plates at a 10,000 cells/well seeding density. A 10-fold serial dilution in seven steps was performed on a pre-dilution microtiter plate using the same cell culture medium, and the suspension was transferred to the corresponding wells of the prepared cell culture plate. Plates were incubated for 5 days at 37 °C with 5 % CO_2. Read-out based on the presence of cytopathic effect (CPE) was performed by crystal violet staining after inactivating and fixing the cell culture monolayer in 10 V/V% formaldehyde-PBS solution.

2.3. SARS-CoV-2 MA10 infection

A group of mice were infected with 10^5 PFU MA10 SARS-CoV-2 viruses intranasally, diluted in 1x phosphate buffered solution (PSB), under ketamine (CP-Ketamin; 100 mg/ml; 0.3 ml; Medicus Partner Ltd.; Biatorbágy, Hungary) + xylazine (CP-Xylazin; 20 mg/ml; 0.1 ml; Medicus Partner Ltd.; Biatorbágy, Hungary) anaesthesia (Fig. 1). 1x PSB were administered intranasally for control animals under the same circumstances. Animals were closely monitored post-treatment, while survival, body weight and general health (eyes, fur, locomotion) were checked daily for 10 days. The scoring system for the evaluation of general health was based on the methodology of Clark et el (Clark et al., 1997). with some modifications: eyes: 0: normal, 1: rheumy, 2: semi-closed, 3: closed; fur: 0: normal, 1: localized, mild disorder on the head, 2: localized disorder throughout the body, 3: generalized disorder; locomotion: 0: normal, 1: calm, slow exploration, 2: inert, not exploring, 3: lethargic, few spontaneous movement. In one-third of the infected mice (n = 10) showed at least 10 % decrease in their starting body weight, that considered successful viral infection.

2.4. JNJ 47965567 treatment

A group of mice, previously infected with SARS-CoV-2, were treated with 30 mg/kg JNJ 47965567 (Tocris Bioscience, Bristol, UK), a specific P2X7 receptor antagonist, administered intraperitoneally (i.p.) once daily, 30 min before each behavioural test, for a period of 5 days (Fig. 1).

2.5. Behaviour experiments

2.5.1. Open field test

Mice were put individually in the centre of an empty arena (35 x 35 × 40 cm) to explore the apparatus for 10 min freely. The movements of the animals were video recorded and analysed using the EthoVision XT 13.0 video tracking system (Noldus Information Technology, Wageningen, Netherlands). After each trial of all behavioural tests, the arenas were cleaned with Mikrozid® AF (Schülke & Mayr GmbH; Norderstedt, Germany).

2.5.2. Splash test

Mice were placed individually in a rectangular arena (40 x 13 × 10 cm, covered by a transparent acrylic sheet) for 5 min habituation. Then, the animals were sprayed with 10 % sucrose solution, and the grooming behaviour was recorded for 5 min. The results were analysed manually using the Solomon Coder event-recording software (András Péter, www.solomoncoder.com) by an experimenter blinded to the treatment.

2.5.3. Tail suspension test

Mice were suspended 10 cm high from the floor using adhesive tape placed 1–2 cm from the end of their tails. The movements of the animals were video recorded for 6 min and manually analysed using the Solomon Coder event-recording software by an experimenter blinded to the treatment.

2.5.4. T-maze

Mice were placed at the base of the longer arm of the T-maze (30 × 20 cm) and video recorded for 10 min. Animal movements were analysed manually by an experimenter blinded to the treatment, and the spontaneous alterations were scored. An arm entry was counted only when all four animal paws crossed the threshold of the corresponding arm.

2.5.5. Novel object recognition

Two wooden objects were placed on the opposite side of the arena (35 x 35 × 40 cm), differing in shape (triangle, cuboid, and cylinder) and colour (blue, green). Mice were put individually in the centre of the arena and allowed to freely explore the two objects for 5 min. Then, the animals were removed from the arena and placed back into their home cage for 3 min. One of the two object were replaced with a different item. Then, the mice were put back into the arena to explore the apparatus for 10 min. Each session was video recorded, and the time spent with the familiar and novel object was manually scored using Solomon Coder event-recording software by an experimenter blinded to the treatment.

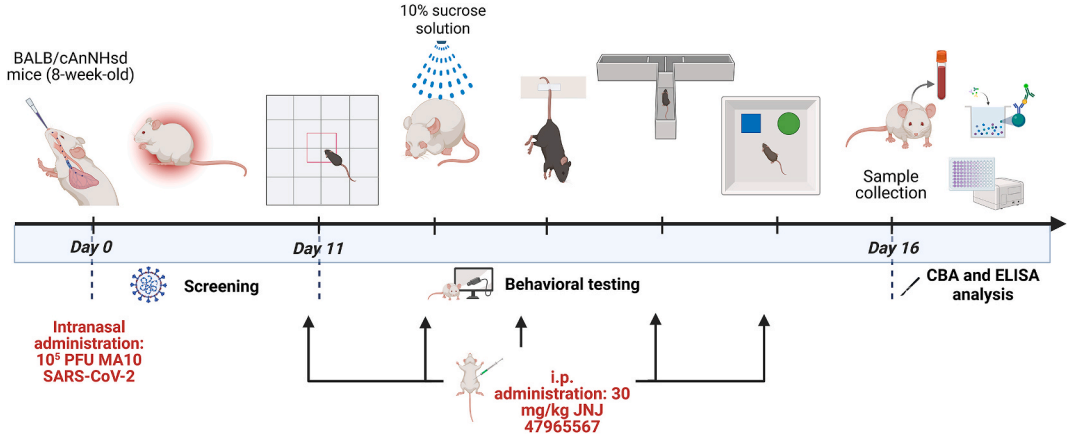


Fig. 1. Experimental design of the study. Timeline of experiments and treatments. We examined the neuropsychiatric consequences of COVID-19 infection following intranasal administration of 10^5 PFU SARS-CoV-2 MA10. 30 min before each behavioural testing, a group of mice received additional P2X7 antagonist (JNJ 47965567) treatment from day 11 to day 15 (one test per day). At 24 h after the end of behavioural testing (day 16), mice were sacrificed under ketamine-xylazine anaesthesia followed by cervical dislocation. Blood sample was collected from the vena cava inferior, pulmonar and brain tissues have been collected and stored at −80 °C until CBA and ELISA analysis.

2.5.6. Termination and tissue collection

Within 24 h after the last behaviour test, the mice were sacrificed under ketamine + xylazine anaesthesia followed by cervical dislocation. Heparin (550 IU/mL, 10 mL/kg) was administered i.p., 2 min before termination (Fig. 1). Blood sample was collected from the vena cava and centrifuged at 6000 rpm for 2 min. Tissue samples were chemically inactivated using lysis buffer containing (mM): 50 Tris-HCl, 150 NaCl, 5 $CaCl_2$; 0.02 % NaN_2, 1 % Triton-X, 1 % protease inhibitor (Protease Inhibitor Cocktail I; Merck KGaA; Darmstadt, Germany). The right inferior lobe of the lung, the prefrontal cortex, the hippocampus, and the striatum have been dissected and stored at −80 °C until further analysis.

2.5.7. Tissue homogenisation and total protein level determination

Tissue samples were homogenized by ultrasonication and incubated for 2 h at room temperature, then centrifuged at 10,000 g for 15 min. The supernatants were collected and stored at −80 °C until further analysis. Total protein levels were determined using Pierce BCA Protein Assay (ThermoFisher Scientific, Waltham, MA, US) according to the manufacturer's instructions. Samples were diluted 10x and used in duplicates.

2.5.8. Soluble and tissue P2X7 level determination

P2X7 levels were measured using a P2X7 receptor ELISA kit (MyBioSource, San Diego, CA, USA) according to the manufacturer's instructions (detection range: 0.156–10 ng/mL). Undiluted plasma and tissue samples were used in duplicates, and the absorbance levels were read at 450 nm using Multiskan Sky microplate spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). Soluble P2X7 (sP2X7) protein levels were expressed as pg/ml, and tissue P2X7 levels were expressed as pg/mg protein.

2.5.9. Cytometric bead array (CBA)

Cytokine levels were assessed using CBA Flex Sets (BD Biosciences, San Jose, CA, USA) in the plasma and tissue samples according to the manufacturer's protocol. The following cytokines were measured: interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, interferon (IFN)-γ, C-X-C motif chemokine receptor 1 (CXCL1), and tumor necrosis factor-α (TNF-α). Two-fold diluted samples were measured in duplicate on a BD FACSVerse flow cytometer (BD Biosciences), and the results were analysed with FCAP Array software (Soft Flow Kft., Pécs, Hungary). Soluble cytokine levels were expressed as pg/ml, and tissue cytokine levels as pg/μg protein.

2.5.10. Statistics

Mice were randomly assigned to three experimental groups prior to SARS-CoV-2 infection and further randomized among the infected animals before JNJ 47965567 treatment. Values were expressed as mean ± 95 % CI or mean ± SEM (error bars). Outliers were identified and excluded using the ROUT method (q = 1 %) (Motulsky and Brown, 2006). The data distribution's normality was analysed using the Kolmogorov-Smirnov test. Survival rates were analysed using the Log-rank (Mantel-Cox) test. In the case of multiple experimental groups, one-way or two-way ANOVA with Sidak or Tukey's post-hoc test was performed with or without repeated measures. Two groups were analysed using unpaired *t*-test or Mann-Whitney *t*-test. Post-hoc tests were run only if F was $P < 0.05$ and considered statistically significant if $p < 0.05$. Behavioural analysis and measurements were performed by an experimenter blinded to the treatment. All data analysis was performed using GraphPad Prism 8.0.2 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. SARS-CoV-2 MA10 infection induced severe morbidity and mortality in male mice

10^5 PFU SARS-CoV-2 MA10 administration induced marked mortality among the infected animals. The survival rate was 0.411 on day 10, the median survival time was 6 days (Fig. 2A). The observed body weight changes were statistically different between the control (PSB) and SARS-CoV-2 MA10 infected mice from day 3 to day 9 (Fig. 2B). The minimum mean body weight change occurred on day 4 (83.19 ± 1.582 %). The general health condition (squinting, matted fur, general weakness, immobility) of the viral infected mice were showed similar delineation, the most severe indications occurred from day 5 to day 8 (Fig. 2C). Further investigations were conducted only in these animals.

