Journal of NeuroVirology
https://doi.org/10.1007/s13365-023-01156-w

Serum of COVID-19 patients changes neuroinflammation and mitochondrial homeostasis markers in hippocampus of aged rats

Yorran Hardman A. Montenegro[1] · Larissa Daniele Bobermin[1] · Patrícia Sesterheim[1,2,3] · Richard Steiner Salvato[3] · Fernando Anschau[1,4,5,6] · Maria José Santos de Oliveira[1] · Angela T. S. Wyse[7] · Carlos Alexandre Netto[1,7] · Carlos-Alberto Saraiva Gonçalves[1,7] · André Quincozes-Santos[1,7] · Guilhian Leipnitz[1,7]

Received: 9 March 2023 / Revised: 18 May 2023 / Accepted: 26 June 2023

Abstract
Patients affected by COVID-19 present mostly with respiratory symptoms but acute neurological symptoms are also commonly observed. Furthermore, a considerable number of individuals develop persistent and often remitting symptoms months after infection, characterizing the condition called long-COVID. Since the pathophysiology of acute and persistent neurological manifestations is not fully established, we evaluated the expression of different genes in hippocampal slices of aged rats exposed to the serum of a post-COVID (sPC) individual and to the serum of patients infected by SARS-CoV-2 [Zeta (sZeta) and Gamma (sGamma) variants]. The expression of proteins related to inflammatory process, redox homeostasis, mitochondrial quality control and glial reactivity was determined. Our data show that the exposure to sPC, sZeta and sGamma differentially altered the mRNA levels of most inflammatory proteins and reduced those of antioxidant response markers in rat hippocampus. Furthermore, a decrease in the expression of mitochondrial biogenesis genes was induced by all serum samples, whereas a reduction in mitochondrial dynamics was only caused by sPC. Regarding the glial reactivity, S100B expression was modified by sPC and sZeta. These findings demonstrate that changes in the inflammatory response and a reduction of mitochondrial biogenesis and dynamics may contribute to the neurological damage observed in COVID-19 patients.

Keywords SARS-CoV-2 · COVID-19 · Neurological symptoms · Inflammation · Mitochondria · Hippocampus

Introduction

A considerable number of individuals infected by SARS-CoV-2 present with neurological manifestations with astrocyte and neuron injury possibly related to a neuroinflammatory process triggered by the so-called cytokine storm (Vabret et al. 2020; Najjar et al. 2020; Monje and Iwasaki 2022). These acute neurological symptoms include anosmia, ageusia, cognitive impairment, depression, and anxiety (Nasserie et al. 2021).

✉ Yorran Hardman A. Montenegro
yorran_montenegro@hotmail.com

✉ Guilhian Leipnitz
guilhian@ufrgs.br

[1] Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, RS, Porto Alegre, Brazil

[2] Programa de Pós-Graduação em Ciências da Saúde: Cardiologia, Instituto de Cardiologia/ Fundação Universitária de Cardiologia, RS, Porto Alegre, Brazil

[3] Centro de Desenvolvimento Científico e Tecnológico, Centro Estadual de Vigilância em Saúde da Secretaria de Saúde do Estado do Rio Grande do Sul, RS, Porto Alegre, Brazil

[4] Setor de Pesquisa da Gerência de Ensino, Pesquisa e Inovação do Grupo Hospitalar Conceição (GHC), RS, Porto Alegre, Brazil

[5] Programa de Pós-Graduação em Avaliação de Tecnologias para o SUS do GHC, Porto Alegre, RS, Brazil

[6] Escola de Medicina, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil

[7] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, RS, Porto Alegre, Brazil

Published online: 27 July 2023

Additionally, mounting evidence has shown that a considerable percentage of individuals previously infected with SARS-CoV-2 develop persistent and often remitting symptoms months after infection (Spudich and Nath 2022). The presence of these lasting neurological sequelae is referred to as post-COVID-19 condition or long COVID (Subramanian et al. 2022; Raveendran et al. 2021; Halpin et al. 2021).

Glial cells actively participate in cognitive function by interacting with neurons, regulating the activity of neural circuits and also by forming and maintaining the integrity of the blood–brain barrier (BBB). Furthermore, these cells have an important role during viral infections since their activation leads to the release of proinflammatory molecules, stimulating both the innate and the adaptive immune systems (Chen and Li 2021). In the context of COVID-19, it has been shown that the immune response to SARS-CoV-2 in the respiratory system may cause neuroinflammation, inducing the activation of resident microglia and astrocytes. In turn, astrocyte reactivity may induce BBB disruption and neural circuit impairment (Zhang et al. 2021). Consistent with these observations, studies have shown high plasma levels of glial fibrillary acidic protein (GFAP) in COVID-19 patients (Kanberg et al., 2020) and the presence of SARS-CoV-2 particles in astrocytes of patients (Crunfli et al. 2020).

In addition, mitochondria have been also suggested to participate in the pathogenesis of COVID-19 owing to their role in the innate immune system (Bhowal et al. 2022). After SARS-CoV-2 invasion in cells, an increase in mitochondrial reactive oxygen species (ROS) generation with subsequent respiratory chain impairment leads to the release of mitochondrial DNA and cardiolipin. These so-called damage-associated molecular patterns (DAMPs) activate Toll-like receptors (TLR) and nuclear factor kappa B (NFκB), as well as the release of inflammatory cytokines. Furthermore, SARS-CoV-2 is known to modulate Mitochondrial Antiviral Signal (MAVS), which further contributes to the production of cytokines and induction of a hyperinflammatory state (Burtscher et al. 2020; Banoth and Cassel 2018).

Little is known about the mechanisms underlying the neurological dysfunction observed in SARS-CoV-2 infection, as well as in long-COVID. Moreover, whether the neuroinflammation occurs via direct viral invasion into the central nervous system (CNS) or by a sustained systemic inflammation that disrupts the BBB is still a matter of debate. Our hypothesis is that pro-inflammatory factors present in the blood of patients may alter the expression of different genes in the brain thereby contributing to neurological manifestations. Therefore, in the present study, we evaluated the expression of genes related to glial reactivity, mitochondrial function, and inflammation in hippocampal slices from aged rats exposed to the serum of COVID-19 patients.

Methods

Serum sample collection and viral variant analysis

Serum from four individuals (three individuals that had SARS-CoV-2 infection and one individual that was not infected) was used in the experiments. Blood was collected from two individuals infected with SARS-CoV-2 at Grupo Hospitalar Conceição, Porto Alegre, RS, Brazil (process number: CEP/GHC 4280802), at the time of admission to the intensive care unit (ICU) during the acute phase of COVID-19, during the period of January 05, 2021, and June 11, 2021. The collection followed the internal protocol of Grupo Hospitalar Conceição. Blood was also collected from an individual previously infected with SARS-CoV-2 but tested negative for SARS-CoV-2 (PCR test) during the blood collection. A pre-COVID control serum (naive) was used as a control for gene expression analysis. The serum of each patient was aliquoted and kept in an ultra-freezer at -80 ºC until the moment of use.

SARS-CoV-2 detection in serum

SARS-CoV-2 detection was conducted through reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The RNA was extracted from samples using a MagMAX Viral/Pathogen II Isolation kit on a KingFisher Flex extractor (Thermo Fisher Scientific, Waltham, USA) and the pathogen detection was performed using the Allplex SARS-CoV-2 Assay (Seegene Inc, Seoul, Republic of Korea) in a CFX Opus Real-Time PCR System (Bio-Rad, Hercules, CA, USA). A CT higher than 30 indicated low levels or absence of viral particles.

