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Lung inflammation induced by silica particles triggers hippocampal inflammation, synapse damage and memory impairment in mice

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Abstract

Background: Considerable evidence indicates that a signaling crosstalk between the brain and periphery plays important roles in neurological disorders, and that both acute and chronic peripheral inflammation can produce brain changes leading to cognitive impairments. Recent clinical and epidemiological studies have revealed an increased risk of cognitive impairment and dementia in individuals with impaired pulmonary function. However, the mechanistic underpinnings of this association remain unknown. Exposure to SiO₂ (silica) particles triggers lung inflammation, including infiltration by peripheral immune cells and upregulation of pro-inflammatory cytokines. We here utilized a mouse model of lung silicosis to investigate the crosstalk between lung inflammation and memory.

Methods: Silicosis was induced by intratracheal administration of a single dose of 2.5 mg SiO₂/kg in mice. Molecular and behavioral measurements were conducted 24 h and 15 days after silica administration. Lung and hippocampal inflammation were investigated by histological analysis and by determination of pro-inflammatory cytokines. Hippocampal synapse damage, amyloid- β (A β) peptide content and phosphorylation of Akt, a proxy of hippocampal insulin signaling, were investigated by Western blotting and ELISA. Memory was assessed using the open field and novel object recognition tests.

Results: Administration of silica induced alveolar collapse, lung infiltration by polymorphonuclear (PMN) cells, and increased lung pro-inflammatory cytokines. Lung inflammation was followed by upregulation of hippocampal pro-inflammatory cytokines, synapse damage, accumulation of the A β peptide, and memory impairment in mice.

Conclusion: The current study identified a crosstalk between lung and brain inflammatory responses leading to hippocampal synapse damage and memory impairment after exposure to a single low dose of silica in mice.

Keywords: Silicosis, Lung inflammation, Brain inflammation, Synapse damage, Cognitive impairment, Dementia

Introduction

Mounting evidence indicates that a crosstalk between peripheral and central inflammation can lead to brain dysfunction and neurodegeneration [1–3]. For example, chronic kidney disease has been associated with cognitive impairment, delirium, encephalopathy, and dementia [4, 5]. Type 2 diabetes and obesity, characterized by

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peripheral inflammation and insulin resistance, are risk factors for dementia [6–8] and for major depressive disorder [9]. Gut microbiota has also been implicated in brain–periphery crosstalk [10]. Exposure to microbial amyloids in the gastrointestinal tract can accelerate alpha-synuclein aggregation in the gut and brain, and lead to enhanced microgliosis and astrogliosis, suggesting that bacterial amyloid may function as a trigger to initiate brain inflammation and alpha-synuclein aggregation in synucleinopathies [11, 12].

Robust evidence further indicates that acute peripheral inflammatory conditions, including viral and bacterial infections, can trigger brain inflammation and dysfunction, resulting in cognitive decline or neuropsychiatric conditions [13, 14]. For example, recent studies demonstrate that systemic inflammation induced by SARS-CoV-2, the etiologic agent of COVID-19, activates brain toll-like receptors (TLRs) and upregulates brain tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) signaling, triggering synapse damage and leading to depressive and cognitive symptoms in COVID-19 patients [15]. Collectively, multiple lines of evidence indicate that both acute and chronic, low grade peripheral inflammation can trigger brain inflammation and progressively lead to brain dysfunction underlying cognitive decline and dementia [13–17].

Silicosis is a major occupational lung disease, with a significant impact on health systems and on the quality of life of workers in many industries [18, 19]. Exposure to silica (SiO₂) particles induces chronic lung inflammation, including immune cell infiltration, macrophage activation and release of pro-inflammatory cytokines, e.g., TNF- α , interleukin-1 β (IL-1 β) and IL-6, resulting in tissue fibrosis, alveolar collapse and lung dysfunction [19–21].

Clinical and epidemiological evidence points to an association between pulmonary function and cognitive impairment. A recent meta-analysis of longitudinal studies of individuals with impaired pulmonary function found an increased risk of dementia in such individuals [22], and this appears more pronounced for restrictive pulmonary impairment than for obstructive lung disease [23]. Recent studies indicate that both neurodegeneration and vascular brain lesions may underlie the association between pulmonary dysfunction, memory impairments and dementia [24, 25]. However, the mechanistic underpinnings of the connection between lung and brain dysfunction remain unclear.

Here, we investigated the crosstalk between lung inflammation, brain inflammation and memory in a mouse model of silicosis. We report that lung inflammation in mice exposed to silica particles is accompanied by hippocampal inflammation, synapse damage and memory impairment.

Methods

Animals

Experiments were performed on 8- to 10-week-old male Swiss mice. Animals were housed in groups of five per cage with free access to food and water, under a 12-h light/dark cycle with controlled room temperature (21 ± 2 °C). Mice were randomly divided into two groups. In the control group (Ctrl), mice received an intratracheal (i.t.) administration of 0.05 mL sterile saline solution (0.9% NaCl). Silica-exposed animals (Si) received an intratracheal administration of 2.5 mg silica (SiO₂; particle size: 500 nm–10 μ m, 80% of the particles between 1 and 5 μ m; S5631, Sigma Chemical Co., St. Louis, USA) suspended in 0.05 mL saline, as previously described in murine models of acute silicosis [26, 27]. Animal behavior was analyzed 24 h or 15 days after saline or silica administration. This study was approved by the Ethics Committee on the Use of Animals, Health Sciences Center, Federal University of Rio de Janeiro.

Tissue collection and preparation

Mice were anesthetized with 1.5 ml/kg of a solution containing 10% ketamine and 2% xylazine immediately after behavioral tests. Bilateral hippocampi and lungs of Ctrl and Si animals were collected, immediately frozen in liquid nitrogen, and stored at -80 °C until use in Western blotting or ELISA assays.

Lung histology

Histology was performed as previously described [20, 26]. Morphometric analysis was performed in granuloma-free tissue areas using an integrating eyepiece with a coherent system of a 100-point and 50 lines (known length) grid coupled to a conventional light microscope (Axioplan; Zeiss). Analysis was performed in 10 random, non-overlapping fields with 200 \times magnification.

For cellularity analysis, total numbers of mononuclear (MN) and polymorphonuclear (PMN) cells in granuloma-free lung tissue areas were counted in each animal across 10 random non-overlapping microscopic fields at 1000 \times magnification. Data are presented as cell count percentages and cell numbers/tissue area.

Hippocampal and lung cytokines

Hippocampi and lungs were homogenized in ice-cold PBS with protease and phosphatase inhibitors (Pierce–Thermo Scientific, Rockford, IL), and were centrifuged for 10 min at 26,500 $\times g$ at 4 °C. Supernatants were collected and assayed in duplicate by ELISA for the following cytokines: Interleukin-1 β (IL-1 β , Thermo Scientific, Rockford, IL), TNF- α (Biolegend, San Diego, CA), interleukin-6 (IL-6; R&D Systems, Minneapolis, MN).

Amyloid- β 42 was quantified using a mouse A β ELISA kit (Invitrogen/Thermo, cat # KMB3441).