3.2. Post-acute infection with SARS-CoV-2 MA10 led to elevated P2X7 receptor expression in mouse plasma and brain samples

We measured P2X7 receptor levels using ELISA to investigate its potential role in various tissues during the post-acute phase of SARS-CoV-2 MA10 infection. The plasma level of the P2X7 receptor was significantly elevated after SARS-CoV2 infection compared to the control group at day 16 (Fig. 3B). However, no difference was observed in the lung at the same time (Fig. 3C). In the brain, the P2X7 receptor levels were markedly increased in the prefrontal cortex and hippocampus (Fig. 3D and E), but no alteration was seen in the striatum (Fig. 3F).

3.3. SARS-CoV-2 MA10 infection led to anxiety- and depression-related neurobehavioral alterations in mice

Open field (OF) test was conducted to evaluate the locomotor activity and anxiety-related behaviour of the SARS-CoV-2 MA10 and SARS-CoV-2 MA10 + JNJ treated groups. SARS-CoV-2 MA10 infection did not affect the velocity or the travelled distance compared to control group. However, JNJ treatment induced a significant increase in both the velocity (SARS-CoV-2 MA10: 3.941 ± 0.21 cm/s vs SARS-CoV-2 MA10 + JNJ: 5.13 ± 0.562 cm/s) and the travelled distance (SARS-CoV-2 MA10: 2256 ± 93.44 cm vs SARS-CoV-2 MA10 + JNJ: 3073 ± 333.5 cm) in comparison to the SARS-CoV-2 MA10 group (Fig. 4A and B). SARS-CoV-2 MA10 infection, or JNJ treatment did not influence the rotational movement of the animals (Fig. 4C). SARS-CoV-2 MA10 infection led to a marked decrease in both the time spent in the centre of the OF arena and the frequency of head orientation toward the centre compared to the control group. Furthermore, JNJ treatment rescued these anxiety-like behaviours: the mice spent a comparable amount of time in the centre as the control animals (control: 56.02 ± 3.99 s, SARS-CoV-2 MA10: 26.4 ± 2.38 s, SARS-CoV-2 MA10 + JNJ: 53.56 ± 4.65 s) and also exhibited restored head-orienting behaviour (control: 63.37 ± 2.32 s, SARS-CoV-2 MA10: 41.77 ± 2.36 s, SARS-CoV-2 MA10 + JNJ: 58.51 ± 3.75 s) (Fig. 4D and E). To characterize the depression-like behaviour, as indicated by neglect of self-care, the splash test was performed according to the protocol described by Hu et al. (2017). The time spent with self-grooming was markedly decreased after viral infection compared to the control group (control: 61.97 ± 11.06 s vs SARS-CoV-2 MA10: 19.97 ± 7.35 s), meanwhile the JNJ treatment did not significantly diminish the effect of SARS-CoV-2 MA10 infection (Fig. 4F). However, we did not find any significant difference in the grooming frequency (Fig. 4G). Although it cannot be ruled out that the current sample size was insufficient to fully assess its potential impact. In the tail suspension test, no change in immobility time or frequency was observed after SARS-CoV-2 MA10 infection, and JNJ treatment did not influence behavioural despair (Fig. 4H and I).

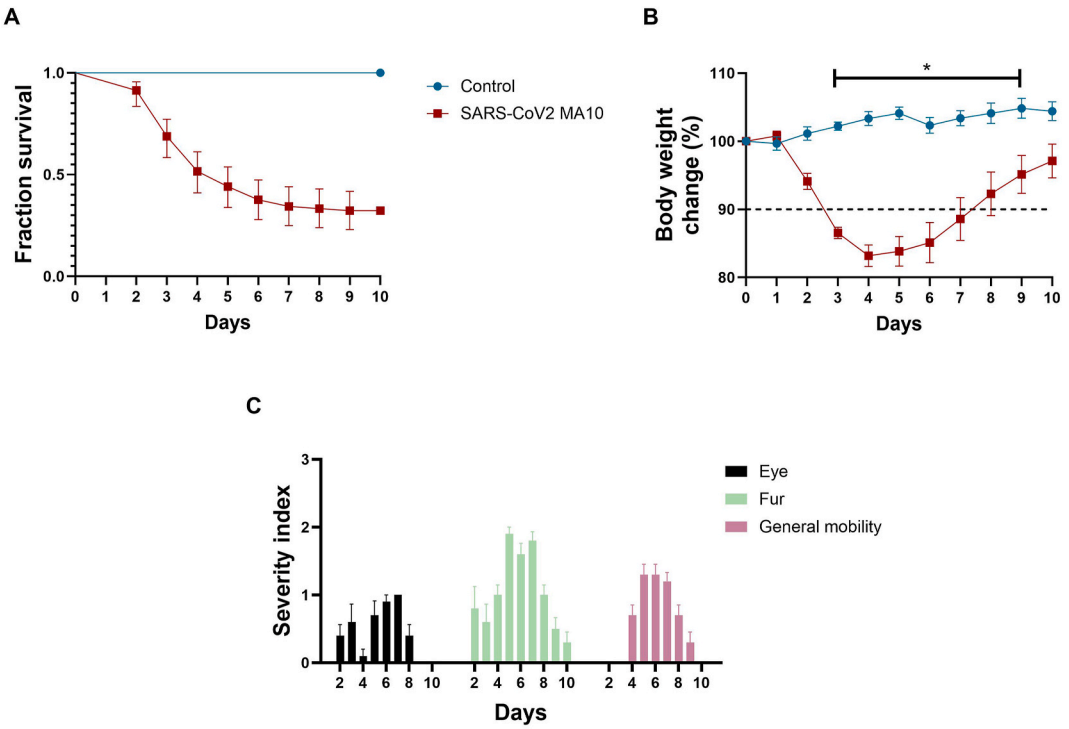


Fig. 2. Survival and general health related conditions after SARS-CoV-2 MA10 infection in mice. SARS-CoV-2 MA10 infection induced high mortality rate and body weight decrease in treated animals. (A) Fractional survival, (B) Relative body weight changes (C) Health condition of the infected animals. Values are presented as (A) mean ± 95 % CI, (B, C) mean ± SEM; A: control: n = 6, SARS-CoV-2 MA10: n = 30; B and C: control: n = 6, SARS-CoV-2 MA10: n = 10. (A) Log-rank (Mantel-Cox) test [Chi square = 7.242, df = 1, p = 0.007], (B) Repeated two-way ANOVA [F (10, 140) = 10.90] with Sidak post-hoc test. *p < 0.05 compared to the control group.

3.4. SARS-CoV-2 MA10 infection did not influence the working memory of the mice

The spontaneous alterations in T-maze and NOR test were used to quantify the effect of SARS-CoV-2 MA10 infection on working memory. No changes were observed in the total number of alternations (Fig. 5A) and the spontaneous correct alternations (Fig. 5B) after SARS-CoV-2 MA10 infection. Furthermore, the SARS-CoV-2 MA10 treated animals showed no difference in the NOR test (Fig. 5C). The pretreatment with JNJ did not affect working memory performance either (Fig. 5A–C).

3.5. SARS-CoV-2 MA10 infection led to decreased IL-1α and CXCL1 levels

Pro-inflammatory (IL-1α, -1β, −2, −6, IFN-γ, CXCL1, and TNF) and anti-inflammatory (IL-4, and -10) levels were determined in the periphery (plasma, lung) and in the central nervous system (prefrontal cortex, hippocampus, and striatum) 16 days after the infection. IL-2 and IL-4 remained below the detection limit in both the plasma and in the lung (data not shown). No changes were observed in the plasma cytokine levels (Fig. 6A). Among the pro-inflammatory cytokines, IL-1α levels were elevated in the SARS-CoV-2 MA10 + JNJ group mice compared to the control animals in the lung (control: 0.0014 ± 0.00026 pg/μg, SARS-CoV-2 MA10 + JNJ: 0.0025 ± 0.001 pg/μg). No alterations were shown in the other investigated pro-inflammatory cytokines and the anti-inflammatory IL-10 levels in the periphery.

3.6. SARS-CoV-2 MA10 infection induced IFN-γ elevation in the striatum

The pro-, and anti-inflammatory cytokine levels were measured in the CNS as well. Among the investigated cytokines, IFN-γ was elevated after 16 days in the striatum in response to SARS-CoV-2 MA10 infection. IL-10 level was markedly increased after JNJ treatment compared to the virus infected group in the striatum (SARS-CoV-2 MA10: 0.000253 ± 0.000064 pg/μg, SARS-CoV-2 MA10 + JNJ: 0.00058 ± 0.00017 pg/μg) (Fig. 7C). Furthermore, JNJ treatment alleviated the increased IFN-γ level (control: 0.000023 ± 0.000012 pg/μg, SARS-CoV-2 MA10: 0.000089 ± 0.00003 pg/μg, SARS-CoV-2 MA10 + JNJ: 0.000033 ± 0.000012 pg/μg) (Fig. 7D). The CXCL1 levels were markedly decreased in the hippocampus after SARS-CoV-2 MA10 infection, whereas JNJ treatment did not reverse the effect of viral infection (control: 0.00018 ± 0.000044 pg/μg, SARS-CoV-2 MA10: 0.000081 ± 0.000022 pg/μg, SARS-CoV-2 MA10 + JNJ: 0.000099 ± 0.000018 pg/μg) (Fig. 7E). The level of IL-2, IL-4, IL-6 and TNF-remained below the detection limit in all groups at this time point (data not shown).