Genome sequencing of SARS-CoV-2 genome in the patients´ serum

Whole genome sequencing of SARS-CoV-2 in the patients' serum was performed using the Illumina COVIDSeq protocol (Illumina Inc, USA) on the Illumina MiSeq platform, according to the manufacturer's instructions. The pipeline ViralFlow v1.0 (https://viralflow.github.io) was used to perform genome assembly, variant calling, and consensus generation. The SARS-CoV-2 lineages assignment was performed using the web-based software Pangolin v4.2 (Phylogenetic Assignment of Named Global Outbreak Lineages), available at https://pangolin.cog-uk.io.

Animals

Male Wistar rats (n = 4–6) were obtained from the Department of Biochemistry, ICBS, Federal University of Rio Grande do Sul (UFRGS), Brazil. Rats were maintained on a 12:12 h light/dark cycle (lights on 7:00 am—7:00 pm) in a constant temperature (22 ± 1 °C) colony room, with free access to water and 20% (w/w) protein commercial chow (SUPRA, RS, Brazil). All animal experiments were performed following the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees of the Federal University of Rio Grande do Sul (process number 42979). Considering that age is a risk factor associated with a greater rate of mortality due to COVID-19 (Kang and Jung 2020), we used 365-day-old rats.

Hippocampal slice preparation

At the age of 365 days, the animals were euthanized by decapitation under anesthesia with isoflurane (1–2%, inhalation). Hippocampi were rapidly dissected and transversely sliced (300 µm) in a McIlwain Tissue Chopper. The slices were separated and incubated individually in culture plates (1 slice per well) at room temperature (25 °C). The incubation medium contained oxygenated saline buffer with 120 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM HEPES, 1 mM KH_2PO_4 and 10 mM glucose, pH 7.4. The medium was changed every 15 min with fresh saline buffer at room temperature. After a stabilization period of 60 min, the slices were incubated under different conditions (see topic 2.4) at 30 °C for 2 h (Bobermin et al. 2020) (Fig. 1a).

Exposure of hippocampal slices to the blood serum

After stabilization, the slices were exposed for 2 h to the following: i) saline solution containing serum (10% v/v) from one individual (naive) that was not infected with SARS-CoV-2; ii) saline solution containing serum (10% v/v) from one individual previously infected with SARS-CoV-2 but negative at the moment of the collection; iii and iv) saline solution containing serum (10% v/v) from two different SARS-CoV-2 positive patients with two different viral variants. At the end of the incubation period, the slices submitted to the same treatment were pooled in tubes containing the Trizol reagent. Incubations with blood serum were carried out in a biosafety level 2 (NB2) laboratory, as recommended by the Pan American Health Organization (PAHO) and the Ministry of Health (Brazil). It should be noted that our approach consists of an acute model (2-h exposure), which was used to guarantee the maintenance of cell viability, as previously shown (Nardin et al. 2009).

RNA extraction and cDNA conversion

Total RNA was isolated from the hippocampal slices using the Trizol reagent (Invitrogen, CA, USA) (Bobermin et al. 2020). RNA concentration and purity were determined spectrophotometrically (Biodrop Duo, Biochrom) at a ratio of 260:280. Total RNA (0.15 g) was reversed transcribed to obtain complementary DNA (cDNA) using the Applied Biosystems™ High Capacity Reverse Transcription kit in a reaction volume of 20 µL, according to the manufacturer's instructions (Applied Biosystems, Thermo Fisher Scientific).

RT-qPCR

RT-qPCR analysis was preceded by a 1:20 cDNA dilution. Subsequently, RT-qPCR reactions were performed using Taqman probes (ThermoFisher, MA, USA) for the following targets: β-Actin; p21 (Rn00589996_m1); citrate synthase (CS) (Rn01774376_g1); dynamin-related protein 1 (DRP1; Rn00586466_m1); GFAP (Rn00566603_m1); heme oxygenase 1 (HO-1; Rn01536933_m1); interferon-induced transmembrane protein 3 (IFITM3; Rn03811114_s1); interleukin-1 beta (IL-1ß; Rn00580432_m1); interleukin 1 receptor type 1 (IL1R1; Rn00565482_m1); p38 mitogen-activated protein kinase (p38 MAPK; Rn00578842_m1); mitofusin 1 (MFN1; Rn00594496_m1); nuclear factor of activated T cells 3 (NFAT; Rn01426728_m1); NFκB p65 (Rn01502266_m1); nuclear factor erythroid-derived 2-like 2 (Nrf2; Rn00582415_m1); peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α; Rn00580241_m1); calcineurin (CaN; Rn00690508_m1); cyclooxygenase 2 (COX-2; Rn01483828_m1); S100 calcium binding protein B (S100B; Rn04219408_m1); signal transducer and activator of transcription 3 (STAT3; Rn00680715_m1); TLR2 (Rn02133647_s1); TLR3 (Rn01488472_g1); TLR4 (Rn00569848_m1); tumor necrosis factor alpha (TNF-α; Rn99999017_m1); tumor necrosis factor receptor 1 (TNFR1; Rn01492348_m1); translocase of outer mitochondrial membrane 70 kDa (Tom70; Rn01444393_g1); voltage dependent anion channel 1 (VDAC1; Rn00821325_g1). Target mRNA levels were normalized by β-actin. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

The results were analyzed by ANOVA followed by Tukey's test if they were normally distributed. Otherwise, the non-parametric Kruskal–Wallis test was used. $p < 0.05$ was considered significant.

