Behavioral and Brain Functions

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Cutibacterium Acnes induces Alzheimer's disease-like pathology in brains of wistar rats through structural changes associated with microtubules

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Abstract

Background *Cutibacterium acnes*(*C. acnes*), a Gram-positive anaerobe and a dominant bacterium species in the sebaceous follicles of the face was detected in the brain of Alzheimer's disease (AD) patients. It has been found that *C. acnes* activates non-specifically the innate immune system by producing proinflammatory cytokines and can participate in brain inflammation. We hypothesise that *C. acnes* could influence the brain through the structural alteration in axons and dendrites of neurons.

Methods In this regard, the hippocampus of rats was infected with *C. acnes*, and memory retention, amyloid-β ($A\beta_{1-42}$) deposition, hyperphosphorylated tau protein (p-Tau) formation, and expression levels of MAP2 and β-tubulin proteins in the hippocampus tissues were investigated.

Results *C. acnes*-infected rats displayed memory deficits and $A\beta_{1-42}$ deposits were detected in their hippocampus tissue up to 7 days post-infection. *C. acnes* was neurotoxic and exerted detrimental effects on MAP2 and β-tubulin proteins, which are required for normal neuronal function. An elevated level of p-Tau was also identified in infected animals.

Conclusion Based on these results, we propose that *C. acnes* infection of the brain participates in the initiation of the pathogenesis of sporadic AD through degeneration of axons and dendrites.

Keywords *Cutibacterium acnes*, Alzheimer's disease, Memory, Amyloid-β deposition, Hyperphosphorylated tau, Neurotoxicity

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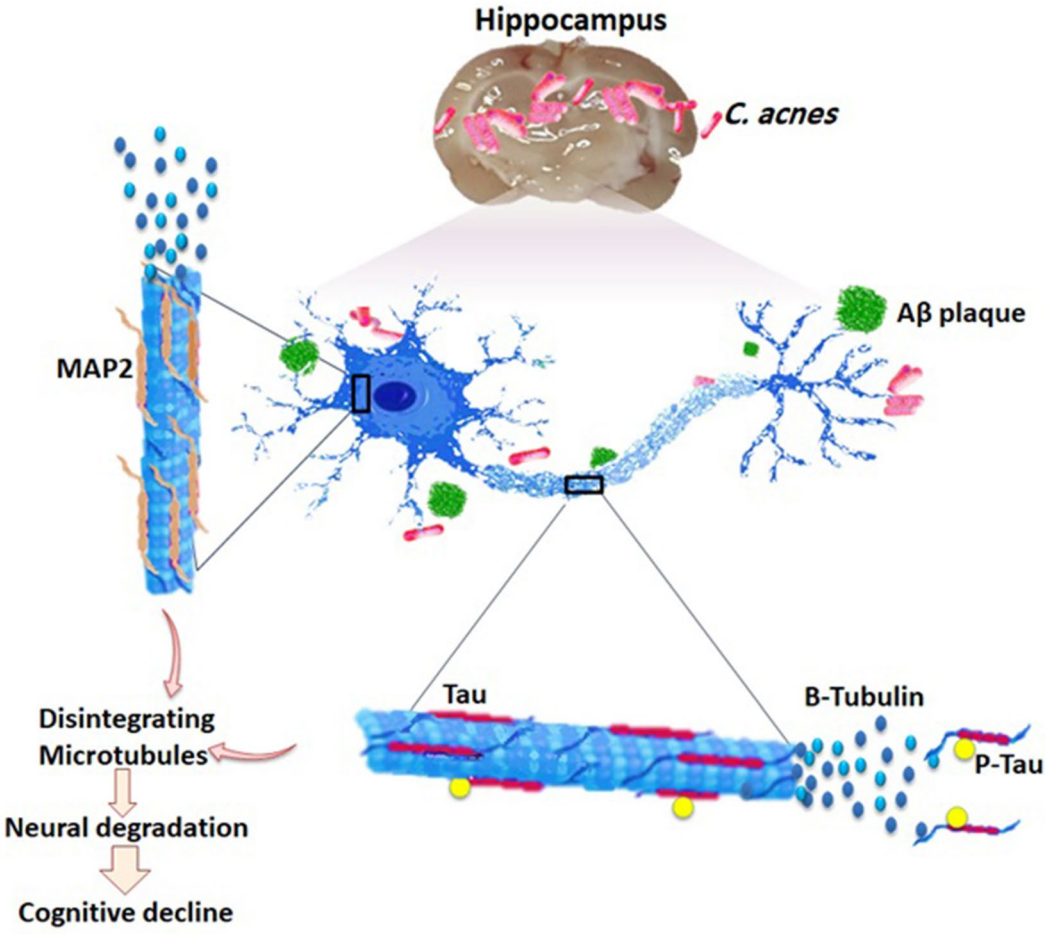
Graphical Abstract