4. Discussion

In the present study, our primary aim was to investigate whether SARS-CoV-2 induce behaviours reminiscent to post-acute COVID depression and anxiety in mice and to determine the role of P2X7 in the development of these symptoms and the underlying neuroinflammation. While the original human SARS-CoV-2 is not virulent in mice due to differences in the ACE2 receptor (Chu et al., 2022), the development of mouse-adapted strains of SARS-CoV-2 has raised significant interest in understanding their impact on neurological and psychological outcomes. Mouse-adapted strains, such as those developed by Leist et al. (2020), have been engineered through serial passaging of the virus in mice, enabling their use in various experimental settings to simulate human COVID-19 pathology. This adaptation process enhances ability of the virus to infect murine models, which have been instrumental in studying the neurological mechanisms of neuropsychological complications similar to those seen in humans. To our knowledge, this is the first published study in the literature employing a mouse-adapted SARS-CoV-2 strain to assess behavioural alterations resembling human neuropsychological symptoms. Following mouse-adapted SARS-CoV-2

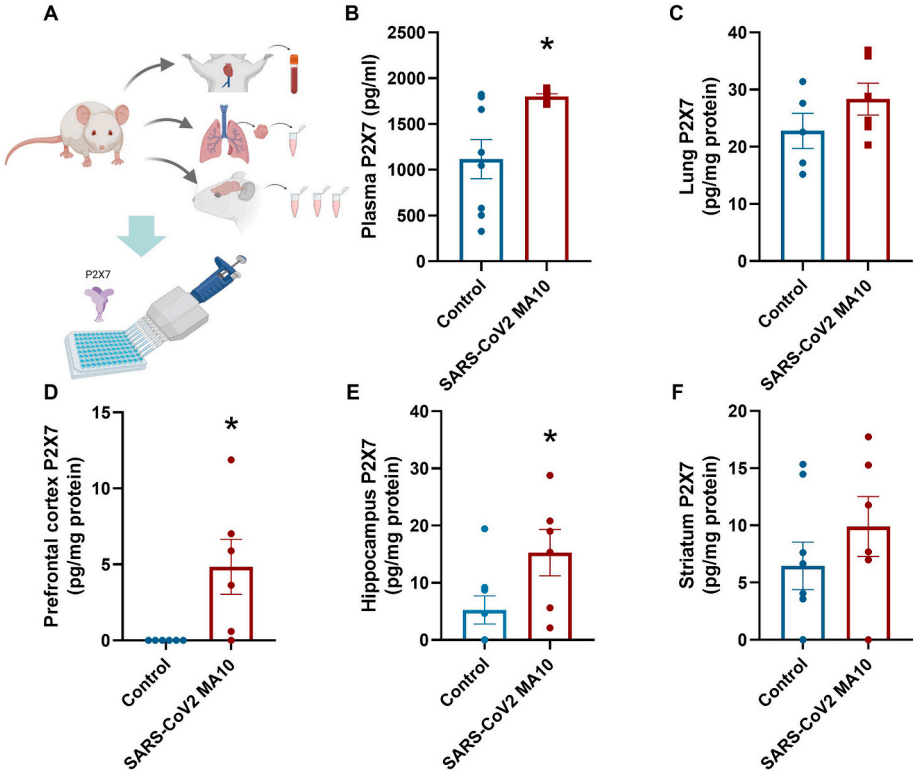


Fig. 3. P2X7 receptor levels in plasma, lung and brain samples of mice following post-acute SARS-CoV-2 MA10 infection. Plasma P2X7 levels were significantly elevated (B), while no change was observed in lung samples two weeks following SARS-CoV-2 MA10 infection. Increased P2X7 expression was detected in the prefrontal cortex (D) and hippocampus (E). Data are presented as mean ± SEM. Control group: n = 8; SARS-CoV-2 MA10 group: n = 6. Unpaired *t*-test (B: p = 0.0181, t = 2,735, df = 12; C: p = 0.2115, t = 1,345, df = 9; E: t = 2.230, df = 12, p = 0.0456; F: p = 0.3163, t = 1,046, df = 12); Mann–Whitney test following log transformation (D: p = 0.0152). $*p < 0.05$. $**p < 0.01$. $***p < 0.001$ compared to the control group.

injection, we found a moderate to strong sickness behaviour, seen in body weight loss and general health conditions, of the mice peaking between days 5 and 7 (Fig. 2C). Infected mice exhibited weight loss up to 10–20 %, peaking at day 4 (Fig. 2B), which is consistent with other studies in the literature (Amruta et al., 2022; Leist et al., 2020).

Although the role of P2X7 receptors in the pathophysiology of acute SARS-CoV-2 infection has been extensively investigated, particularly in relation to inflammation and immune response (García-Villalba et al., 2022; Lécuyer et al., 2023; Ribeiro et al., 2021), less is known about their potential contribution to the neuropsychiatric symptoms of post-acute sequelae of COVID-19. To address this, we examined P2X7 concentration in brain regions implicated in anxiety and depression (prefrontal cortex, striatum and hippocampus), as well as in plasma and lungs of mice following post-acute SARS-CoV-2 MA10 infection. Our findings revealed a significant elevation of P2X7 levels in plasma (Fig. 3B), which may reflect residual effects from acute infection (García-Villalba et al., 2022), while unchanged lung expression suggests resolution of acute pulmonary inflammation two weeks post-infection, supported by the lack of elevated pro-inflammatory cytokine levels. Notably, increased P2X7 expression was detected in the prefrontal cortex (Fig. 3D) and hippocampus (Fig. 3E), supporting the hypothesis of persistent P2X7 activation contributing to prolonged neuropsychiatric symptoms. These observations raise the possibility that P2X7 receptors may serve as a mechanistic link between acute infection and long-term neurological manifestations in COVID-19.

To assess neurobehavioral alterations and the potential role of P2X7, behavioural testing began on day 11 post-infection (Fig. 1), after the resolution of overt signs of acute infection. This aligns with previous studies in murine models, where anxiety-like behaviour and cognitive decline typically emerge around two weeks following SARS-CoV-2 infection and may persist for extended periods thereafter (Oh et al., 2022; Singh et al., 2024). We found a significant change in part of the anxiety- and depression-related behaviour tests confirming the post-COVID neurobehavioral alterations in mice (Fig. 4). Specifically, SARS-CoV-2-infected mice spent significantly less time in the centre of the open field arena and showed reduced head orientation toward the centre (Fig. 4D and E), indicating anxiety-like behaviour. Furthermore, these mice exhibited significantly reduced time spent grooming during the splash test (Fig. 4F), suggesting a reduction in motivational behaviour usually seen in depression-like phenotypes, although no change in the immobility time or frequency was detected in the tail suspension test (Fig. 4H and I). The behavioural changes observed in our mouse model, tested starting on day 10 post-SARS-CoV-2 infection, align with emerging clinical data suggesting that anxiety and depressive symptoms are common features of post-acute sequelae of SARS-CoV-2 infection, which may evolve into post-COVID condition in the later stages (Jiao et al., 2024).

Due to the biosafety constraints associated with live virus handling in BSL-3 conditions, the behavioural test battery was necessarily limited; however, the observed anxiety-related and motivational alterations support the emergence of an affective behavioural phenotype, potentially skewed toward anxiety-dominant features rather than classical depressive states. One additional limitation of our study is the relatively small sample size in certain experimental groups, which was primarily due to the unexpectedly high mortality rate (~59 %) in adult male BALB/c mice following SARS-CoV-2 MA10 infection, as reported by Leist et al. (2020). While this reduced statistical power in some comparisons, the overall trends observed across behavioural and molecular measures remain informative and align with known features of post-acute viral neuroinflammation.

Assessment of cognitive behaviours revealed no significant differences between the infected and the PBS-treated control group (Fig. 5).