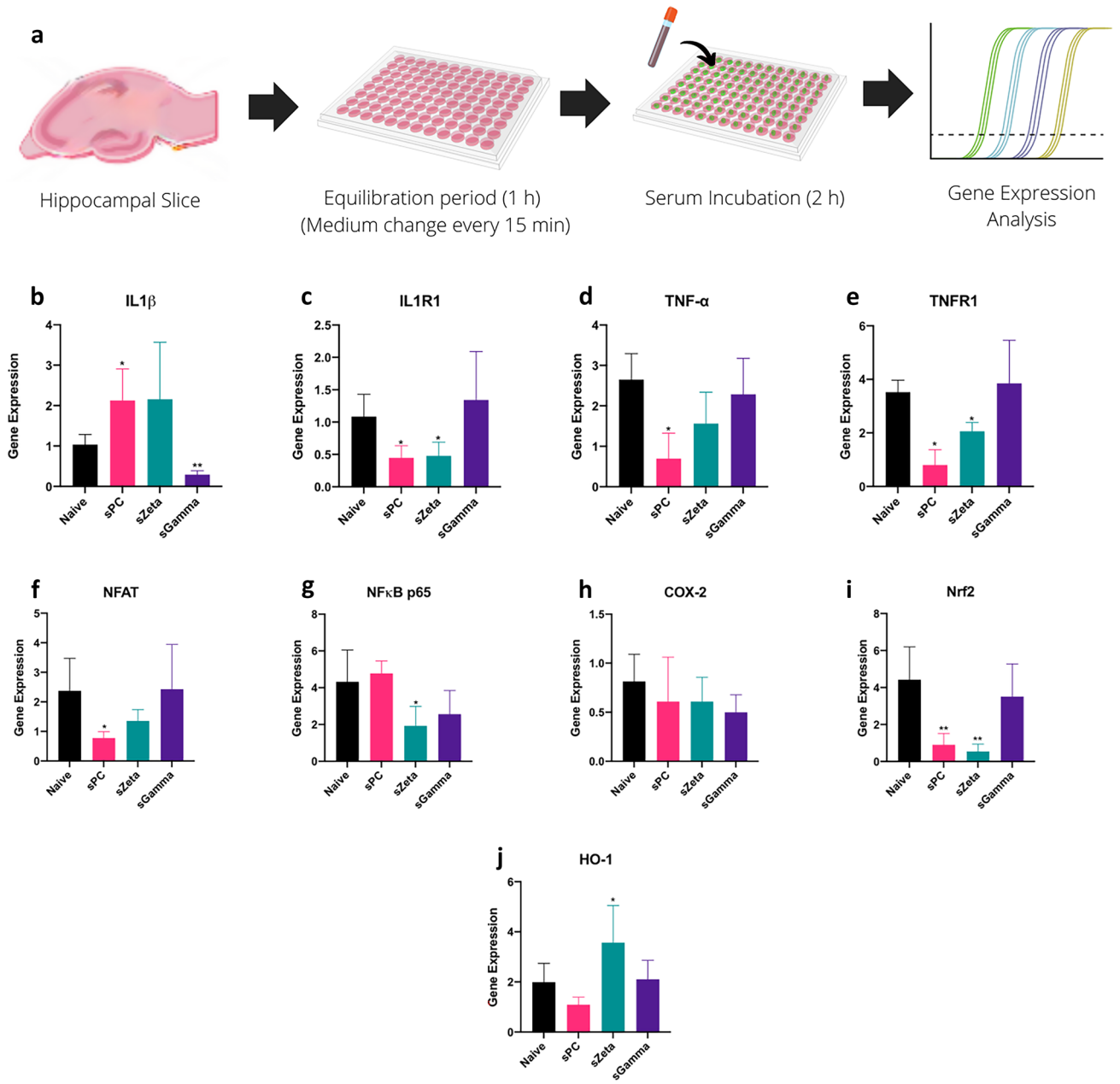

Fig. 1 **a** Schematic representation of the experimental approach used to expose rat hippocampal slices to different treatments. Hippocampal slices (300 mm) were obtained from 365-day-old rats and maintained for an equilibration period of 1 h (cellular recovery). After this period, the slices were treated with different sera for 2 h: serum from an individual not infected with SARS-CoV-2 (naïve), serum from a post-COVID patient (sPC), serum of a patient infected with Zeta variant (sZeta), and serum of a patient infected with the Gamma variant (sGamma). **b**-**j** mRNA expression of IL-1b **b**, IL1R1 **c**, TNFa **d**, TNFR1 **e**, NFAT **f**, NF-kB **g**, COX-2 h, Nrf2 **i** and HO-1 **j**. In black, expression levels in slices exposed to naïve serum; in pink, expression levels in slices exposed to sPC; in green, expression levels in slices exposed to sZeta; and, in purple, expression levels in slices exposed to sGamma. Data represent means ± SD of at least four experimental determinations performed in triplicate

Results

Viral variant determination

Two SARS-CoV-2 positive patients with different variants were recruited at Grupo Hospitalar Conceição, Brazil. The profiles of these individuals are described in Table 1. The analysis of the variants in the blood of these patients showed that the first patient had Zeta variant (also known as B.1.1.28), whereas the second patient had Gamma variant (also known as B.1.1.248). Zeta variant carries the lineage-defining mutations ORF1ab:L3468V, ORF1ab:synC11824U,

Table 1 Serum and Patient Profile

Viral Variant	Sex	Age	C-reactive protein (mg/L)	Hospitalization
Naïve	Woman	60 y.o.	< 5mg/L	N/A
Post-COVID-19	Man	61 y.o.	< 5mg/L	N/A
Zeta	Woman	65 y.o.	> 5mg/L	Dec, 12th 2020
Gamma	Man	61 y.o.	> 5mg/L	Jun, 11th 2021

N/A: Not Applicable; Normal C-reactive protein levels: < 5mg/L

N:A119S, and S:E484K, and was described for the first time in the city of Rio de Janeiro, Brazil (Varela et al. 2021; Faria et al. 2021; Voloch et al. 2021). Gamma variant has twelve mutations in the S protein (three were identified in our analysis: K417N, E484K, N501Y) and was described for the first time in the city of Manaus, Brazil. Viral load was determined in the sera of patients before the experimental treatment; however, low levels or no virus was detected (CT > 30; data not shown).

We also evaluated the presence of SARS-CoV-2 copies in the hippocampal slices after 2-h incubation, but low levels or no viral copies were detected in the slices (CT > 30; data not shown).

Serum of COVID-19 patients induces changes in the expression of genes related to inflammatory response, mitochondrial function, and glial reactivity in the hippocampus of aged rats

Our results showed variable alterations in gene expression of hippocampal slices of 365-day-old rats incubated with the serum of an individual previously infected with SARS-CoV-2 (post-COVID: PC; serum of PC individual: sPC) and with the serum of patients infected with the variants Zeta (sZeta) and Gamma (sGamma) as well as. First, we determined the mRNA levels of inflammatory genes, including cytokines, receptors and signaling factors. Figure 1b demonstrates an increase in the expression of IL-1β in the slices exposed to sPC and a reduction when exposed to sGamma ($p < 0.05$). Furthermore, a trend to an increase in the levels of IL-1β by sPC and sZeta ($p < 0.05$) can be also seen. On the other hand, IL1R1 expression was decreased by sPC and sZeta ($p < 0.05$) (Fig. 1c). We further verified that the mRNA levels of TNF-α ($p < 0.05$) (Fig. 1d), TNFR1 ($p < 0.05$) (Fig. 1e), and NFAT ($p < 0.05$) (Fig. 1f) were reduced or had a tendency towards a reduction in slices incubated with sPC and sZeta.

The expression of NFκB p65, a transcriptional factor that regulates the expression of cytokines, chemokines, and immunoreceptors (Dresselhaus and Meffert 2019), was significantly reduced by sZeta ($p < 0.05$) and had a tendency towards a reduction by sGamma ($p < 0.05$) (Fig. 1f). Moreover, reduced mRNA levels of Nrf2 (Fig. 1i), a regulator of antioxidant and inflammatory gene expression (Seminotti et al. 2021), were observed in slices exposed to sPC and sZeta ($p < 0.01$). On the other hand, the expression of HO-1 (Fig. 1j), a downstream target of Nrf2 and an upstream modulator of NFκB p65, had a trend towards a decrease by sPC and a significant increase by sZeta ($p < 0.05$). In contrast, COX-2 expression was not modified ($p > 0.05$) (Fig. 1h).

Next, we evaluated the expression of genes related to classical receptors of the immune response. We verified that the expression of TLR2 was significantly increased in hippocampal slices exposed to sPC, sZeta and sGamma ($p < 0.05$) (Fig. 2a). Furthermore, the expression of TLR3 was significantly increased by sZeta and had a tendency towards an increase by sGamma, whereas it was decreased by sPC ($p < 0.05$) (Fig. 2b). TLR4 was markedly downregulated after the exposition to sGamma ($p < 0.05$) (Fig. 2c). Additionally, IFITM3 expression was significantly decreased by sPC ($p < 0.05$) (Fig. 2).