Western immunoblotting

Samples were thawed and homogenized in 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40 (Invitrogen), 1% sodium deoxycholate, 0.1% SDS, 5 mM EDTA, 1% Triton X-100 and phosphatase and protease inhibitor cocktail. Protein concentrations were determined using the BCA kit (Pierce-Thermo Scientific, Rockford, IL). Samples containing 30 μ g protein were resolved in 4–20% polyacrylamide Tris-glycine gels (Novex; Invitrogen, Grand Island, NY) and transferred to nitrocellulose membranes at 300 mA for 1 h. Blots were incubated with Odyssey® blocking buffer (Li-Cor; Lincoln, NE) at room temperature for 1 h and were incubated with primary antibody diluted in blocking buffer at 4 °C overnight. Primary antibodies used were anti-PSD-95 (1:1000; Santa Cruz, Dallas, TX), anti-synaptophysin (1:1000; Sigma, St Louis, MO), anti- β -actin (1:15,000; Abcam, Cambridge, UK), anti-cyclophilin (1:10,000; Abcam, Cambridge, UK), anti-amyloid precursor protein (APP) (1:1000 Zymed- Thermo Scientific, Rockford, IL), anti-Bace1 (1:2,000; Millipore-sigma, Burlington, MA #5940), and anti-p-AKT-Ser473 (1:1500; Cell Signaling, Danvers, MA). Membranes were incubated with IRDye-conjugated secondary antibodies (1:10,000; LI-COR Biosciences, Lincoln, NE) at room temperature for 1 h, imaged on an Odyssey Imaging System (LI-COR Biosciences, Lincoln, NE), and analyzed using NIH Image J.

Behavioral tests

Open field

Mice were placed at the center of an open field arena (30 cm \times 30 cm \times 45 cm) for habituation, and their activity was recorded for five minutes. Total distance traveled and time spent in the central square were automatically quantified using Any-maze® video-tracking system (Stoelting Inc., Kiel, WI). The arena was cleaned with 20% ethanol between trials to eliminate olfactory cues.

Novel object recognition test (NOR)

The novel object recognition test was performed in the open field arena with objects fixed to the box using tape. The test was video recorded for behavior readout [28]. During training and test sessions, animals were placed at the center of the arena, and exploratory behavior toward both objects was recorded for 5 min. The arena was cleaned with 20% ethanol between trials to eliminate olfactory cues. The training session was performed in the presence of two identical objects. One of the two objects used in the training session was then replaced by

a novel object for the test session, carried out one and a half hours after training. Sniffing and touching the object were considered exploratory behavior, and the amount of time spent exploring each object was registered by a trained researcher [28].

Statistical analyses

All datasets were submitted to the Shapiro–Wilk normality test. Specific statistical tests employed are mentioned in figure legends. Datasets showing normal distribution were analyzed by ANOVA or Student's *t*-test. Histological datasets were analyzed by ANOVA and differences were considered statistically significant with $p < 0.05$. Data from the NOR task were analyzed using a one-sample Student's *t*-test, comparing the exploration time of the novel object to the fixed value of 50% (chance level) as previously described [28–31]. All analyses were performed using GraphPad Prism 8 (GraphPad Software; La Jolla, CA). Results are expressed as means \pm SEM, and corresponding *t*-values.

Results

Intratracheal administration of silica induces alveolar collapse and lung infiltration by polymorphonuclear (PMN) cells

Lung histology showed that intratracheal administration of SiO₂ (silica) particles caused a significant increase in the percentage of collapsed alveoli compared to control, saline-instilled mice ($F(3, 25) = 22.2908$, $p = 0.000003$), both 24 h ($p = 0.00007$) and 15 d ($p = 0.00007$) after administration of silica (Fig. 1A–E). The number of polymorphonuclear (PMN) cells in the lungs was significantly increased 24 h after administration of silica ($F(6, 48) = 13.452$, $p = 0.000166$), and returned to baseline values 15 d after administration ($p = 0.000066$). No differences were observed in lung mononuclear cell numbers in silica-treated mice.

Intratracheal administration of silica induces lung inflammation

Lung pro-inflammatory cytokines IL-1 β [$t(1,16) = 2.656$; $p = 0.0172$] and IL-6 [$t(1,16) = 5.997$; $p = 0.0001$] were significantly increased 24 h after administration of silica (Fig. 2A, B). Cytokine levels were markedly reduced 15 days after administration of silica, compared to the levels found 24 h after instillation. Compared to Ctrl mice, lung IL-1 β remained elevated 15 d after administration of silica [$t(1,15) = 2.653$; $p = 0.0181$], whereas IL-6 returned to baseline levels 15 days after instillation of silica (Fig. 2D, E). Intriguingly, lung TNF- α showed an initial decrease 24 h after administration of silica [$t(1,16) = 4.529$; $p = 0.0003$] followed by a trend of elevation at 15 days [$t(1,15) = 1.605$; $p = 0.1294$] (Fig. 2C, F).