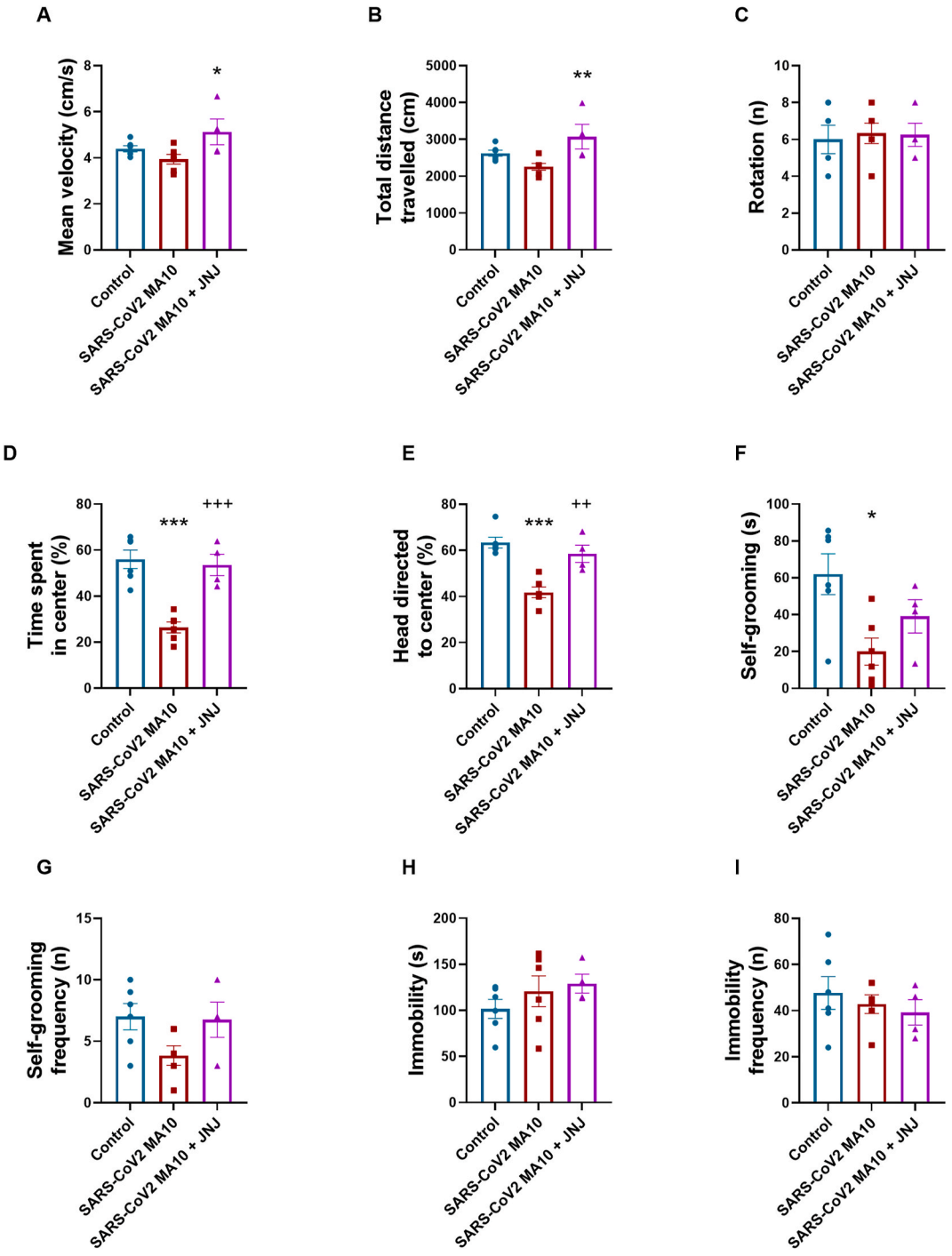


Fig. 4. Assessment of behaviour in the open field (OF), splash test (SPT) and tail suspension tests (TST). SARS-CoV-2 MA10 infection induced anxiety-like behaviour, as reflected by decreased time spent in the centre of the open field and reduced head-orientation toward the centre. JNJ treatment elevated basal locomotor activity and effectively reversed these anxiety-like effects. In the SPT, infection led to reduced grooming duration indicative of motivational impairment; however, JNJ treatment did not significantly alleviate these changes. In contrast, no significant behavioural alterations were observed in the TST across groups. OF: (A) Mean velocity, (B) Total distance travelled, (C) Rotational movement, (D) Time spent in centre, (E) Head directed to centre; SPT: (F) Self-grooming total time, (G) Self-grooming frequency; TST: (H): Immobility time, (I) Immobility frequency. Values are presented as mean ± SEM; control: n = 6, SARS-CoV-2 MA10: n = 6, SARS-CoV-2 MA10 + JNJ: n = 4. One-way ANOVA (A: $F[(2, 13) = 3912]$, $p = 0.047$; C: $F[(2, 13) = 0{,}07175]$, $p = 0.931$; D: $F[(2, 13) = 22{,}15]$, $p < 0.001$; E: $F[(2, 13) = 19.68)]$, $p = 0.0001$, F: $F[(2, 13) = 5498)]$, $p = 0.019$; G: $F[(2, 13) = 2.967$, $p = 0.0868$, H: $F[(2, 13) = 1038)$, $p = 0.382)$, (I) $F[(2,13) = 0.4903]$, $p = 0.6233$ or one-way ANOVA after log transformation (B: $F[(2, 13) = 6720]$, $p = 0.01$) with Tukey's post hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to the control group; $^{+++}$ $p < 0.001$ compared to the SARS-CoV-2 MA10 treated group.

Specifically, no statistically significant differences were observed in spatial working memory, as measured by the T-maze test (Fig. 5A and B), recognition memory, assessed using the novel object recognition test (Fig. 5C), which was performed using a time-restricted protocol adapted from Drayson et al. (2023) due to BSL-3 constraints, which may have limited the sensitivity of the task. Similarly, JNJ 47965567 treatment did not significantly alter these specific behavioural outcomes in SARS-CoV-2-infected mice. According to the literature, cognitive or

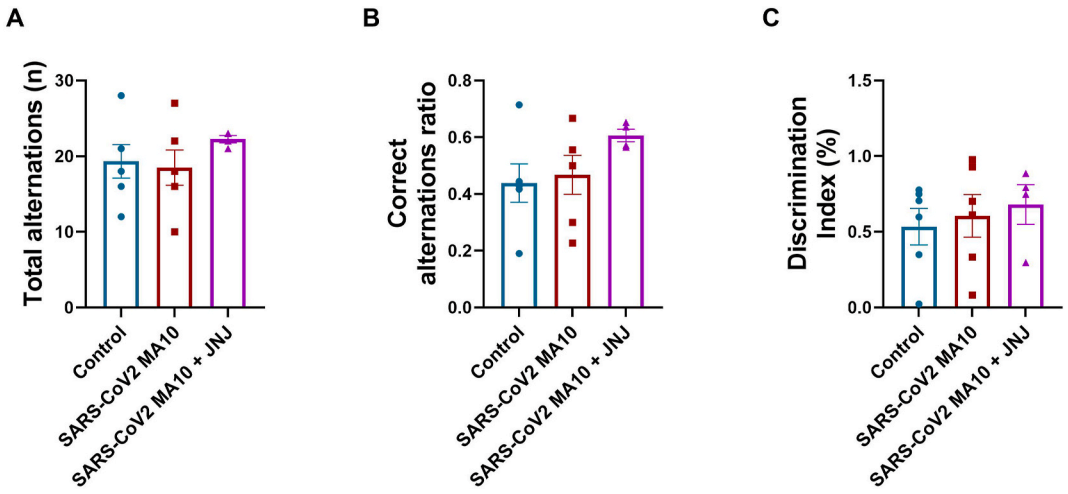


Fig. 5. Assessment of the working memory after SARS-CoV-2 MA10 administration. The viral infection did not alter the working memory of the animals. T-maze (A) Total number of alternations, (B) spontaneous correct alternations number, (C) Discrimination index in the novel object recognition test. Values are presented as mean ± SEM; control: n = 6, SARS-CoV-2 MA10: n = 6, SARS-CoV-2 MA10 + JNJ: n = 4. One-way ANOVA (A: F[(2, 13) = 0,8595], p = 0.446; :B: F[(2, 13) = 1657], p = 0.229, C: F[(2, 13) = 0,2746], p = 0.764) with Tukey's post hoc test.

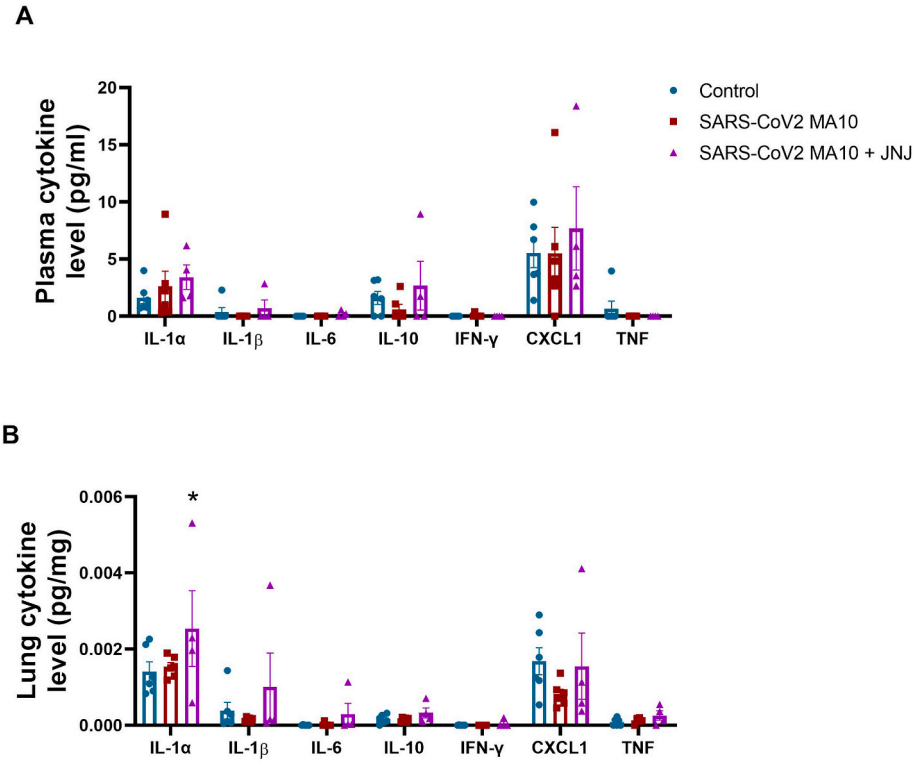


Fig. 6. Cytokine levels in the periphery 16 days after SARS-CoV-2 MA10 infection. Cytokine levels in the (A) plasma, (B) lung. No alterations were observed in the plasma, however, JNJ treatment induced elevation in the lung IL-1α level compared to the control animals. Values are presented as mean ± SEM; control: n = 6, SARS-CoV-2 MA10: n = 6, SARS-CoV-2 MA10 + JNJ: n = 4. Two-way ANOVA (A: F [(16, 117) = 0.3450], p = 0.991; B: interaction: F [(16, 117) = 0.9799], p = 0.483, treatment: F[(2, 117) = 4122], p = 0.019) with Tukey's post hoc test. *p < 0.05 compared to the control group.