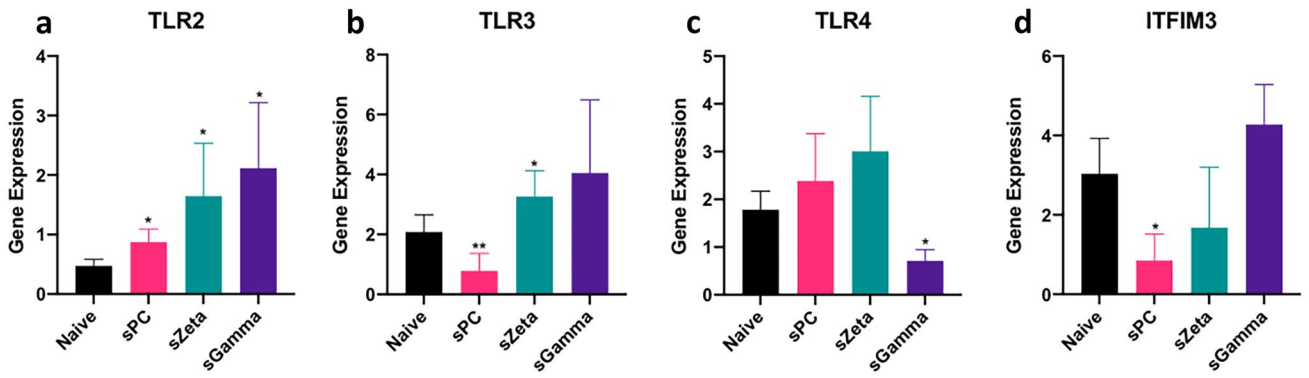

Fig. 2 mRNA expression of TLR2 **a**, TLR3 **b**, TLR4 **c** and ITFIM3 **d**. In black, expression levels in slices exposed to naïve serum; in pink, expression levels in slices exposed to sPC; in green, expression levels in slices exposed to sZeta; and, in purple, expression levels in slices exposed to sGamma. Data represent means ± SD of at least four experimental determinations performed in triplicate

Considering that mitochondria participate in the innate immune response and are key drivers of inflammation (Vringer and Tait 2022), we also evaluated the expression of genes regulating mitochondrial function (Fig. 3). Our results demonstrated that VDAC1 expression was significantly reduced in slices exposed to sPC as well as to sZeta and sGamma ($p < 0.05$) (Fig. 3a). CS expression was reduced by sPC and had a trend toward a decrease by sZeta ($p < 0.05$) (Fig. 3b). However, Tom70 expression was not significantly modified in any group ($p > 0.05$) (Fig. 3c). We also found that the expression of PGC-1α gene was decreased in the hippocampus exposed to sPC and sZeta ($p < 0.05$) and that the expression of MFN1 and DRP1 was reduced by sPC ($p < 0.05$) (Fig. 3d).

We further studied the expression levels of CaN, p21, p38 MAPK and STAT3, classical proteins involved in cellular regulatory and signaling functions, and did not verify any significant alteration ($p > 0.05$) (Fig. 4a-d). Finally, since inflammation and mitochondrial dysfunction are often associated with glial reactivity induction (Sanz and Garcia-Gimeno 2020), we determined the expression of S100B and GFAP. Figure 4e demonstrates that S100B expression was increased only by sZeta and had a slight tendency to be decreased by sPC ($p < 0.05$), whereas GFAP was not significantly modified in any condition ($p > 0.05$) (Fig. 4f).

Discussion

Mounting evidence has shown that neuroinflammation is involved in the pathophysiology of the neurological manifestations observed in COVID-19 patients (Monje and Iwasaki 2022). Nevertheless, little is known about the exact mechanisms leading to the inflammatory response in the CNS, so we evaluated here the acute effects of serum from COVID-19 patients on the expression of genes related to inflammatory response, mitochondrial biogenesis and dynamics, as well as glial reactivity in the hippocampus of aged rats. Noteworthy, since age is a known factor of COVID-19 severity (Mueller et al. 2020), we used 365-day-old rats to better mimic the aged neural circuitry. It is important to note that we also tested the serum of individuals infected with two different viral variants of SARS-CoV-2 to evaluate the impact of these mutations on neural parameters associated with brain homeostasis.

First, we decided to use hippocampus in our experimental model because this brain structure is crucial for learning and long-term memory consolidation, and its physiological processes are susceptible to endogenous and exogenous factors that can interfere with synaptic signaling (Bartsch and Wulff 2015; Shivarama and Sajikumar 2017). Hippocampus

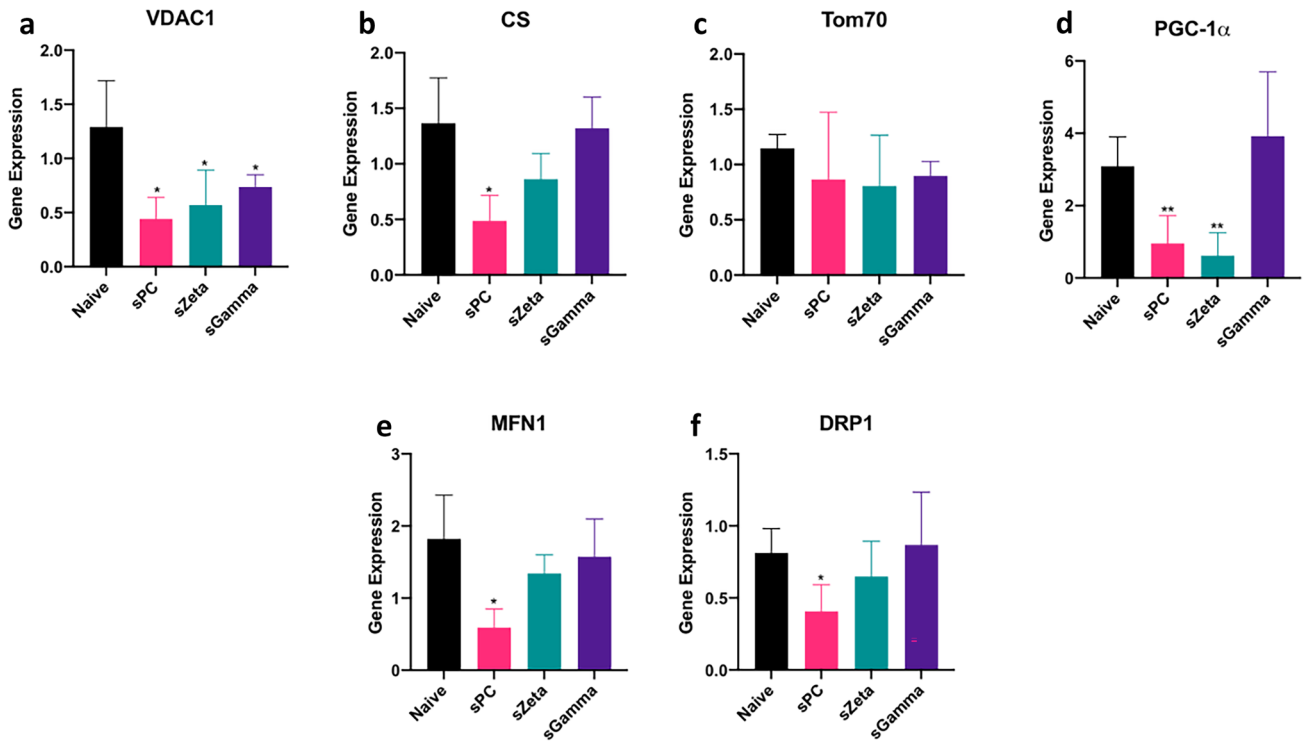

Fig. 3 mRNA expression of VDAC **a**, citrate synthase (CS) **b**, Tom70 **c**, PGC-1a **d**, MFN1 (e) and DRP1 (f). In black, expression levels in slices exposed to naïve serum; in pink, expression levels in slices exposed to sPC; in green, expression levels in slices exposed to sZeta; and, in purple, expression levels in slices exposed to sGamma. Data represent means ± SD of at least four experimental determinations performed in triplicate