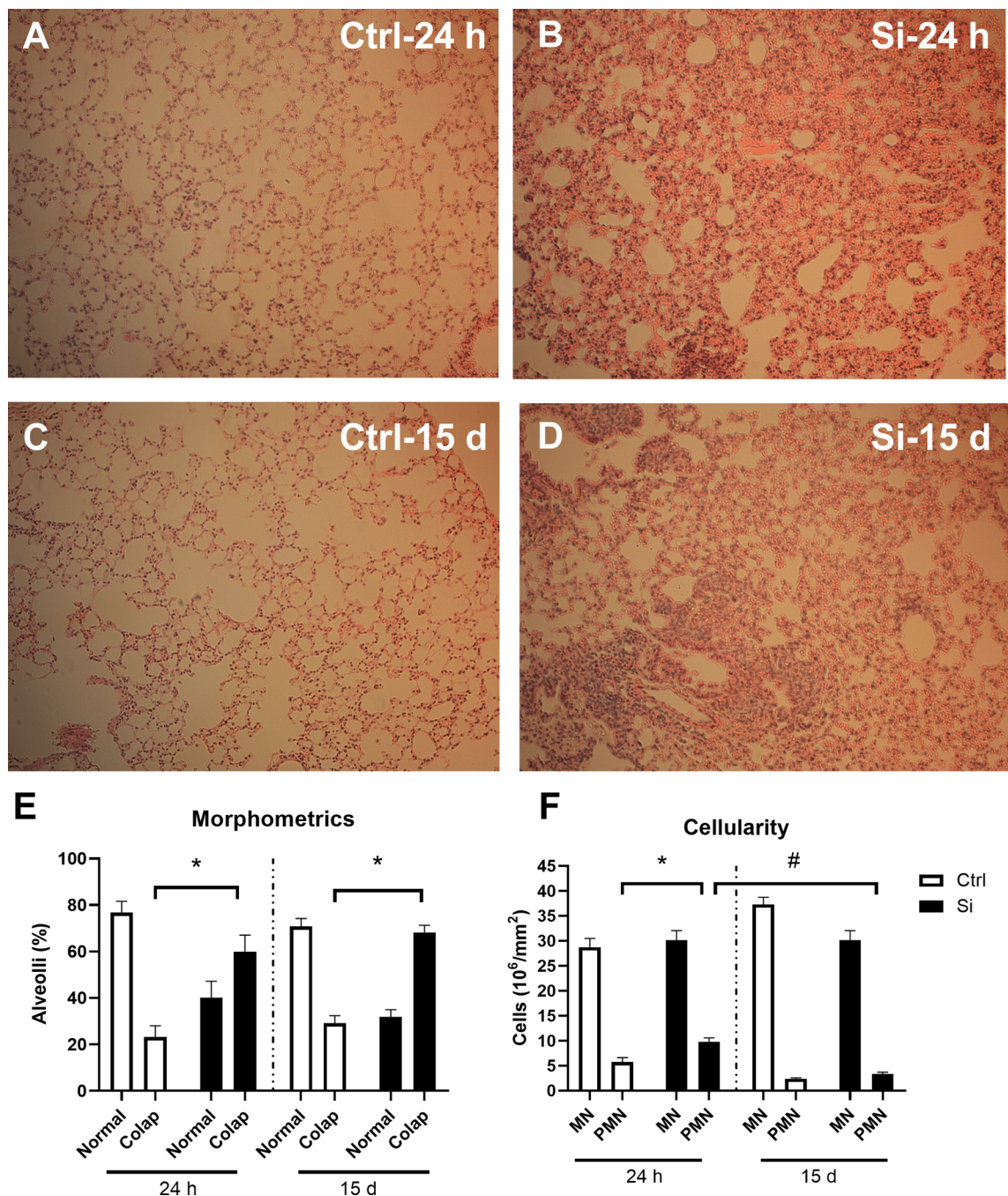


Fig. 1 Alveolar collapse and lung infiltration by PMN cells in silica-instilled mice. Representative photomicrographs of lungs from control (Ctrl) or silica-instilled (Si) animals. **A** Ctrl 24 h; **B** Si 24 h; **C** Ctrl 15 d; **D** Si 15 d. **E** Percentage of collapsed alveoli in Ctrl (white bars) or Si (black bars) animals. **F** Cellularity expressed as total cell counts per area. Data are expressed as mean \pm SEM; white bars represent Ctrl mice, and black bars represent silica-instilled (Si) mice. Two-way ANOVA with Duncan post hoc test. * $p < 0.05$ between Ctrl and Si; # $p < 0.05$ between 24 h and 15 d. $N = 7-10$ mice per experimental group

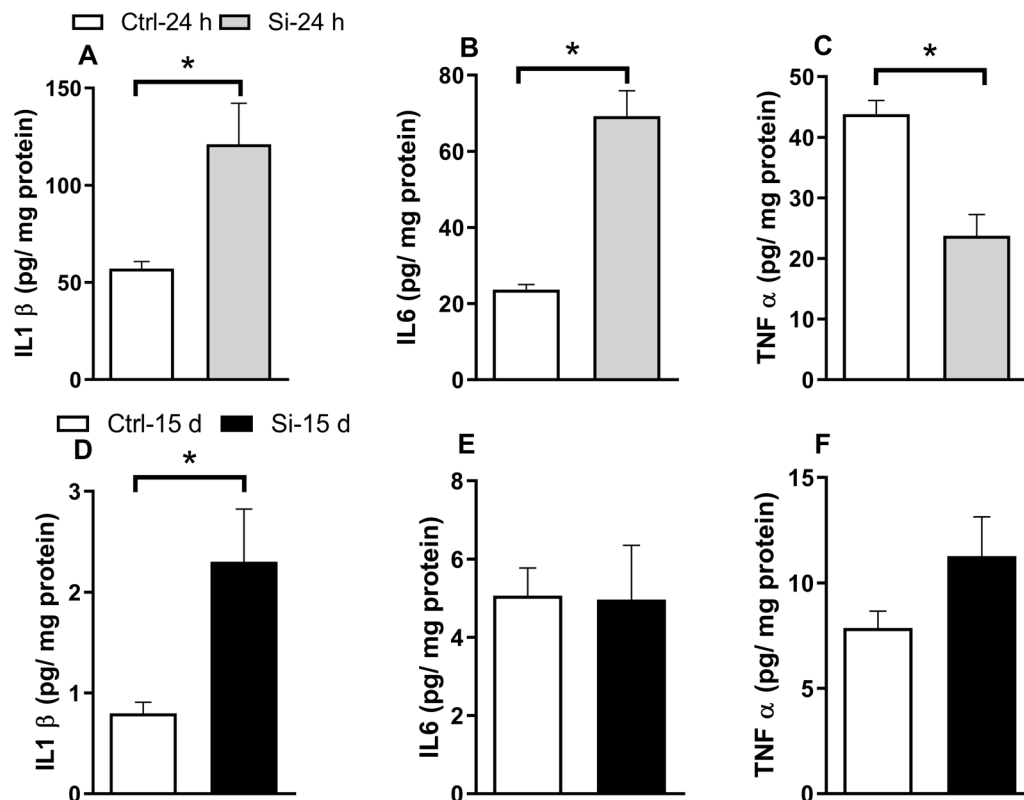


Fig. 2 Lung pro-inflammatory cytokines in silica-instilled mice. Lung IL-1 β , IL-6, and TNF- α were determined by ELISA 24 h (A–C; $N=8$ Ctrl mice, 10 Si mice) or 15 d (D–F; $N=8$ Ctrl mice, 9 Si mice) after intratracheal administration of saline (Ctrl) or silica (Si). Data are expressed as means \pm SEM. * $p < 0.05$, Student's t -test

Intratracheal administration of silica induces hippocampal inflammation in mice

No differences in hippocampal pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) were detected 24 h after intratracheal administration of silica in mice (Fig. 3A–C). Interestingly, however, hippocampal IL-1 β [$t(1,13)=2.358$; $p=0.0347$], and IL-6 [$t(1,15)=3.543$; $p=0.0030$], but not TNF- α [$t(1,15)=1.668$; $p=0.1175$], were significantly increased (compared to Ctrl mice) 15 days after intratracheal administration of silica (Fig. 3D–F).

Intratracheal administration of silica induces hippocampal synapse damage and increases A β 42 levels in mice

We next investigated whether the induction of pro-inflammatory cytokines induced by lung silicosis could entail synapse damage in the hippocampus. Pre- and post-synaptic markers, synaptophysin [$t(1,14)=2.310$; $p=0.0413$] and PSD-95 [$t(1,10)=2.326$; $p=0.0423$], respectively, were significantly reduced in hippocampal homogenates from silica-instilled mice (Fig. 4A, B).

The decreases in pre- and post-synaptic marker proteins in the hippocampi of silica-instilled mice were similar to previous observations in Alzheimer's disease (AD) mouse models [31]. This led us to investigate if hippocampal inflammation in silica-instilled mice could be associated with changes in levels of the amyloid- β peptide (A β), a neurotoxin that accumulates in AD brains and is implicated in brain inflammation and synapse damage in AD [8, 30, 32–34]. A β was indeed elevated in the hippocampi of Si mice compared to Ctrl animals [$t(1,14)=2.157$; $p=0.0488$] (Fig. 4C).

To examine a possible mechanism underlying increased hippocampal A β in silica-instilled mice, we measured protein levels of β -secretase (BACE 1), a key protease involved in the cleavage of the amyloid precursor protein (APP) to release A β [35, 36]. No difference was observed in BACE1 immunoreactivity between Ctrl and Si mice [$t(1,12)=0.2663$; $p=0.410$] (Fig. 4D). However, APP was significantly increased in the hippocampus 15 days after administration of silica in mice [$t(1,11)=2.174$; $p=0.05$] (Fig. 4E), suggesting that the increase in A β might be related to increased substrate (APP) availability.