memory deficits are not universally observed following SARS-CoV-2 infection (Francis and Thunell, 2023; Wild et al., 2022). Nalbandian et al. provide a comprehensive review of post-acute COVID-19 syndrome, noting that while persistent symptoms can occur following acute COVID-19, studies specifically investigating behavioural and cognitive impacts in animal models remain limited. The review indicates that the multifaceted nature of post-acute COVID-19 syndrome does not definitively establish a consistent association between SARS-CoV-2 infection and long-term depression or cognitive deficits observed in humans (Nalbandian et al., 2021). It is also important to acknowledge the inherent limitations of behavioural tests in animal models. For example, the tail suspension test relies on immobility time as a proxy for depression-like behaviour and may not fully capture the complexity of this phenotype in humans. Moreover, the absence of significant differences in these tests does not preclude the possibility of more subtle behavioural changes or alterations in other behavioural domains. The lack of observed effect on cognitive performance is particularly noteworthy given previous reports of SARS-CoV-2-induced cognitive

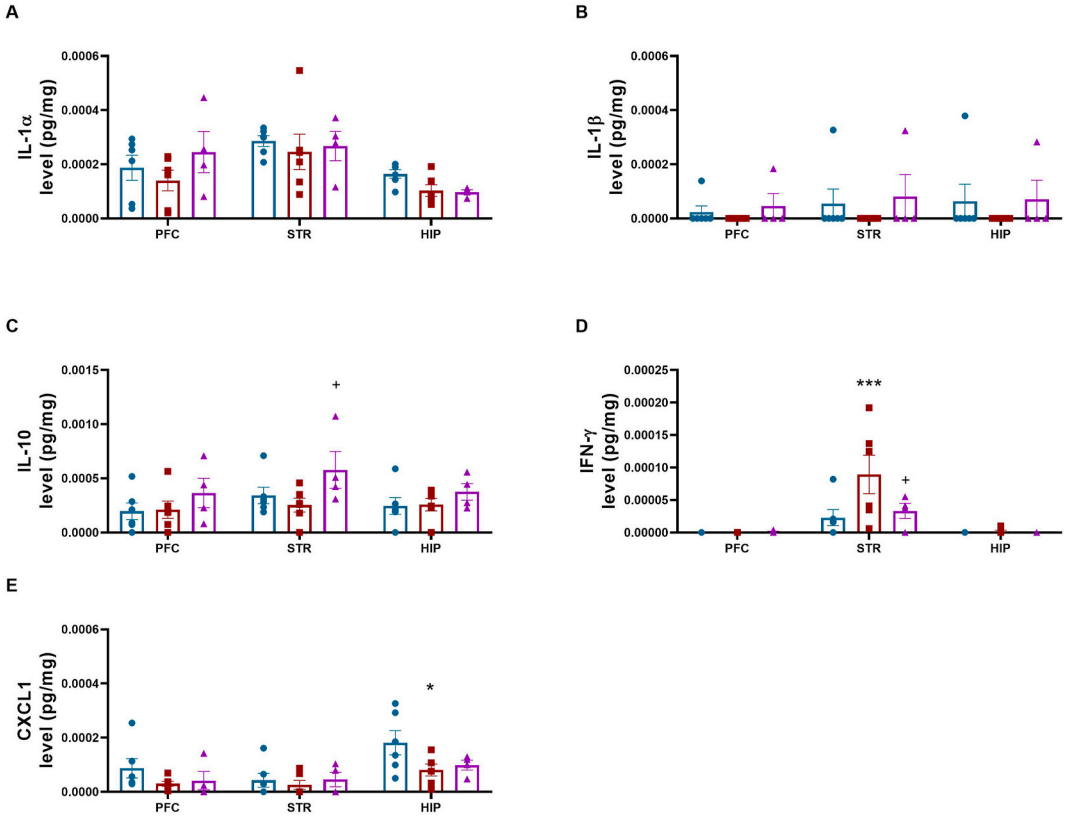


Fig. 7. Cytokine levels in the central nervous system 16 days after SARS-CoV-2 MA10 infection. JNJ treatment increased the IL-10 level in the STR, but viral infection did not alter compared to the control treatment. IFN-γ level was elevated after SARS-CoV-2 MA10 infection, while JNJ treatment markedly decreased its level in the STR. In contrary, CXCL1 was decreased after viral infection in the HIP, and JNJ treatment did not influenced its level. Values are presented as mean ± SEM; control: n = 6, SARS-CoV-2 MA10: n = 6, SARS-CoV-2 MA10 + JNJ: n = 4. Two-way ANOVA (Interaction: A: F[(4, 39) = 0,5839], p = 0.6762 (B) IL-1β F[(4, 39) = 0,08208], p = 0.9874, (D): F[(4, 39) = 2889], p = 0.0346, treatment: (C) F[(2, 39) = 4001], p = 0.0263, (E): F[(2, 39) = 3655], p = 0.0351) with Tukey's post hoc test. *p < 0.05, ***p < 0.001 compared to the control group, + p < 0.05 compared to the SARS-CoV-2 MA10 treated group.

impairments in mice (Jiang et al., 2025; Oh et al., 2022). This discrepancy underscores the variability in outcomes observed across different studies, which may stem from variations in experimental design, such as viral strain, mouse model, or assessment time points (Li et al., 2023). Such methodological variability is a common challenge in preclinical research and has been particularly evident in the rapidly evolving field of COVID-19 research, where standardized protocols are still under development (Dai et al., 2025).

JNJ 47965567 is a high-affinity, selective CNS-penetrant P2X7 antagonist (Bhattacharya et al., 2013) that demonstrated efficacy in preclinical models of psychiatric disorders, including depression (Bhattacharya and Ceusters, 2020), schizophrenia (Koványi et al., 2016) and autism spectrum disorders (Szabó et al., 2022). Infected mice treated with JNJ 47965567 spent significantly more time in the centre of the open field (Fig. 4D), showed increased head orientation toward the centre (Fig. 4E), and had significantly increased locomotor activity (Fig. 4A and B) compared to the infected, untreated group. Thus, in the open field test, but not in the others, JNJ 47965567 prevented the anxiety-like symptoms of SARS-CoV-2 infection (Fig. 4), suggesting endogenous activation of P2X7 receptors and their participation in mediating these virus-induced behavioural changes. These results implicate P2X7 as a potential therapeutic target for managing the anxiety-related sequelae of SARS-CoV-2 infection.

Research highlighted by Amruta et al. demonstrated that infection with mouse-adapted SARS-CoV-2 (MA10) resulted in significant neuroinflammation, suggesting potential mechanisms underlying cognitive deficits and depressive symptoms (Amruta et al., 2022). The presence of viral antigens in the brains of infected mice implies a direct neurotropic effect, further supporting the hypothesis of psychological disturbances following COVID-19 infection (Oladunni et al., 2020). Neuroinvasion by SARS-CoV-2 leads to disruptions at the blood-brain barrier, potentially facilitating the neuroinflammatory processes responsible for post-COVID anxiety and depression (Zhang et al., 2021). Long COVID studies have suggested that chronic effects of the virus can lead to persistent brain inflammation, which has been linked to cognitive impairments and mood disorders (Sriram et al., 2022). The P2X7 and its downstream signalling pathway might also play a crucial role in the immune dysregulation caused by the SARS-CoV-2 infection (García-Villalba et al., 2022; Lécuyer et al., 2023).

To investigate whether pro-inflammatory cytokine activation is present at this stage of post-infection in mice, we conducted a cytokine bead array analysis. Interestingly, plasma and brain samples showed no elevation in pro-inflammatory cytokines such as IL-1α, IL-1β, IL-2, and IL-6 (Fig. 7A, B, C, E), indicating the absence of acute inflammation. Interestingly, in lung samples, IL-1α levels were significantly elevated only in the SARS-CoV-2-infected group treated with JNJ47965567 compared to controls (Fig. 6B). This finding raises several mechanistic possibilities. IL-1α can be induced independently of the canonical P2X7–IL-1β axis and may be upregulated via alternative inflammatory pathways such as TLR signalling or DAMP/PAMP-mediated activation, especially when the P2X7–NLRP3 pathway is pharmacologically blocked (Rider et al., 2011). Although the animals had already recovered from the acute phase, viral antigens or DAMPs may persist in the pulmonary mucosa, sustaining local IL-1α expression - not sufficient to induce systemic inflammation but still capable of promoting focal immune responses, especially via epithelial or non-classical immune cell sources. Moreover, IL-1α is not exclusively pro-inflammatory; it also plays a reparative role when produced by epithelial cells or fibroblasts,

suggesting that its elevated levels may indicate ongoing tissue regeneration in the lung (Chen et al., 2007; Rider et al., 2011).