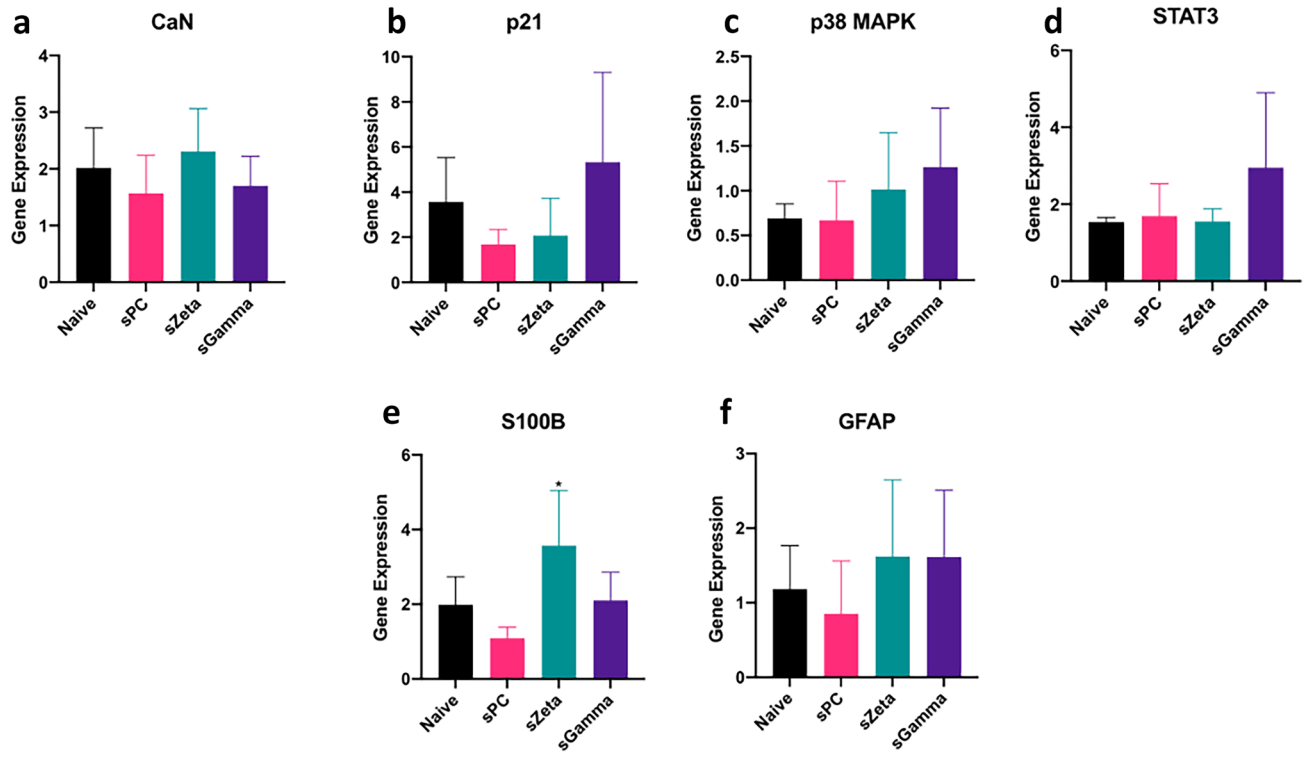

Fig. 4 mRNA expression of CaN **a**, p21 **b**, p38 MAPK **c**, STAT3 **d**, S100B (e) and GFAP (f). In black, expression levels in slices exposed to naïve serum; in pink, expression levels in slices exposed to sPC; in green, expression levels in slices exposed to sZeta; and, in purple, expression levels in slices exposed to sGamma. Data represent means ± SD of at least four experimental determinations performed in triplicate

is also a region of great interest to evaluate the effects of viruses with tropism for neural cells. Previous studies showed alterations in neurons and astrocytes caused by different viruses, such as Zika virus (Bobermin et al. 2020), Herpes simplex virus type I (Yong et al. 2021), and Influenza A virus (Hosseini et al. 2021). Interestingly, a study recently showed that SARS-CoV-2 is able to infect the hippocampus of mice after intranasal administration (Song et al. 2021). Additionally, the hippocampus is a key structure for understanding the aging process and its impact on cognition since significant changes are observed in this region, such as volume reduction, neuronal loss, vascular degeneration, reduction of synaptic plasticity and inflammation with up-regulation of cytokines and microglial activation (Cribbs et al. 2012; von Bernhardi et al. 2010; von Bettio et al. 2017).

Our findings showed that the exposure of hippocampal slices to all sera evaluated caused changes in the inflammatory response. A decrease in the expression of IL1R1 and TNFR1 was verified after incubation with sZeta and sPC, whereas a decrease in IL-1β was seen with sGamma. In this regard, data from the literature demonstrated that the reduction in IL1R1 levels is associated with the persistence of the viral infection, allowing an immune escape from activated T cells (Kim et al. 2012; Orzalli et al. 2018). These observations are also in line with findings showing that SARS-CoV-2 causes a delay in the type I interferon (IFN-1) response and consequently in the expression of pattern recognition receptors and cytokine production (Lowery et al. 2021). Noteworthy, although SARS-CoV-2 was not detected in hippocampal slices, the sera triggered a relevant inflammatory process.

While TNF-α is important for synaptic signaling processes (Beattie et al. 2002), neurotransmitter receptor trafficking (Stellwagen et al. 2005), and regulation of gliotransmission (Santello et al. 2011), NFAT regulates the expression of genes involved in Ca^{2+} homeostasis and axonal growth (Nguyen and Di Giovanni 2008; Kraner and Nossis 2018). Therefore, the downregulation of TNF-α and NFAT caused by the sera reinforces that a reduction in the inflammatory process occurred in rat hippocampus. Consistent with this, the expression of NFκB p65, a crucial regulator of the immune response (Mitchell et al. 2016), was also reduced. Furthermore, it should be noted that in the CNS, NFκB p65 presents specific roles in synaptic processing and neuron-glia communication (Dresselhaus and Meffert 2019).

In addition, a decreased expression of Nrf2 was observed after exposition to sZeta and sPC, suggesting that the ability

of cells to upregulate the antioxidant defenses is compromised (Ma 2013). This is in line with a previous work showing a reduction of Nrf2 levels in the plasma of COVID-19 patients (Gümüs et al. 2022). Consistent with this, it has been demonstrated that a reduction in Nrf2 expression may be associated with an increase in the efficiency of the viral reproduction process (Olagnier et al. 2020; Khan et al. 2021; Bousquet et al. 2020). It should be also noted that the NFκB subunit p65, which is decreased in our model, negatively regulates the transcriptional activation of Nrf2 (van der Horst et al. 2022).