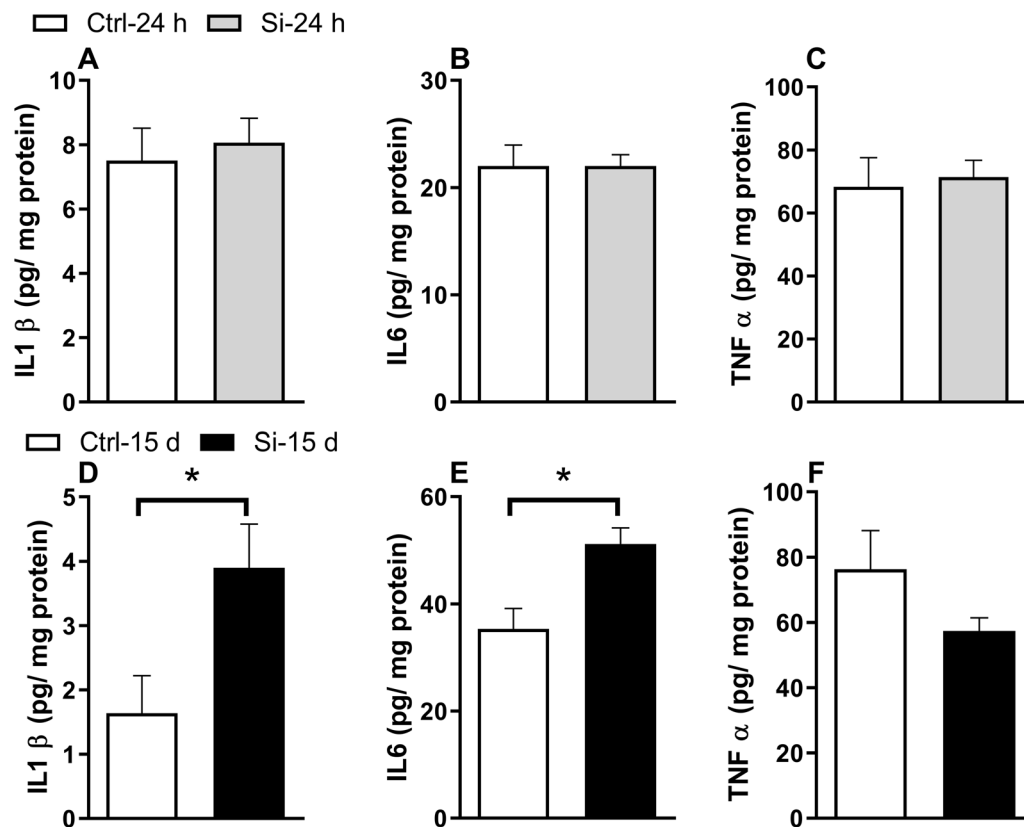


Fig. 3 Hippocampal pro-inflammatory cytokines in silica-instilled mice. Hippocampal IL-1β, IL-6 and TNF-α were determined by ELISA 24 h (A–C; $N=8$ Ctrl mice, 10 Si mice) or 15 d (D–F; $N=8$ Ctrl mice, 9 Si mice) after intratracheal administration of saline (Ctrl) or silica (Si). Data are expressed as mean \pm SEM. * $p < 0.05$, Student's t -test

Recent studies have established that inflammation-associated inhibition of brain insulin signaling plays an important role in neurodegenerative mechanisms leading to synapse damage and cognitive impairments in AD [8] and sepsis [13, 37]. To determine whether a similar mechanism might be induced by silicosis-induced hippocampal inflammation, we examined pSer473-Akt levels as a proxy of activity of the insulin signaling pathway. pSer473-Akt was reduced in Si mice compared to Ctrl animals [$t(1,12)=2.124$; $p=0.0551$], indicating that brain inflammation induced by intratracheal administration of silica is accompanied by inhibition of hippocampal insulin signaling.

Intratracheal administration of silica induces memory impairment in mice

Finally, we hypothesized that the impact of silicosis on hippocampal pro-inflammatory cytokines, synaptic markers, and phosphorylation of Akt could result in memory impairments in mice. Control open field tests revealed no differences in total distance traveled or time spent at the center of the arena between Ctrl and Si mice,

indicating that silica instillation did not affect locomotor/exploratory behavior or induced anxiety in mice (Fig. 5A, B, D, E).

As expected, Ctrl mice exhibited a clear preference for the novel object in the novel object recognition (NOR) test session, both 24 h [$t(1,6)=7.227$; $p=0.0004$] and 15 days [$t(1,7)=4.713$; $p=0.0022$] after saline administration. In contrast, silica-instilled mice failed to recognize the familiar object as such, and spent comparable amounts of time exploring familiar and novel objects, both 24 h [$t(1,7)=1.276$; $p=0.2425$] and 15 days [$t(1,8)=1.180$; $p=0.2720$] after administration of silica (Fig. 5C, F).

Discussion

We initially established that a single dose of silica (2.5 mg/kg; particle size range: 500 nm–10 μ m) administered into the trachea induced alveolar collapse, lung infiltration by PMN cells, and increased lung pro-inflammatory cytokines, all indicative of the induction of silicosis in mice. We further found that lung silicosis induced by this low dose of silica [38] was followed by an increase