While acute COVID-19 is often marked by elevated pro-inflammatory cytokines, long COVID may involve a more complex, dysregulated immune response that is not solely characterised by persistent hypercytokinemia (Queiroz et al., 2022). Specifically, a long COVID cytokine profile may involve high IL-17 and IL-2 levels with low IL-4 and IL-10 levels, rather than simply reflecting the cytokine storm of acute infection (Queiroz et al., 2022). This shift in immune profile, combined with potential neuroinflammation driven by persistent viral proteins and vascular changes, may underlie the persistent symptoms of long COVID (Ganesh et al., 2024). CXCL1 levels were significantly decreased in the hippocampus (Fig. 7E), which may reflect persistent neuroimmune activation. During chronic inflammation, diminished CXCL1 production may result from chemokine system exhaustion or negative feedback regulation of inflammatory pathways.

In our study, P2X7 receptor antagonism induced a marked shift toward an anti-inflammatory cytokine profile in the striatum: IL-10 levels were significantly increased in the SARS-CoV-2 + JNJ group compared to untreated infected animals, while IFN-γ levels were concurrently reduced (Fig. 7C and D). This suggests that P2X7 blockade not only suppresses pro-inflammatory signalling but may actively promote anti-inflammatory responses in brain regions implicated in mood regulation. IFN-γ can suppress IL-10 production in macrophages by interfering with the MAPK signalling pathway and through GSK3-mediated inhibition of IL-10 transcription factors such as CREB and AP-1, particularly under TLR2 stimulation (Hu et al., 2006; Saraiva and O'Garra, 2010). The inverse pattern of these cytokines in the striatum observed in our study (Fig. 7C and D) - reduced IFN-γ and increased IL-10 levels following P2X7 blockade - may reflect the relief of IFN-γ–mediated suppression of IL-10. Although IL-10 has also been reported to suppress IFN-γ expression in macrophages (Shibata et al., 1998), this mechanism is less well-characterized in the CNS context. Taken together, these findings suggest that IL-10 and IFN-γ may act as part of a dynamic regulatory loop, and P2X7 signalling appears to influence this interaction during post-acute neuroinflammation. This supports the hypothesis that targeting P2X7 receptors may restore immune homeostasis and mitigate the neuroinflammatory mechanisms underlying PASC.

Ruiz-Rodríguez et al. show that changes in P2X7 expression are possible to impact $CD8^+$ T cell maturation and subsequent production of IFN-γ (Ruiz-Rodríguez et al., 2020), although further research is needed to fully elucidate the interplay between P2X7 and IFN-γ within the striatum following SARS-CoV-2 infection. Interestingly, the selective elevation of IFN-γ in the striatum, but not in the prefrontal cortex or hippocampus, suggests a region-specific inflammatory response, which - although unexpected - is not without precedent in infection models. Mukherjee et al. found elevated IFN-γ levels in the striatum of mice following *Citrobacter rodentium* infection, which contributed to blood-brain barrier disruption and glial activation, processes often seen in viral encephalitis and other inflammation-related conditions within the brain (Mukherjee et al., 2024). This localized IFN-γ response of our study, despite the absence of other pro- and anti-inflammatory cytokine changes, may indicate a distinct inflammatory process within the striatum, a brain region implicated in motor control and reward processing, which can contribute to anxiety and depressive-like behaviours (Lago et al., 2017).

5. Conclusion

In conclusion, here we report for the first time that the activation of P2X7 receptors following SARS-CoV-2 infection is involved in the induction of anxiety and mood alterations through inflammatory pathways. Thus, targeting these pathways may represent a novel therapeutic approach to alleviate post-COVID neuropsychiatric symptoms. However, the complexity of post-acute COVID-19 syndrome makes it difficult to draw definitive conclusions about the direct causal link between SARS-CoV-2 and long-term cognitive or behavioural changes; our study supports the hypothesis that P2X7 antagonism may represent a promising therapeutic avenue for the alleviation post-COVID anxiety and depression by sustaining neuroinflammation. This aligns with the perspective highlighted in the review by Pacheco and Faria, which discusses the potential therapeutic benefits of targeting P2X7-mediated inflammatory responses that could contribute to post-COVID symptoms (Pacheco and Faria, 2021). However, further research is needed to explore the potential of P2X7 antagonism as a therapeutic strategy for post-COVID neurological complications or other viral infections leading to long-term neurobehavioral alterations and to fully elucidate the interplay between P2X7 and IFN-γ within the striatum following SARS-CoV-2 infection.

CRediT authorship contribution statement

Dorottya Szabó: Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Pál Tod:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Fruzsina Maácz:** Investigation, Data curation. **Flóra Gölöncsér:** Investigation. **Bernadett Iring-Varga:** Investigation. **Bibiána Török:** Investigation. **Gyula Zsidei:** Investigation. **Bernadett Pályi:** Methodology. **Zoltán Kis:** Resources, Methodology. **Beáta Sperlágh:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Funding sources

This work was supported by the Hungarian Brain Research Program (grant number NAP2022-I-1/2022 to BS), Recovery and Resilience Facility of the European Union within the framework of Programme Széchenyi Plan Plus (grant number RRF-2.3.1-21-2022-00011, National Laboratory of Translational Neuroscience and grant number RRF-2.3.1-21-2022-00015, National Laboratory of Drug Research and Development to BS). This article is based upon work from COST Action CA21130, supported by COST (European Cooperation in Science and Technology).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful for István Mészáros, Zoltán Zádori and Miklós Gyuranecz from the HUN-REN Veterinary Medical Research Institute (Budapest, Hungary) for initial assistance with virus propagation and the basics of BSL-3 laboratory work, as well as the support of the HUN-REN IEM Cell Biology Center (Flow Cytometry Core Facility).

During the preparation of this work the authors used Jenni.ai in order to improve readability and language. Jenni.ai was used to refine grammar, and sentence structure. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data availability

Data will be made available on request.

References

Alves, V.S., Santos, S., Leite-Aguiar, R., Paiva-Pereira, E., Dos Reis, R.R., Calazans, M.L., Fernandes, G.G., Antônio, L.S., de Lima, E.V., Kurtenbach, E., Silva, J.L., Fontes-Dantas, F.L., Passos, G.F., Figueiredo, C.P., Coutinho-Silva, R., Savio, L.E.B., 2023.