Previous data suggested that some viruses can modulate TLR to support their metabolism (Carty and Bowie 2010). Our observations showed that the sera modulated mRNA levels of TLR2 and TLR3, suggesting that these receptors may play an important role in the response to SARS-CoV-2 infection and an adaptation of the hippocampus to cope with inflammatory mediators that are present in the sera. Consistent with this hypothesis, a study demonstrated that TLR2 leads to a pro-inflammatory response against SARS-CoV-2 infection that is independent on viral entry into the cells (Zheng et al. 2021). Furthermore, it was demonstrated that the intranasal administration of a TLR3/MDA5 agonist initially caused upregulation of innate immunity-related genes in the lungs of mice infected with a lethal dose of SARS-CoV-2 followed by a reduction of viral load and expression of cytokines, chemokines and interferon in the brain of these animals (Tamir et al. 2022). It should be further noted that there were very low or no viral particles in the cells of hippocampal slices, at least after a 2 h incubation, which might have facilitated the induction of adaptive mechanisms by neural cells via mitochondrial response.

As for the mitochondrial markers, VDAC1 expression was reduced by the sZeta and sGamma whereas PGC-1α was decreased by sZeta. Since VDAC1 is the most abundant protein in the mitochondrial outer membrane and usually reflects mitochondrial mass (Fang and Maldonado 2018), and PGC-1α is considered the master regulator of mitochondrial biogenesis, these results indicate a decrease in mitochondrial biogenesis. It should be also noted that PGC-1α may be associated with NFκB (Alvarez-Guardia et al. 2010), which is in accordance with the decreased expression of this transcription factor. In contrast, the exposure to sGamma did not change PGC-1α. In line with this, it was shown that the levels of some cytokines may even take days during or after the infection to change in the serum of COVID-19 patients, thus implying that sGamma may take longer periods to modulate this protein (Tan et al. 2021). Moreover, it is also known that VDAC1 mediates the mitochondrial release of ROS (Shoshan-Barmatz et al. 2010). Thus, the reduction in the expression of VDAC1 as well as of Nrf2 reinforce that oxidative stress is induced by the sera. Conversely, a different study demonstrated an increase in VDAC1 levels in a specific population of T cells from acutely ill COVID-19 patients that is more susceptible to apoptosis. These differential effects may be explained by the different approaches used to evaluate VDAC1 expression as well as by the different tissue/cell type evaluated in each study (rat hippocampal slices *versus* peripheral blood mononuclear cells) (Thompson et al. 2020).

Regarding the alterations caused specifically by sPC, we observed more pronounced effects on the expression of genes related to mitochondrial homeostasis. Not only the expression of mitochondrial biogenesis markers (PGC-1α, VDAC1, and CS) was markedly reduced by sPC but also the mRNA levels of mitochondrial dynamics proteins were decreased, suggesting that both fusion and fission processes are disturbed. The balance between mitochondrial fusion and fission is necessary to maintain mitochondrial integrity and is intimately involved in the regulation of cell death (Chen et al. 2020). These observations indicate that disturbances in mitochondrial quality control may play a key role in the pathophysiology of neurological dysfunction in long-COVID. Nevertheless, it is difficult to explain why only sPC caused these effects, but we speculate that longer periods of exposure to sZeta and sGamma might be necessary to induce alterations in these mitochondrial processes. Moreover, we should consider that the pre-existence of neuropsychiatric conditions and psychosocial effects caused by the pandemic may differentially modulate molecular markers involved in COVID-19 pathophysiology (Quincozes-Santos et al. 2021).

Additionally, the exposure to sZeta caused a marked increase in the expression of the glial marker S100B, indicative of brain damage (Angelopoulou et al. 2021). In line with this, elevated S100B levels were demonstrated in the plasma of COVID-19 patients that did not survive (Kokkoris et al. 2022).

Conclusion

Our findings provide evidence that the sera of patients infected with the SARS-CoV-2 variants gamma and zeta induce alterations in the inflammatory profile in the hippocampus of aged rats. It should be emphasized that no viral particles were verified in the hippocampal slices, so the effects observed here were possibly induced by pro-inflammatory molecules associated with viral infection, and not by the direct action of the virus. However, the present data must be taken with caution since we evaluated the effects of the serum of one patient affected by each variant. Moreover, we found that the serum of the post-COVID-19 individual (sPC) caused pronounced alterations in the mitochondrial function, especially dynamics, suggesting that these may be key factors underlying the neural cell injury seen in long-COVID.

Data Availability Data will be made available upon request.

References

Alvarez-Guardia D, Palomer X, Coll T, Davidson MM, Chan TO, Feldman AM, Laguna JC, Vázquez-Carrera M (2010) The p65 subunit of NF-kappaB binds to PGC-1alpha, linking inflammation and metabolic disturbances in cardiac cells. Cardiovasc Res 87(3):449–458. https://doi.org/10.1093/cvr/cvq080

Angelopoulou E, Paudel YN, Piperi C (2021) Emerging role of S100B protein implication in Parkinson's disease pathogenesis. Cell Mol Life Sci CMLS 78(4):1445–1453. https://doi.org/10.1007/s00018-020-03673-x

Banoth B, Cassel SL (2018) Mitochondria in innate immune signaling. Translational Research. J Lab Clin Med 202:52–68. https://doi.org/10.1016/j.trsl.2018.07.014

Bartsch T, Wulff P (2015) The hippocampus in aging and disease: From plasticity to vulnerability. Neuroscience 309:1–16. https://doi.org/10.1016/j.neuroscience.2015.07.084

Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC (2002) Control of synaptic strength by glial TNFalpha. Science (New York, N.Y.), 295(5563): 2282–2285. https://doi.org/10.1126/science.1067859

Bhowal C, Ghosh S, Ghatak D, De R (2022) Pathophysiological involvement of host mitochondria in SARS-CoV-2 infection that causes COVID-19: a comprehensive evidential insight. Mol cell biochem 1–19. Advance online publication. https://doi.org/10.1007/s11010-022-04593-z

Bobermin LD, Quincozes-Santos A, Santos CL, Varela APM, Teixeira TF, Wartchow KM, Lissner LJ, da Silva A, Thomaz NK, Santi L, Beys-da-Silva WO, Roehe PM, Sesterheim P, Guimarães JA, Gonçalves CA, Souza DO (2020) Zika virus exposure affects neuron-glia communication in the hippocampal slices of adult rats. Sci Rep 10(1):21604. https://doi.org/10.1038/s41598-020-78735-y

Bousquet J, Cristol JP, Czarlewski W, Anto JM, Martineau A, Haahtela T, Fonseca SC, Iaccarino G, Blain H, Fiocchi A, Canonica GW, Fonseca JA, Vidal A, Choi HJ, Kim HJ, Le Moing V, Reynes J, Sheikh A, Akdis CA, Zuberbier T, ARIA group (2020) Nrf2-interacting nutrients and COVID-19: time for research to develop adaptation strategies. Clin transl allergy 10(1):58. https://doi.org/10.1186/s13601-020-00362-7

Burtscher J, Cappellano G, Omori A, Koshiba T, Millet GP (2020) Mitochondria: In the Cross Fire of SARS-CoV-2 and Immunity. iScience 23(10):101631. https://doi.org/10.1016/j.isci.2020.101631

Carty M, Bowie AG (2010) Recent insights into the role of Toll-like receptors in viral infection. Clin Exp Immunol 161(3):397–406. https://doi.org/10.1111/j.1365-2249.2010.04196.x