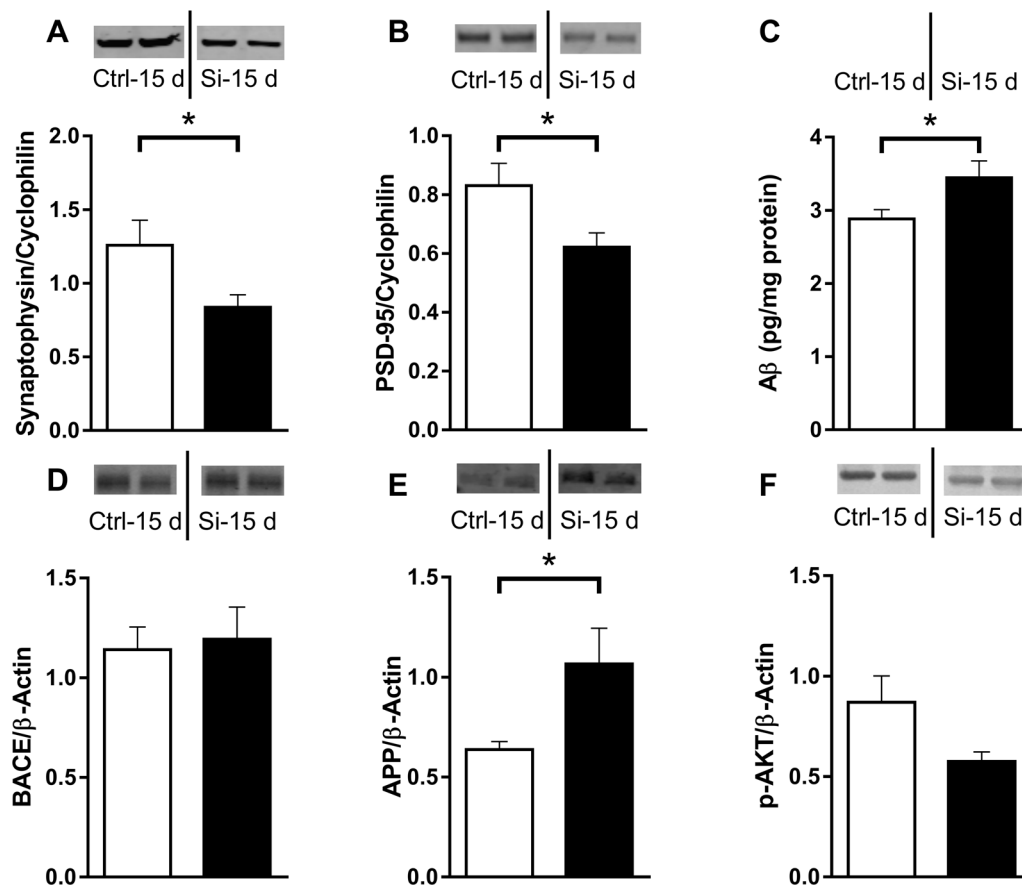


Fig. 4 Intratracheal administration of silica induced hippocampal synapse damage and increased Aβ levels. Pre- and post-synaptic marker proteins (synaptophysin and PSD-95, respectively; **A, B**), Aβ42 (**C**), BACE1 (**D**), APP (**E**), and pSer473-Akt (**F**) were determined in hippocampal homogenates 15 days after intratracheal administration of silica ($N=9$ Ctrl mice, 7 Si mice). Actin and cyclophilin were used as loading controls, as shown (bottom of the representative figures). Data are expressed as mean \pm SEM. * $p < 0.05$, Student's t -test

in hippocampal pro-inflammatory cytokines, synapse damage, accumulation of the Aβ peptide, and impaired memory.

In addition to an acute (24 h) examination of the impact of intratracheal administration of silica particles on the lung and brain, we analyzed lung and brain inflammation 15 days after administration of silica. The 15-day time window is frequently used to study the outcomes of exposure to SiO₂ (e.g., Reiser et al. [39]; Faffe et al. [26]; Yang et al. [40]. Numerous studies have demonstrated significant pathological alterations induced by silica particles using a 15-day experimental window. The experimental design in our current study was thus based on a validated model in the silicosis field.

Silicosis is a restrictive disease that causes alveolar collapse, an increase in lung elastic tissue and lung infiltration by inflammatory cells [26]. The increase in collapsed alveoli indicates tissue damage and impairment of pulmonary function [41]. As a result, cytokines are secreted

into the lung, triggering pulmonary remodeling processes, and resulting in the production of connective tissue fibers [41]. As expected, we found morphometric and cellularity alterations in the lung following intratracheal administration of silica. The percentage of collapsed alveoli was increased 24 h after instillation and remained elevated 15 days later, indicating that tissue damage was persistent over time. We further observed an increase in the number of PMN cells, but not in MN cells, in the lung 24 h after silica instillation (but not 15 days after instillation), indicating an acute lung infiltration by peripheral immune/inflammatory cells.

Previous studies employing administration of higher doses of silica (20 mg/kg) demonstrated that histological alterations in the lung were accompanied by robust increases in pro-inflammatory cytokines [42, 43]. Thus, we sought to determine whether the lower dose of silica employed here elicited a similar response. In accordance with previous results [3], both IL-1β and IL-6 were

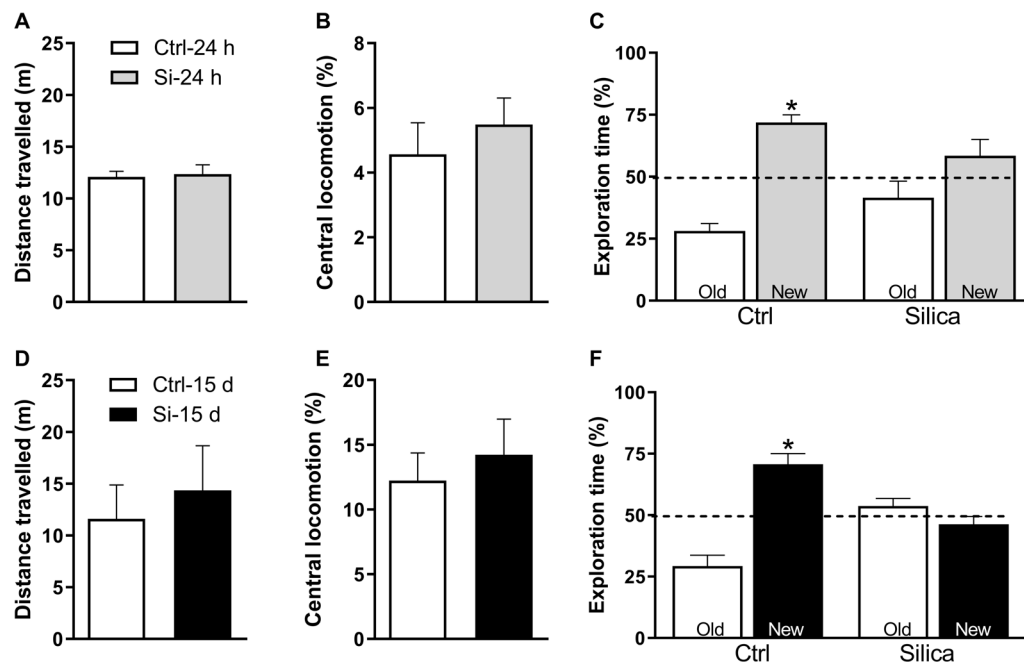


Fig. 5 Intratracheal administration of silica induces memory impairment in mice. Mice were tested 24 h (**A–C**) or 15 d (**D–F**) after intratracheal administration of saline (Ctrl) or silica (Si). Open field tests showed no difference between groups in total distance traveled (**A** 24 h post-instillation, **D** 15 d post-instillation) or time spent at the center of the arena (**B** 24 h post-instillation, **E** 15 d post-instillation) (**A, B** $N = 7$ Ctrl mice, 8 Si mice; **D, E** $N = 8$ Ctrl mice, 9 Si mice). **C, F** Memory performance was assessed in the NOR paradigm 24 h (**C**; $N = 7$ Ctrl mice, 8 Si mice) or 15 d after administration of silica or saline (**F**; $N = 8$ Ctrl mice, 9 Si mice). Data are expressed as mean \pm SEM. * $p < 0.05$, one-sample Student's t -test compared to the fixed value of 50%. Old = familiar object; New = novel object

significantly increased in the lung 24 h after administration of silica. However, in contrast with the increase in lung TNF- α observed in mice instilled with a higher dose of silica [3], under our conditions TNF- α was reduced at 24 h in Si mice compared to Ctrl animals. The different TNF- α response may be related to the low dose of silica administered or to the use of Swiss mice in the current work, as opposed to a higher silica dose and BALB/c mice in previous studies [3]. In addition, evidence suggests that TNF- α release is time-dependent in silica-induced toxicity [44], and lipoxins may regulate this release as discussed next. In line with the reduction in PMN cell number, IL-6 and TNF- α returned to baseline levels 15 days after silica instillation.