SARS-CoV-2 spike protein alters microglial purinergic signaling. Front. Immunol. 14, 1158460. https://doi.org/10.3389/fimmu.2023.1158460.
Amruta, N., Ismael, S., Leist, S.R., Gressett, T.E., Srivastava, A., Dinnon 3rd, K.H., Engler-Chiurazzi, E.B., Maness, N.J., Qin, X., Kolls, J.K., Baric, R.S., Bix, G., 2022. Mouse adapted SARS-CoV-2 (MA10) viral infection induces neuroinflammation in standard laboratory mice. Viruses 15 (1). https://doi.org/10.3390/v15010114.
Bhattacharya, A., Ceusters, M., 2020. Targeting neuroinflammation with brain penetrant P2X7 antagonists as novel therapeutics for neuropsychiatric disorders. Neuropsychopharmacology 45 (1), 234–235. https://doi.org/10.1038/s41386-019-0502-9.
Bhattacharya, A., Derecki, N.C., Lovenberg, T.W., Drevets, W.C., 2016. Role of neuro-immunological factors in the pathophysiology of mood disorders. Psychopharmacology (Berl) 233 (9), 1623–1636. https://doi.org/10.1007/s00213-016-4214-0.
Bhattacharya, A., Wang, Q., Ao, H., Shoblock, J.R., Lord, B., Aluisio, L., Fraser, I., Nepomuceno, D., Neff, R.A., Welty, N., Lovenberg, T.W., Bonaventure, P., Wickenden, A.D., Letavic, M.A., 2013. Pharmacological characterization of a novel centrally permeable P2X7 receptor antagonist: JNJ-47965567. Br. J. Pharmacol. 170 (3), 624–640. https://doi.org/10.1111/bph.12314.
Bidula, S., Dhuna, K., Helliwell, R., Stokes, L., 2019. Positive allosteric modulation of P2X7 promotes apoptotic cell death over lytic cell death responses in macrophages. Cell Death Dis. 10 (12), 882. https://doi.org/10.1038/s41419-019-2110-3.
Burnstock, G., 2016. P2X ion channel receptors and inflammation. Purinergic Signal. 12 (1), 59–67. https://doi.org/10.1007/s11302-015-9493-0.
Chen, C.-J., Kono, H., Golenbock, D., Reed, G., Akira, S., Rock, K.L., 2007. Identification of a key pathway required for the Sterile inflammatory response triggered by dying cells. Nat. Med. 13 (7), 851–856. https://doi.org/10.1038/nm1603.
Chippa, V., Aleem, A., Anjum, F., 2025. Postacute coronavirus (COVID-19) syndrome. In: StatPearls. StatPearls Publishing Copyright © 2025, StatPearls Publishing LLC.
Chu, H., Chan, J.F.-W., Yuen, K.-Y., 2022. Animal models in SARS-CoV-2 research. Nat. Methods 19 (4), 392–394. https://doi.org/10.1038/s41592-022-01447-w.
Clark, W., Nikola, L., Michael, D., Eckenstein, F., 1997. Monofilament intraluminal middle cerebral artery occlusion in the mouse. Neurol. Res. 19 (6), 641–648. https://doi.org/10.1080/01616412.1997.11740874.
Csölle, C., Baranyi, M., Zsilla, G., Kittel, A., Gölöncsér, F., Illes, P., Papp, E., Vizi, E.S., Sperlágh, B., 2013. Neurochemical changes in the mouse hippocampus underlying the antidepressant effect of genetic deletion of P2X7 receptors. PLoS One 8 (6), e66547. https://doi.org/10.1371/journal.pone.0066547.
D'Hondt, S., Gisle, L., De Pauw, R., Van Cauteren, D., Demarest, S., Drieskens, S., Cornelissen, L., De Ridder, K., Charafeddine, R., Smith, P., 2024. Anxiety and depression in people with post-COVID condition: a Belgian population-based cohort study three months after SARS-CoV-2 infection. Soc. Psychiatr. Psychiatr. Epidemiol. 59 (11), 2083–2092. https://doi.org/10.1007/s00127-024-02655-9.
Dai, J., He, F., Chen, Q., Li, Q., Zhao, L., Du, Y., 2025. Animal models of post-acute COVID-19 syndrome: a call for longitudinal animal studies. Front. Immunol. 16. https://doi.org/10.3389/fimmu.2025.1521029.
Drayson, O.G.G., Vozenin, M.C., Limoli, C.L., 2023. A rigorous behavioral testing platform for the assessment of radiation-induced neurological outcomes. Methods Cell Biol. 180, 177–197. https://doi.org/10.1016/bs.mcb.2023.02.015.
Edouard Mathieu, H.R., Rodés-Guirao, Lucas, Appel, Cameron, Gavrilov, Daniel, Giattino, Charlie, Hasell, Joe, Macdonald, Bobbie, Dattani, Saloni, Beltekian, Diana, Ortiz-Ospina, Esteban, Roser, Max, 2024. Excess mortality during the coronavirus pandemic (COVID-19). https://ourworldindata.org/excess-mortality-covid.
Francis, G., Thunell, E., 2023. COVID-19 infection does not seem to affect cognition in college students. Conscious. Cognit. 108, 103464. https://doi.org/10.1016/j.concog.2023.103464.
Frontera, J.A., Simon, N.M., 2022. Bridging knowledge gaps in the diagnosis and management of neuropsychiatric sequelae of COVID-19. JAMA Psychiatry 79 (8), 811–817. https://doi.org/10.1001/jamapsychiatry.2022.1616.
Ganesh, R., Yadav, S., Hurt, R.T., Mueller, M.R., Aakre, C.A., Gilman, E.A., Grach, S.L., Overgaard, J., Snyder, M.R., Collins, N.M., Croghan, I.T., Badley, A.D., Razonable, R. R., Salonen, B.R., 2024. Pro inflammatory cytokines profiles of patients with long COVID differ between variant epochs. Journal of Primary Care & Community Health 15, 21501319241254751. https://doi.org/10.1177/21501319241254751.
García-Villalba, J., Hurtado-Navarro, L., Peñín-Franch, A., Molina-López, C., Martínez-Alarcón, L., Angosto-Bazarra, D., Baroja-Mazo, A., Pelegrin, P., 2022. Soluble P2X7 receptor is elevated in the plasma of COVID-19 patients and correlates with disease severity. Front. Immunol. 13, 894470. https://doi.org/10.3389/fimmu.2022.894470.
Hu, C., Luo, Y., Wang, H., Kuang, S., Liang, G., Yang, Y., Mai, S., Yang, J., 2017. Re-evaluation of the interrelationships among the behavioral tests in rats exposed to chronic unpredictable mild stress. PLoS One 12 (9), e0185129. https://doi.org/10.1371/journal.pone.0185129.
Hu, X., Paik, P.K., Chen, J., Yarilina, A., Kockeritz, L., Lu, T.T., Woodgett, J.R., Ivashkiv, L.B., 2006. IFN-Gamma suppresses IL-10 production and synergizes with TLR2 by regulating GSK3 and CREB/AP-1 proteins. Immunity 24 (5), 563–574. https://doi.org/10.1016/j.immuni.2006.02.014.
Iqbal, P., Ata, F., Chaudhry, H., Muthanna, B., Waqas Younas, H., Munamm, S.A.U., Sharma, R., Fadah, K., Elazzazy, S., Hamad, A., Said Abu Tabar, O., Omar, N.E., 2023. Post-COVID-19-associated multiorgan complications or "long COVID" with literature review and management strategy discussion: a meta-analysis. Health Sci. Rep. 6 (4), e1211. https://doi.org/10.1002/hsr2.1211.
Jiang, Q., Li, G., Wang, H., Chen, W., Liang, F., Kong, H., Chen, T.S.R., Lin, L., Hong, H., Pei, Z., 2025. SARS-CoV-2 spike S1 protein induces microglial NLRP3-dependent neuroinflammation and cognitive impairment in mice. Exp. Neurol. 383, 115020. https://doi.org/10.1016/j.expneurol.2024.115020. (Accessed 1 January 2025).
Jiao, T., Huang, Y., Sun, H., Yang, L., 2024. Research progress of post-acute sequelae after SARS-CoV-2 infection. Cell Death Dis. 15 (4), 257. https://doi.org/10.1038/s41419-024-06642-5.
Klein, R., Soung, A., Sissoko, C., Nordvig, A., Canoll, P., Mariani, M., Jiang, X., Bricker, T., Goldman, J., Rosoklija, G., Arango, V., Underwood, M., Mann, J.J., Boon, A., Dowrk, A., Boldrini, M., 2021. COVID-19 induces neuroinflammation and loss of hippocampal neurogenesis. Res Sq. https://doi.org/10.21203/rs.3.rs-1031824/v1.
Koványi, B., Csölle, C., Calovi, S., Hanuska, A., Kató, E., Köles, L., Bhattacharya, A., Haller, J., Sperlágh, B., 2016. The role of P2X7 receptors in a rodent PCP-Induced schizophrenia model. Sci. Rep. 6 (1), 36680. https://doi.org/10.1038/srep36680.
Lago, T., Davis, A., Grillon, C., Ernst, M., 2017. Striatum on the anxiety map: small detours into adolescence. Brain Res. 1654 (Pt B), 177–184. https://doi.org/10.1016/j.brainres.2016.06.006.
Lécuyer, D., Nardacci, R., Tannous, D., Gutierrez-Mateyron, E., Deva Nathan, A., Subra, F., Di Primio, C., Quaranta, P., Petit, V., Richetta, C., Mostefa-Kara, A., Del Nonno, F., Falasca, L., Marlin, R., Maisonnasse, P., Delahousse, J., Pascaud, J., Deprez, E., Naigeon, M., Chaput, N., Paci, A., Saada, V., Ghez, D., Mariette, X., Costa, M., Pistello, M., Allouch, A., Delelis, O., Piacentini, M., Le Grand, R., Perfettini, J.L., 2023. The purinergic receptor P2X7 and the NLRP3 inflammasome are druggable host factors required for SARS-CoV-2 infection. Front. Immunol. 14, 1270081. https://doi.org/10.3389/fimmu.2023.1270081.
Leeson, H.C., Chan-Ling, T., Lovelace, M.D., Brownlie, J.C., Gu, B.J., Weible, M.W., 2019. P2X7 receptor signaling during adult hippocampal neurogenesis. Neural Regen Res 14 (10), 1684–1694. https://doi.org/10.4103/1673-5374.257510.
Leist, S.R., Dinnon 3rd, K.H., Schäfer, A., Tse, L.V., Okuda, K., Hou, Y.J., West, A., Edwards, C.E., Sanders, W., Fritch, E.J., Gully, K.L., Scobey, T., Brown, A.J., Sheahan, T.P., Moorman, N.J., Boucher, R.C., Gralinski, L.E., Montgomery, S.A., Baric, R.S., 2020. A mouse-adapted SARS-CoV-2 induces acute lung injury and mortality in standard laboratory mice. Cell 183 (4), 1070. https://doi.org/10.1016/j.cell.2020.09.050, 1085.e1012.
Li, J., Wang, J., Wang, H., 2023. Emerging landscape of preclinical models for studying COVID-19 neurologic diseases. ACS Pharmacol. Transl. Sci. 6 (10), 1323–1339. https://doi.org/10.1021/acsptsci.3c00127.
Miras-Portugal, M.T., Sebastián-Serrano, Á., de Diego García, L., Díaz-Hernández, M., 2017. Neuronal P2X7 receptor: involvement in neuronal physiology and pathology. J. Neurosci. 37 (30), 7063–7072. https://doi.org/10.1523/jneurosci.3104-16.2017.
Monje, M., Iwasaki, A., 2022. The neurobiology of long COVID. Neuron 110 (21), 3484–3496. https://doi.org/10.1016/j.neuron.2022.10.006. (Accessed 2 November 2022).
Motulsky, H.J., Brown, R.E., 2006. Detecting outliers when fitting data with nonlinear regression - a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinf. 7, 123. https://doi.org/10.1186/1471-2105-7-123.
Mukherjee, S., Grouza, V., Tchung, A., Even, A., Yaqubi, M., Tuznik, M., Canon, T., Recinto, S., Gavino, C., Bourque, M.-J., McBride, H., Desjardins, M., Gruenheid, S., Stratton, J., Rudko, D., Trudeau, L.-E., 2024. A single Citrobacter rodentium infection in Pink1 knockout and wild type mice leads to regional blood-brain-barrier perturbation and glial activation without dopamine neuron axon terminal loss. https://doi.org/10.1101/2024.12.24.630165.
Nalbandian, A., Sehgal, K., Gupta, A., Madhavan, M.V., McGroder, C., Stevens, J.S., Cook, J.R., Nordvig, A.S., Shalev, D., Sehrawat, T.S., Ahluwalia, N., Bikdeli, B., Dietz, D., Der-Nigoghossian, C., Liyanage-Don, N., Rosner, G.F., Bernstein, E.J., Mohan, S., Beckley, A.A., Seres, D.S., Choueiri, T.K., Uriel, N., Ausiello, J.C., Accili, D., Freedberg, D.E., Baldwin, M., Schwartz, A., Brodie, D., Garcia, C.K., Elkind, M.S.V., Connors, J.M., Bilezikian, J.P., Landry, D.W., Wan, E.Y., 2021. Post-acute COVID-19 syndrome. Nat. Med. 27 (4), 601–615. https://doi.org/10.1038/s41591-021-01283-z.
Oh, J., Cho, W.-H., Barcelon, E., Kim, K.H., Hong, J., Lee, S.J., 2022. SARS-CoV-2 spike protein induces cognitive deficit and anxiety-like behavior in mouse via non-cell autonomous hippocampal neuronal death. Sci. Rep. 12 (1), 5496. https://doi.org/10.1038/s41598-022-09410-7.
Oladunni, F.S., Park, J.-G., Pino, P.A., Gonzalez, O., Akhter, A., Allué-Guardia, A., Olmo-Fontánez, A., Gautam, S., Garcia-Vilanova, A., Ye, C., Chiem, K., Headley, C., Dwivedi, V., Parodi, L.M., Alfson, K.J., Staples, H.M., Schami, A., Garcia, J.I., Whigham, A., Platt, R.N., Gazi, M., Martinez, J., Chuba, C., Earley, S., Rodriguez, O. H., Mdaki, S.D., Kavelish, K.N., Escalona, R., Hallam, C.R.A., Christie, C., Patterson, J.L., Anderson, T.J.C., Carrion, R., Dick, E.J., Hall-Ursone, S., Schlesinger, L.S., Alvarez, X., Kaushal, D., Giavedoni, L.D., Turner, J., Martinez-Sobrido, L., Torrelles, J.B., 2020. Lethality of SARS-CoV-2 infection in K18 human angiotensin-converting enzyme 2 transgenic mice. Nat. Commun. 11 (1), 6122. https://doi.org/10.1038/s41467-020-19891-7.
Oliveira-Giacomelli, Á., Petiz, L.L., Andrejew, R., Turrini, N., Silva, J.B., Sack, U., Ulrich, H., 2021. Role of P2X7 receptors in immune responses during neurodegeneration. Front. Cell. Neurosci. 15, 662935. https://doi.org/10.3389/fncel.2021.662935.
Oliveira, Á., Illes, P., Ulrich, H., 2016. Purinergic receptors in embryonic and adult neurogenesis. Neuropharmacology 104, 272–281. https://doi.org/10.1016/j.neuropharm.2015.10.008.
Organization, W.H., 2025. Post-COVID-19 condition (long COVID). https://www.who.int/news-room/fact-sheets/detail/post-covid-19-condition-(long-covid.
Pacheco, P.A.F., Faria, R.X., 2021. The potential involvement of P2X7 receptor in COVID-19 pathogenesis: a new therapeutic target? Scand. J. Immunol. 93 (2), e12960. https://doi.org/10.1111/sji.12960.
Queiroz, M.A.F., Neves, P., Lima, S.S., Lopes, J.D.C., Torres, M., Vallinoto, I., Bichara, C. D.A., Dos Santos, E.F., de Brito, M., da Silva, A.L.S., Leite, M.M., da Costa, F.P., Viana, M., Rodrigues, F.B.B., de Sarges, K.M.L., Cantanhede, M.H.D., da Silva, R.,