Chen Y, Guo S, Tang Y, Mou C, Hu X, Shao F, Yan W, Wu Q (2020) Mitochondrial Fusion and Fission in Neuronal Death Induced by Cerebral Ischemia-Reperfusion and Its Clinical Application: A Mini-Review. Medical science monitor: Int Med J Experimen Clin Res 26:e928651. https://doi.org/10.12659/MSM.928651

Chen Z, Li G (2021) Immune response and blood-brain barrier dysfunction during viral neuroinvasion. Innate Immun 27(2):109–117. https://doi.org/10.1177/1753425920954281

Cribbs DH, Berchtold NC, Perreau V, Coleman PD, Rogers J, Tenner AJ, Cotman CW (2012) Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study. J Neuroinflammation 9:179. https://doi.org/10.1186/1742-2094-9-179

Crunfi F et al (2020) SARS-CoV-2 infects brain astrocytes of COVID-19 patients and impairs neuronal viability. medRxiv. https://doi.org/10.1101/2020.10.09.20207464

Dresselhaus EC, Meffert MK (2019) Cellular Specificity of NF-κB Function in the Nervous System. Front Immunol 10:1043. https://doi.org/10.3389/fimmu.2019.01043

Fang D, Maldonado EN (2018) VDAC Regulation: A Mitochondrial Target to Stop Cell Proliferation. Adv Cancer Res 138:41–69. https://doi.org/10.1016/bs.acr.2018.02.002

Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, Crispim MAE, Sales FCS, Hawryluk I, McCrone JT, Hulswit RJG, Franco LAM, Ramundo MS, de Jesus JG, Andrade PS, Coletti TM, Ferreira GM, Silva CAM, Manuli ER, Pereira RHM, Sabino EC (2021) Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science 372(6544):815–821. https://doi.org/10.1126/science.abh2644

Gümüş H, Erat T, Öztürk İ, Demir A, Koyuncu I (2022) Oxidative stress and decreased Nrf2 level in pediatric patients with COVID-19. J Med Virol 94(5):2259–2264. https://doi.org/10.1002/jmv.27640

Halpin S, O'Connor R, Sivan M (2021) Long COVID and chronic COVID syndromes. J Med Virol 93(3):1242–1243. https://doi.org/10.1002/jmv.26587

Hosseini S, Michaelsen-Preusse K, Schughart K, Korte M (2021) Long-Term Consequence of Nonneurotropic H3N2 Influenza A Virus Infection for the Progression of Alzheimer's Disease Symptoms. Front Cell Neurosci 15:643650. https://doi.org/10.3389/fncel.2021.643650

Kanberg N, Ashton NJ, Andersson LM, Yilmaz A, Lindh M, Nilsson S, Price RW, Blennow K, Zetterberg H, Gisslén M (2020) Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. Neurology 95(12):e1754–e1759. https://doi.org/10.1212/WNL.0000000000010111

Kang SJ, Jung SI (2020) Age-Related Morbidity and Mortality among Patients with COVID-19. Infect Chemother 52(2):154–164. https://doi.org/10.3947/ic.2020.52.2.154

Kim BS, Jin YH, Meng L, Hou W, Kang HS, Park HS, Koh CS (2012) IL-1 signal affects both protection and pathogenesis of virus-induced chronic CNS demyelinating disease. J Neuroinflammation 9:217. https://doi.org/10.1186/1742-2094-9-217

Khan H, Patel S, Majumdar A (2021) Role of NRF2 and Sirtuin activators in COVID-19. Clin Immun (Orlando, Fla.) 233:108879. https://doi.org/10.1016/j.clim.2021.108879

Kokkoris S, Stamataki E, Emmanouil G, Psachoulia C, Ntaidou T, Maragouti A, Kanavou A, Malachias S, Christodouli F, Papachatzakis I, Markaki V, Katsaros D, Vasileiadis I, Glynos C, Routsi C (2022) Serum inflammatory and brain injury biomarkers in COVID-19 patients admitted to intensive care unit: A pilot study. eNeurological Sci, 29:100434. https://doi.org/10.1016/j.ensci.2022.100434

Kraner SD, Norris CM (2018) Astrocyte Activation and the Calcineurin/NFAT Pathway in Cerebrovascular Disease. Front Aging Neurosci 10:287. https://doi.org/10.3389/fnagi.2018.00287

Lowery SA, Sariol A, Perlman S (2021) Innate immune and inflammatory responses to SARS-CoV-2: Implications for COVID-19. Cell Host Microbe 29(7):1052–1062. https://doi.org/10.1016/j.chom.2021.05.004

Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Mitchell S, Vargas J, Hoffmann A (2016) Signaling via the NFκB system. Wiley Interdiscipl Rev Syst Biol Med 8(3):227–241. https://doi.org/10.1002/wsbm.1331

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

Mueller AL, McNamara MS, Sinclair DA (2020) Why does COVID-19 disproportionately affect older people. Aging 12(10):9959–9981. https://doi.org/10.18632/aging.103344

Najjar S, Najjar A, Chong DJ, Pramanik BK, Kirsch C, Kuzniecky RI, Pacia SV, Azhar S (2020) Central nervous system complications

associated with SARS-CoV-2 infection: integrative concepts of pathophysiology and case reports. J Neuroinflammation 17(1):231. https://doi.org/10.1186/s12974-020-01896-0

Nardin P, Tortorelli L, Quincozes-Santos A, de Almeida LM, Leite MC, Thomazi AP, Gottfried C, Wofchuk ST, Donato R, Gonçalves CA (2009) S100B secretion in acute brain slices: modulation by extracellular levels of Ca(2+) and K (+). Neurochem Res 34(9):1603–1611. https://doi.org/10.1007/s11064-009-9949-0

Nasserie T, Hittle M, Goodman SN (2021) Assessment of the Frequency and Variety of Persistent Symptoms Among Patients With COVID-19: JAMA network open. Syst Rev 4(5):e2111417. https://doi.org/10.1001/jamanetworkopen.2021.11417

Nguyen T, Di Giovanni S (2008) NFAT signaling in neural development and axon growth: the Official Journal of the International Society for Developmental Neuroscience. Int J Dev Neurosci 26(2):141–145. https://doi.org/10.1016/j.ijdevneu.2007.10.004

Olagnier D, Farahani E, Thyrsted J, Blay-Cadanet J, Herengt A, Idorn M, Hait A, Hernaez B, Knudsen A, Iversen MB, Schilling M, Jørgensen SE, Thomsen M, Reinert LS, Lappe M, Hoang HD, Gilchrist VH, Hansen AL, Ottosen R, Nielsen CG, Holm CK (2020) SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. Nature Commun 11(1):4938. https://doi.org/10.1038/s41467-020-18764-3

Orzalli MH, Smith A, Jurado KA, Iwasaki A, Garlick JA, Kagan JC (2018) An Antiviral Branch of the IL-1 Signaling Pathway Restricts Immune-Evasive Virus Replication. Mol Cell 71(5):825-840.e6. https://doi.org/10.1016/j.molcel.2018.07.009

Quincozes-Santos A, Rosa RL, Tureta EF, Bobermin LD, Berger M, Guimarães JA, Santi L, Beys-da-Silva WO (2021) COVID-19 impacts the expression of molecular markers associated with neuropsychiatric disorders. Brain Behav Immun Health 11: 100196. https://doi.org/10.1016/j.bbih.2020.100196

Raveendran AV, Jayadevan R, Sashidharan S (2021) Long COVID: An overview. Diabetes Metab Syndr15(3):869–875. https://doi.org/10.1016/j.dsx.2021.04.007