Modulation of cytokine levels in silicosis may be further be related to the upregulation of lipoxin A4 (LXA4) signaling. An increase in LXA4 has been found to contribute to the protective effect of apolipoprotein A1 (ApoA1) against fibrosis in an experimental model of lung silicosis [45]. The mechanism of LXA4 protection has been reported to involve attenuation of the release of pro-inflammatory cytokines and chemokines, such as TNF- α and macrophage inflammatory protein-2 [46]. In addition to its anti-inflammatory activity, LXA4 stimulates macrophages to perform phagocytosis of apoptotic

immune cells without the release of pro-inflammatory cytokines [47].

Administration of micro- and nano-sized particulate matter to animals via the lung has been shown to trigger systemic inflammation and lesions to the spleen, heart, and kidney [40, 48], as well as autoimmunity [49]. Humans exposed to silica displayed increased levels of systemic inflammatory markers [50], rheumatoid arthritis or systemic sclerosis [51]. The central nervous system can also be impacted by silica. After inhalation, SiO₂ nanoparticles penetrate the epithelium of the respiratory tract and are translocated to the brain via either the circulatory system or the olfactory nerve [52]. Following exposure to silica nanoparticles, mice showed neuropathology, degeneration, and synapse damage [3]. Silica nanoparticles were found to be primarily deposited in the frontal cortex and hippocampus [3]. Because the hippocampus is centrally implicated in memory and cognition, and pathological deregulation of hippocampal function underlies cognitive and memory impairments in neurological disorders, we investigated the possibility that lung inflammation might be followed by hippocampal inflammation and memory loss in silicosis.

Increasing evidence supports the notion that both acute and chronic peripheral inflammation can trigger

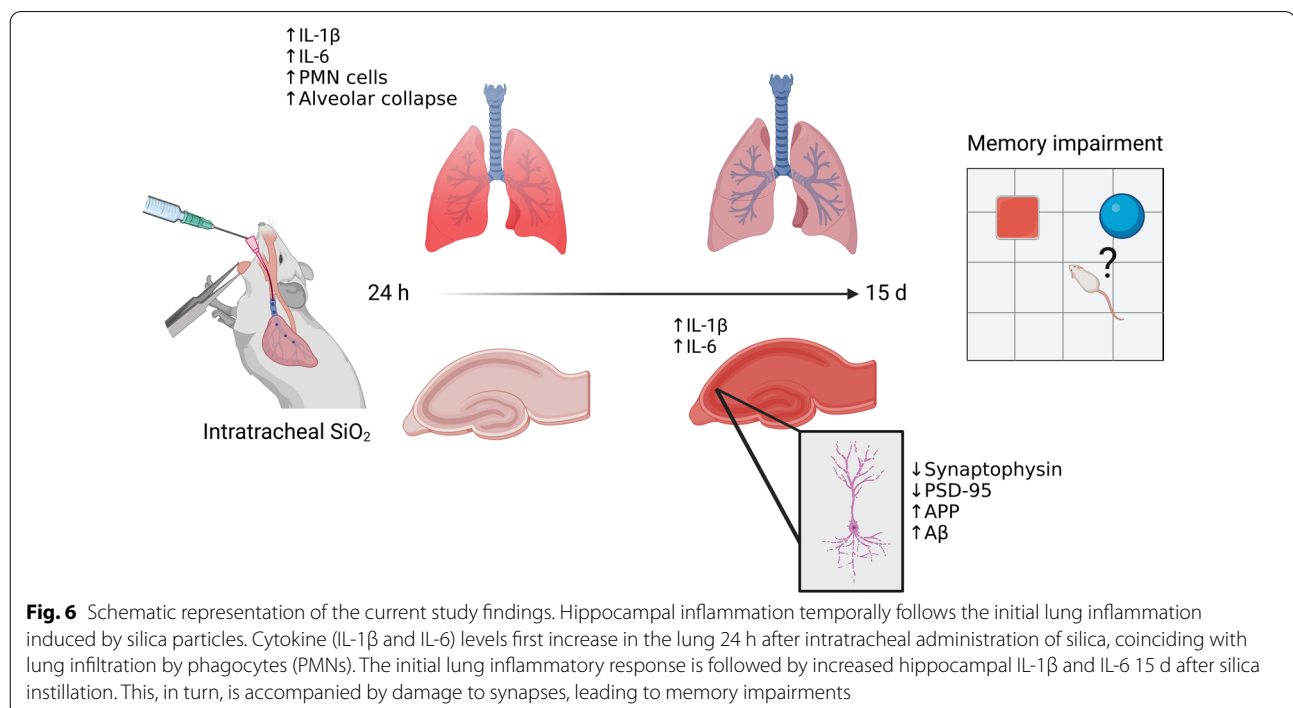
brain inflammation, neurodegeneration, and cognitive deficits [2, 30, 37]. Significantly, recent studies have revealed that restrictive pulmonary diseases, such as silicosis, are risk factors for cognitive impairment and dementia [23]. However, the mechanisms underlying the association between lung and brain dysfunction remain unknown.

The potential crosstalk between lung inflammation and brain dysfunction in the mouse silicosis model was initially evaluated by measuring hippocampal cytokines. Results showed no significant differences in hippocampal IL-1 β , IL-6, or TNF- α 24 h after administration of silica. However, hippocampal IL-1 β and IL-6 were significantly increased 15 days after instillation, suggesting that hippocampus inflammation was triggered by and temporally followed the initial lung inflammation caused by silica.

Increased brain levels of pro-inflammatory cytokines trigger synapse damage and affect neuroplasticity [6], resulting in cognitive impairments [2, 32]. We found that hippocampal pre- and post-synaptic markers (synaptophysin and PSD-95, respectively) were significantly decreased 15 days after administration of silica, indicating synapse damage and loss. Numerous studies have established synapse loss as a hallmark of AD [33]. Soluble forms of the A β peptide accumulate in the AD brain [35, 36] and activate pro-inflammatory pathways leading to synapse loss and neuronal damage [7, 53]. This prompted us to investigate whether hippocampal inflammation in silica-instilled mice was associated

with elevated A β levels. Hippocampal A β levels were indeed higher in silica-instilled than in control mice. While we did not detect any changes in BACE1, one of the secretases responsible for cleavage of APP and production of A β [54], APP was found to be elevated in the hippocampi of mice that received silica. It is, thus, possible that the increase in hippocampal A β resulted from increased substrate (APP) availability for cleavage by secretases in silica-instilled mice. Results suggest that inflammatory signaling from the lung triggers a deleterious process involving elevated hippocampal cytokines and A β and leading to synapse damage in the hippocampus. Communication via the vagus nerve might be an additional mechanism involved in the crosstalk between peripheral and central inflammation in silicosis, and further studies appear warranted to investigate this communication.

Inhibition of brain insulin signaling is thought to play a major role in the pathogenesis of neurodegenerative disorders [7, 8, 37] and in sepsis [24, 25], and to be a leading mechanism underlying cognitive impairment. A key component of the insulin signaling pathway is Akt, which becomes phosphorylated at Ser473 in response to insulin [8]. Disruption of Akt signaling is detrimental to insulin response and may lead to brain dysfunction [7]. Our results showed a decrease in hippocampal pSer473-Akt in silica-instilled mice, suggesting disruption of insulin signaling such as observed in AD and other neurodegenerative disorders.



Finally, our findings of hippocampal inflammation, synapse damage and impaired insulin signaling prompted us to assess the impact of the administration of silica on memory. Remarkably, we found that intratracheal instillation of a low-dose of silica impaired memory in the NOR test.