Bichara, C.N.C., van den Berg, A.V.S., Veríssimo, A.O.L., Carvalho, M.D.S., Henriques, D.F., Dos Santos, C.P., Nunes, J.A.L., Costa, I.B., Viana, G.M.R., Carneiro, F.R.O., Palacios, V., Quaresma, J.A.S., Brasil-Costa, I., Dos Santos, E.J.M., Falcão, L.F.M., Vallinoto, A.C.R., 2022. Cytokine profiles associated with acute COVID-19 and long COVID-19 syndrome. Front. Cell. Infect. Microbiol. 12, 922422. https://doi.org/10.3389/fcimb.2022.922422.

Ribeiro, D.E., Oliveira-Giacomelli, Á., Glaser, T., Arnaud-Sampaio, V.F., Andrejew, R., Dieckmann, L., Baranova, J., Lameu, C., Ratajczak, M.Z., Ulrich, H., 2021. Hyperactivation of P2X7 receptors as a culprit of COVID-19 neuropathology. Mol. Psychiatr. 26 (4), 1044–1059. https://doi.org/10.1038/s41380-020-00965-3.

Rider, P., Carmi, Y., Guttman, O., Braiman, A., Cohen, I., Voronov, E., White, M.R., Dinarello, C.A., Apte, R.N., 2011. IL-1α and IL-1β recruit different myeloid cells and promote different stages of sterile inflammation. J. Immunol. 187 (9), 4835–4843. https://doi.org/10.4049/jimmunol.1102048.

Ruiz-Rodríguez, V.M., Turiján-Espinoza, E., Guel-Pañola, J.A., García-Hernández, M.H., Zermeño-Nava, J.J., López-López, N., Bernal-Silva, S., Layseca-Espinosa, E., Fuentes-Pananá, E.M., Estrada-Sánchez, A.M., Portales-Pérez, D.P., 2020. Chemoresistance in breast cancer patients associated with changes in P2X7 and A2A purinergic receptors in CD8(+) T lymphocytes. Front. Pharmacol. 11, 576955. https://doi.org/10.3389/fphar.2020.576955.

Saraiva, M., O'Garra, A., 2010. The regulation of IL-10 production by immune cells. Nat. Rev. Immunol. 10 (3), 170–181. https://doi.org/10.1038/nri2711.

Shibata, Y., Foster, L.A., Kurimoto, M., Okamura, H., Nakamura, R.M., Kawajiri, K., Justice, J.P., Van Scott, M.R., Myrvik, Q.N., Metzger, W.J., 1998. Immunoregulatory roles of IL-10 in innate immunity: IL-10 inhibits macrophage production of IFN-gamma-inducing factors but enhances NK cell production of IFN-Gamma. J. Immunol. 161 (8), 4283–4288.

Singh, A., Adam, A., Aditi, Peng, B.H., Yu, X., Zou, J., Kulkarni, V.V., Kan, P., Jiang, W., Shi, P.Y., Samir, P., Cisneros, I., Wang, T., 2024. A murine model of post-acute neurological sequelae following SARS-CoV-2 variant infection. Front. Immunol. 15, 1384516. https://doi.org/10.3389/fimmu.2024.1384516.

Sperlágh, B., Illes, P., 2014. P2X7 receptor: an emerging target in central nervous system diseases. Trends Pharmacol. Sci. 35 (10), 537–547. https://doi.org/10.1016/j.tips.2014.08.002.

Sriram, S., Parekh, R., Theobald, D., Akula, S., O'Rourke, D., Eells, J., 2022. Long COVID in K18-hACE2 mice causes persistent brain inflammation and cognitive impairment. https://doi.org/10.21203/rs.3.rs-1818930/v1.

Szabó, D., Tod, P., Gölöncsér, F., Román, V., Lendvai, B., Otrokocsi, L., Sperlágh, B., 2022. Maternal P2X7 receptor inhibition prevents autism-like phenotype in Male mouse offspring through the NLRP3-IL-1β pathway. Brain Behav. Immun. 101, 318–332. https://doi.org/10.1016/j.bbi.2022.01.015. (Accessed 1 March 2022).

Takao, M., Ohira, M., 2023. Neurological post-acute sequelae of SARS-CoV-2 infection. Psychiatr. Clin. Neurosci. 77 (2), 72–83. https://doi.org/10.1111/pcn.13481.

Vultaggio-Poma, V., Sanz, J.M., Amico, A., Violi, A., Ghisellini, S., Pizzicotti, S., Passaro, A., Papi, A., Libanore, M., Di Virgilio, F., Giuliani, A.L., 2023. The shed P2X7 receptor is an index of adverse clinical outcome in COVID-19 patients. Front. Immunol. 14, 1182454. https://doi.org/10.3389/fimmu.2023.1182454.

Wild, C.J., Norton, L., Menon, D.K., Ripsman, D.A., Swartz, R.H., Owen, A.M., 2022. Disentangling the cognitive, physical, and mental health sequelae of COVID-19. Cell Rep. Med. 3 (10), 100750. https://doi.org/10.1016/j.xcrm.2022.100750. (Accessed 18 October 2022).

Yin, W., Godbout, J.P., Sheridan, J.F., 2024. Chapter 7 - interleukin-1 beta in psychosocial stress. In: Fink, G. (Ed.), Stress: Immunology and Inflammation. Academic Press, pp. 53–63. https://doi.org/10.1016/B978-0-12-817558-3.00007-X.

Zeng, N., Zhao, Y.-M., Yan, W., Li, C., Lu, Q.-D., Liu, L., Ni, S.-Y., Mei, H., Yuan, K., Shi, L., Li, P., Fan, T.-T., Yuan, J.-L., Vitiello, M.V., Kosten, T., Kondratiuk, A.L., Sun, H.-Q., Tang, X.-D., Liu, M.-Y., Lalvani, A., Shi, J., Bao, Y.-P., Lu, L., 2023. A systematic review and meta-analysis of long term physical and mental sequelae of COVID-19 pandemic: call for research priority and action. Mol. Psychiatr. 28 (1), 423–433. https://doi.org/10.1038/s41380-022-01614-7.

Zhang, L., Zhou, L., Bao, L., Liu, J., Zhu, H., Lv, Q., Liu, R., Chen, W., Tong, W., Wei, Q., Xu, Y., Deng, W., Gao, H., Xue, J., Song, Z., Yu, P., Han, Y., Zhang, Y., Sun, X., Yu, X., Qin, C., 2021. SARS-CoV-2 crosses the blood–brain barrier accompanied with basement membrane disruption without tight junctions alteration. Signal Transduct. Targeted Ther. 6 (1), 337. https://doi.org/10.1038/s41392-021-00719-9.