Santello M, Bezzi P, Volterra A (2011) TNFα controls glutamatergic gliotransmission in the hippocampal dentate gyrus. Neuron 69(5):988–1001. https://doi.org/10.1016/j.neuron.2011.02.003

Sanz P, Garcia-Gimeno MA (2020) Reactive Glia Inflammatory Signaling Pathways and Epilepsy. Int J Mol Sci 21(11):4096. https://doi.org/10.3390/ijms21114096

Seminotti B, Grings M, Tucci P, Leipnitz G, Saso L (2021) Nuclear Factor Erythroid-2-Related Factor 2 Signaling in the Neuropathophysiology of Inherited Metabolic Disorders. Front Cell Neurosci 15:785057. https://doi.org/10.3389/fncel.2021.785057

Shivarama Shetty M, Sajikumar S (2017) "Tagging" along memories in aging: Synaptic tagging and capture mechanisms in the aged hippocampus. Ageing Res Rev 35:22–35. https://doi.org/10.1016/j.arr.2016.12.008

Shoshan-Barmatz V, De Pinto V, Zweckstetter M, Raviv Z, Keinan N, Arbel N (2010) VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Aspects Med 31(3):227–285. https://doi.org/10.1016/j.mam.2010.03.002

Song E, Zhang C, Israelow B, Lu-Culligan A, Prado AV, Skriabine S, Lu P, Weizman OE, Liu F, Dai Y, Szigeti-Buck K, Yasumoto Y, Wang G, Castaldi C, Heltke J, Ng E, Wheeler J, Alfajaro MM, Levavasseur E, Fontes B, Iwasaki A (2021) Neuroinvasion of SARS-CoV-2 in human and mouse brain. J Exptl Med 218(3):e20202135. https://doi.org/10.1084/jem.20202135

Spudich S, Nath A (2022) Nervous system consequences of COVID-19. Science (New York, N.Y.) 375(6578):267–269. https://doi.org/10.1126/science.abm2052

Stellwagen D, Beattie EC, Seo JY, Malenka RC (2005) Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha: the Official Journal of the Society for Neuroscience. J Neurosci 25(12):3219–3228. https://doi.org/10.1523/JNEUROSCI.4486-04.2005

Subramanian A, Nirantharakumar K, Hughes S, Myles P, Williams T, Gokhale KM, Taverner T, Chandan JS, Brown K, Simms-Williams N, Shah AD, Singh M, Kidy F, Okoth K, Hotham R, Bashir N, Cockburn N, Lee SI, Turner GM, Gkoutos GV, Haroon S (2022) Symptoms and risk factors for long COVID in non-hospitalized adults. Nature Med 28(8):1706–1714. https://doi.org/10.1038/s41591-022-01909-w

Tamir H, Melamed S, Erez N, Politi B, Yahalom-Ronen Y, Achdout H, Lazar S, Gutman H, Avraham R, Weiss S, Paran N, Israely T (2022) Induction of Innate Immune Response by TLR3 Agonist Protects Mice against SARS-CoV-2 Infection. Viruses 14(2):189. https://doi.org/10.3390/v14020189

Tan Y, Zhang W, Zhu Z, Qiao N, Ling Y, Guo M, Yin T, Fang H, Xu X, Lu G, Zhang P, Yang S, Fu Z, Liang D, Xie Y, Zhang R, Jiang L, Yu S, Lu J, Jiang F, Chen S (2021) Integrating longitudinal clinical laboratory tests with targeted proteomic and transcriptomic analyses reveal the landscape of host responses in COVID-19. Cell Discov 7(1):42. https://doi.org/10.1038/s41421-021-00274-1

Thompson EA, Cascino K, Ordonez AA, Zhou W, Vaghasia A, Hamacher-Brady A, Brady NR, Sun IH, Wang R, Rosenberg AZ, Delannoy M, Rothman R, Fenstermacher K, Sauer L, Shaw-Saliba K, Bloch EM, Redd AD, Tobian AA, Horton M, Smith K, Powell JD (2020) Metabolic programs define dysfunctional immune responses in severe COVID-19 patients. preprint server for health sciences. medRxiv 2020.09.10.20186064. https://doi.org/10.1101/2020.09.10.20186064

Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, Levantovsky R, Malle L, Moreira A, Park MD, Pia L, Risson E, Saffern M, Salomé B, Esai Selvan M, Spindler MP, Tan J, van der Heide V, Gregory JK, Alexandropoulos K (2020) Sinai Immunology Review Project Immunology of COVID-19: Current State of the Science. Immunity 52(6):910–941. https://doi.org/10.1016/j.immuni.2020.05.002

van der Horst D, Carter-Timofte ME, van Grevenynghe J, Laguette N, Dinkova-Kostova AT, Olagnier D (2022) Regulation of innate immunity by Nrf2. Curr Opinion Immun 78:102247. https://doi.org/10.1016/j.coi.2022.102247

Varela APM, Prichula J, Mayer FQ, Salvato RS, Sant'Anna FH, Gregianini TS, Martins LG, Seixas A, Veiga ABGD (2021) SARS-CoV-2 introduction and lineage dynamics across three epidemic peaks in Southern Brazil: Massive spread of P.1. Infect. Genet Evol 96:105144

Voloch CM, da Silva Francisco R, Jr de Almeida LGP, Cardoso CC, Brustolini OJ, Gerber AL, Guimarães APC, Mariani D, da Costa RM, Ferreira OC, Jr, Adriana Cony Cavalcanti, Frauches TS, de Mello CMB, Leitão IC, Galliez RM, Faffe DS, Castiñeiras TMPP, Tanuri A, de Vasconcelos ATR (2021) Covid 19-UFRJ Workgroup, Genomic characterization of a novel SARS-CoV-2 lineage from Rio de Janeiro, Brazil. J Virol 95(10):e00119–21.https://doi.org/10.1128/JVI.00119-21. Advance Online Publication

von Bernhardi R, Tichauer JE, Eugenín J (2010) Aging-dependent changes of microglial cells and their relevance for neurodegenerative disorders. J Neurochem 112(5):1099–1114. https://doi.org/10.1111/j.1471-4159.2009.06537.x

von Bettio LEB, Rajendran L, Gil-Mohapel J (2017) The effects of aging in the hippocampus and cognitive decline. Neurosci Biobehav Rev 79:66–86. https://doi.org/10.1016/j.neubiorev.2017.04.030

Vringer E, Tait SWG (2022) Mitochondria and cell death-associated inflammation. Cell Death Differ. https://doi.org/10.1038/s41418-022-01094-w. Advance Online Publication

Yong SJ, Yong MH, Teoh SL, Soga T, Parhar I, Chew J, Lim WL (2021) The Hippocampal Vulnerability to Herpes Simplex Virus Type I Infection: Relevance to Alzheimer's Disease and Memory Impairment. Front Cell Neurosci 15:695738. https://doi.org/10.3389/fncel.2021.695738

ZhangL, 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 Tar Ther 6(1):337. https://doi.org/10.1038/s41392-021-00719-9

Zheng M, Karki R, Williams EP, Yang D, Fitzpatrick E, Vogel P, Jonsson CB, Kanneganti TD (2021) TLR2 senses the SARS-CoV-2 envelope protein to produce inflammatory cytokines. Nat Immunol 22(7):829–838. https://doi.org/10.1038/s41590-021-00937-x