In conclusion, the current study identified a crosstalk between lung and brain leading to hippocampal inflammation, synapse damage and cognitive impairment after a single intratracheal instillation of a low dose of silica in mice. Figure 6 schematically represent this sequence of events. Interestingly, a recent study showed a link between intranasal exposure to silica, α -synuclein aggregation and neurodegeneration in a Parkinson's disease model [55]. In light of recent clinical and epidemiological studies connecting pulmonary dysfunction with cognitive impairments and dementia [22–25], and considering the prevalence of silicosis as an occupational disease, investigation of potential neurological outcomes in patients appears warranted. Our findings further suggest that early intervention to attenuate peripheral pro-inflammatory signaling may be important to preserve brain health in silicosis.

Abbreviations

AD: Alzheimer's disease; APP: Amyloid precursor protein; A β : Amyloid- β peptide; IL-1 β : Interleukin-1 β ; IL-6: Interleukin-6; i.t.: Intratracheal; LXA4: Lipoxin A4; MN: Mononuclear cells; NOR: Novel object recognition test; PMN: Polymorphonuclear cells; TLRs: Toll-like receptors; TNF- α : Tumor necrosis factor- α ; BACE 1: β -Secretase.

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Author contributions

All authors have contributed significantly to this work. STF, FGDF, and WAZ supervised the study. GCK and MNM designed and performed experiments. HMM performed the A β ELISA. LS and PRS analyzed the data, and wrote an initial version of the manuscript. GMCC performed the lung histology quantification. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset(s) supporting the conclusions of this article are available in the OSF repository on <https://osf.io/rp4kq/>.

Declarations

Ethics approval and consent to participate

All procedures involving animal research were approved by the Ethics Committee on the Use of Animals, Health Sciences Center, Federal University of Rio de Janeiro. Consent to participate is not applicable.

Consent for publication

All authors agree with publication of the current manuscript.

Competing interests

Authors declare no competing interests.

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References

1. Ferreira ST, Clarke JR, Bomfim TR, De Felice FG. Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. *Alzheimers Dement*. 2014;10(1 Suppl):S76–83.
2. Lyra ESNM, Gonçalves RA, Pascoal TA, Lima-Filho RAS, Resende EPF, Vieira ELM, et al. Pro-inflammatory interleukin-6 signaling links cognitive impairments and peripheral metabolic alterations in Alzheimer's disease. *Transl Psychiatry*. 2021;11(1):251.
3. You R, Ho YS, Hung CH, Liu Y, Huang CX, Chan HN, et al. Silica nanoparticles induce neurodegeneration-like changes in behavior, neuropathology, and affect synapse through MAPK activation. *Part Fibre Toxicol*. 2018;15(1):28.
4. Lu R, Kiernan MC, Murray A, Rosner MH, Ronco C. Kidney–brain crosstalk in the acute and chronic setting. *Nat Rev Nephrol*. 2015;11(12):707–19.
5. Kurella Tamura M, Yaffe K. Dementia and cognitive impairment in ESRD: diagnostic and therapeutic strategies. *Kidney Int*. 2011;79(1):14–22.
6. Bobbo VC, Engel DF, Jara CP, Mendes NF, Haddad-Tovoli R, Prado TP, et al. Interleukin-6 actions in the hypothalamus protects against obesity and is involved in the regulation of neurogenesis. *J Neuroinflammation*. 2021;18(1):192.
7. De Felice FG, Gonçalves RA, Ferreira ST. Impaired insulin signalling and allostatic load in Alzheimer disease. *Nat Rev Neurosci*. 2022;23(4):215–30.
8. Ferreira ST. Brain insulin, insulin-like growth factor 1 and glucagon-like peptide 1 signalling in Alzheimer's disease. *J Neuroendocrinol*. 2021;33(4):e12959.
9. Watson KT, Simard JF, Henderson VW, Nutkiewicz L, Lamers F, Nasca C, et al. Incident major depressive disorder predicted by three measures of insulin resistance: a Dutch cohort study. *Am J Psychiatry*. 2021;178(10):914–20.
10. Friedland RP. Mechanisms of molecular mimicry involving the microbiota in neurodegeneration. *J Alzheimer's Dis*. 2015;45(2):349–62.
11. Chen SG, Stribinskis V, Rane MJ, Demuth DR, Gozal E, Roberts AM, et al. Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged Fischer 344 rats and *Caenorhabditis elegans*. *Sci Rep*. 2016;6:34477.
12. Sampson TR, Challis C, Jain N, Moiseyenko A, Ladinsky MS, Shastri GG, et al. A gut bacterial amyloid promotes alpha-synuclein aggregation and motor impairment in mice. *Elife*. 2020. <https://doi.org/10.7554/eLife.53111>.
13. De Sousa VL, Araujo SB, Antonio LM, Silva-Queiroz M, Colodeti LC, Soares C, et al. Innate immune memory mediates increased susceptibility to Alzheimer's disease-like pathology in sepsis surviving mice. *Brain Behav Immun*. 2021;95:287–98.

14. Figueiredo CP, Barros-Aragao FGQ, Neris RLS, Frost PS, Soares C, Souza INO, et al. Zika virus replicates in adult human brain tissue and impairs synapses and memory in mice. *Nat Commun*. 2019;10(1):3890.
15. Lyra ESNM, Barros-Aragao FGQ, De Felice FG, Ferreira ST. Inflammation at the crossroads of COVID-19, cognitive deficits and depression. *Neuropharmacology*. 2022;209: 109023.
16. Tahira AC, Verjovski-Almeida S, Ferreira ST. Dementia is an age-independent risk factor for severity and death in COVID-19 inpatients. *Alzheimers Dement*. 2021;17(11):1818–31.
17. De Felice FG, Tovar-Moll F, Moll J, Munoz DP, Ferreira ST. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the central nervous system. *Trends Neurosci*. 2020;43(6):355–7.
18. Quaintance PA. Silicosis: a study of 106 pottery workers. *Am J Public Health Nations Health*. 1934;24(12):1244–51.
19. Krefft S, Wolff J, Rose C. Silicosis: an update and guide for clinicians. *Clin Chest Med*. 2020;41(4):709–22.
20. Machado MN, Schmidt AC, Saldiva PH, Faffe DS, Zin WA. Pulmonary functional and morphological damage after exposure to tripoli dust. *Respir Physiol Neurobiol*. 2014;196:17–24.
21. Piguet PF, Collart MA, Grau GE, Sappino AP, Vassalli P. Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. *Nature*. 1990;344(6263):245–7.
22. Russ TC, Kivimaki M, Batty GD. Respiratory disease and lower pulmonary function as risk factors for dementia: a systematic review with meta-analysis. *Chest*. 2020;157(6):1538–58.
23. Lutsey PL, Chen N, Mirabelli MC, Lakshminarayan K, Knopman DS, Vossel KA, et al. Impaired lung function, lung disease, and risk of incident dementia. *Am J Respir Crit Care Med*. 2019;199(11):1385–96.
24. Wang J, Dove A, Song R, Qi X, Ma J, Bennett DA, et al. Poor pulmonary function is associated with mild cognitive impairment, its progression to dementia, and brain pathologies: a community-based cohort study. *Alzheimers Dement*. 2022. <https://doi.org/10.1002/alz.12625>.
25. Wang J, Song R, Dove A, Qi X, Ma J, Laukka EJ, et al. Pulmonary function is associated with cognitive decline and structural brain differences. *Alzheimers Dement*. 2022;18(7):1335–44.
26. Faffe DS, Silva GH, Kurtz PM, Negri EM, Capelozzi VL, Rocco PR, et al. Lung tissue mechanics and extracellular matrix composition in a murine model of silicosis. *J Appl Physiol*. 2001;90(4):1400–6.
27. Borges VM, Falcao H, Leite-Junior JH, Alvim L, Teixeira GP, Russo M, et al. Fas ligand triggers pulmonary silicosis. *J Exp Med*. 2001;194(2):155–64.
28. Figueiredo CP, Clarke JR, Ledo JH, Ribeiro FC, Costa CV, Melo HM, et al. Memantine rescues transient cognitive impairment caused by high-molecular-weight abeta oligomers but not the persistent impairment induced by low-molecular-weight oligomers. *J Neurosci*. 2013;33(23):9626–34.
29. Lourenco MV, Frozza RL, de Freitas GB, Zhang H, Kincheski GC, Ribeiro FC, et al. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. *Nat Med*. 2019;25(1):165–75.
30. Ledo JH, Azevedo EP, Beckman D, Ribeiro FC, Santos LE, Razolli DS, et al. Cross talk between brain innate immunity and serotonin signaling underlies depressive-like behavior induced by Alzheimer's amyloid-beta oligomers in mice. *J Neurosci*. 2016;36(48):12106–16.
31. Lourenco MV, Clarke JR, Frozza RL, Bomfim TR, Forny-Germano L, Batista AF, et al. TNF-alpha mediates PKR-dependent memory impairment and brain IRS-1 inhibition induced by Alzheimer's beta-amyloid oligomers in mice and monkeys. *Cell Metab*. 2013;18(6):831–43.
32. Bauer ME, Teixeira AL. Inflammation in psychiatric disorders: what comes first? *Ann N Y Acad Sci*. 2019;1437(1):57–67.
33. Forny-Germano L, Lyra e Silva NM, Batista AF, Brito-Moreira J, Gralle M, Boehnke SE, et al. Alzheimer's disease-like pathology induced by amyloid-beta oligomers in nonhuman primates. *J Neurosci*. 2014;34(41):13629–43.
34. LaRocca TJ, Cavalier AN, Roberts CM, Lemieux MR, Ramesh P, Garcia MA, et al. Amyloid beta acts synergistically as a pro-inflammatory cytokine. *Neurobiol Dis*. 2021;159: 105493.
35. Ferreira ST, Klein WL. The Abeta oligomer hypothesis for synapse failure and memory loss in Alzheimer's disease. *Neurobiol Learn Mem*. 2011;96(4):529–43.
36. Mucke L, Selkoe DJ. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med*. 2012;2(7): a006338.
37. Neves FS, Marques PT, Barros-Aragao F, Nunes JB, Venancio AM, Cozachenko D, et al. Brain-defective insulin signaling is associated to late cognitive impairment in post-septic mice. *Mol Neurobiol*. 2018;55(1):435–44.
38. Mayeux JM, Kono DH, Pollard KM. Development of experimental silicosis in inbred and outbred mice depends on instillation volume. *Sci Rep*. 2019;9(1):14190.
39. Reiser KM, Hesterberg TW, Haschek WM, Last JA. Experimental silicosis. I. Acute effects of intratracheally instilled quartz on collagen metabolism and morphologic characteristics of rat lungs. *Am J Pathol*. 1982;107(2):176–85.
40. Yang M, Jing L, Wang J, Yu Y, Cao L, Zhang L, et al. Macrophages participate in local and systemic inflammation induced by amorphous silica nanoparticles through intratracheal instillation. *Int J Nanomedicine*. 2016;11:6217–28.
41. Knudsen L, Ruppert C, Ochs M. Tissue remodelling in pulmonary fibrosis. *Cell Tissue Res*. 2017;367(3):607–26.
42. Santana PT, Luna-Gomes T, Rangel-Ferreira MV, Tamara AS, Da Graca C, Machado MN, et al. P2Y12 receptor antagonist clopidogrel attenuates lung inflammation triggered by silica particles. *Front Pharmacol*. 2020;11:301.
43. Moncao-Ribeiro LC, Faffe DS, Santana PT, Vieira FS, da Graca CL, Marques-da-Silva C, et al. P2X7 receptor modulates inflammatory and functional pulmonary changes induced by silica. *PLoS ONE*. 2014;9(10): e110185.
44. Kawasaki H. A mechanistic review of silica-induced inhalation toxicity. *Inhal Toxicol*. 2015;27(8):363–77.
45. Lee E, Lee EJ, Kim H, Jang A, Koh E, Uh ST, et al. Overexpression of apolipoprotein A1 in the lung abrogates fibrosis in experimental silicosis. *PLoS ONE*. 2013;8(2): e55827.
46. Hachicha M, Pouliot M, Petasis NA, Serhan CN. Lipoxin (LX)A4 and aspirin-triggered 15-epi-LXA4 inhibit tumor necrosis factor 1alpha-initiated neutrophil responses and trafficking: regulators of a cytokine-chemokine axis. *J Exp Med*. 1999;189(12):1923–30.
47. Mitchell S, Thomas G, Harvey K, Cottell D, Reville K, Berlasconi G, et al. Lipoxins, aspirin-triggered epi-lipoxins, lipoxin stable analogues, and the resolution of inflammation: stimulation of macrophage phagocytosis of apoptotic neutrophils in vivo. *J Am Soc Nephrol*. 2002;13(10):2497–507.
48. Sasai F, Rogers KL, Orlicky DJ, Stem A, Schaeffer J, Garcia G, et al. Inhaled silica nanoparticles cause chronic kidney disease in rats. *Am J Physiol Renal Physiol*. 2022;323(1):F48–58.
49. Pollard KM. Silica, silicosis, and autoimmunity. *Front Immunol*. 2016;7:97.
50. Sauni R, Oksa P, Lehtimäki L, Toivio P, Palmroos P, Nieminen R, et al. Increased alveolar nitric oxide and systemic inflammation markers in silica-exposed workers. *Occup Environ Med*. 2012;69(4):256–60.
51. Gomez-Puerta JA, Gedmintas L, Costenbader KH. The association between silica exposure and development of ANCA-associated vasculitis: systematic review and meta-analysis. *Autoimmun Rev*. 2013;12(12):1129–35.
52. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, et al. Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol*. 2004;16(6–7):437–45.
53. De Felice FG, Wu D, Lambert MP, Fernandez SJ, Velasco PT, Lacor PN, et al. Alzheimer's disease-type neuronal tau hyperphosphorylation induced by A beta oligomers. *Neurobiol Aging*. 2008;29(9):1334–47.
54. Vassar R. BACE1: the beta-secretase enzyme in Alzheimer's disease. *J Mol Neurosci*. 2004;23(1–2):105–14.
55. Yuan X, Yang Y, Xia D, Meng L, He M, Liu C, et al. Silica nanoparticles promote alpha-synuclein aggregation and Parkinson's disease pathology. *Front Neurosci*. 2021;15: 807988.

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