Introduction

Infectious agents, such as bacteria, viruses, and fungi potentially contribute to systemic inflammation and can trigger the onset of sporadic AD [1]. A growing body of evidence suggests that systemic inflammation potentially promotes chronic neurodegenerative disorders. Pro-inflammatory agents produced in chronic peripheral inflammation can circulate in the central nervous system (CNS). These factors can penetrate the CNS through the blood-brain barrier (BBB) or circumventricular areas of the brain without passing the BBB [2]. These microbes may directly infect the CNS either through oral-olfactory routes or the trigeminal nerve [2]. Various pathogens, such as Herpes simplex virus type 1 (HSV1), *Borrelia burgdorferi*, *Porphyromonas gingivalis*, *Chlamydia pneumoniae*, the periodontal *Treponemal spp*, and *Cutibacterium acnes (C. acnes)* have been identified in the brains of AD patients [3]. The infectious hypothesis of AD is a very new idea and suggests that microbes and pathogens are the causative agents in AD pathology, including accumulation of amyloid beta (Aβ), p-Tau, and inflammation in the brain [4, 5]. Animals infected by bacterial lipopolysaccharide (*Pg*-LPS) or *Porphyromonas gingivalis* (*P. gingivalis*) illustrated the pathogenesis of AD, such as Aβ overproduction, stimulation of complement cascade, and overexpression of pro-inflammatory cytokines, leading to age-dependent brain inflammation, neuroinflammation, and neurodegeneration [6]. Infection of the CNS by dysbiosis-associated oral bacteria was also repeatedly reported in patients with AD and elevated amounts of pro-inflammatory cytokines were also identified [7]. According to recent findings, Aβ peptides have potent antimicrobial properties (antibacterial, antiviral, and antifungal), which protect the brain against pathogens and are generated even under normal conditions [4]. Although, in the case of prolonged overactivation of a senescent immune system, accumulation and aggregation of Aβ contribute to synapse disruption, and induce cytotoxicity and neuronal cell death leading to neurodegeneration disorder [4]. *Cutibacterium acnes* (*C. acnes)*,

an aerotolerant anaerobic Gram-positive bacterium, is found in the pilosebaceous follicles of the human skin, hair follicles, external ear canal, conjunctiva, oral cavity, and intestinal tract [8]. *C. acnes* is the dominant bacterium in the most common human skin disorder, acne vulgaris, which afflicts up to 80% of people at some point during their lives. High local concentrations of *C. acnes* can cross the BBB and invade the brain through the transcellular traversal pathway [9]. It was reported that a patient with severe chronic sinusitis had developed a brain abscess due to an infection of the brain with *C. acnes* [10]. Another patient with metastatic melanoma had developed meningitis due to infection with *C. acnes* [11]. *C. acnes* has been detected in the frontal cortex of AD patients [12, 13] and may promote brain inflammation leading to AD and also Parkinson's disease (PD) [9]. In two AD case reports, combination treatment with cephalosporine, estrogen and enalapril against *C. acnes* resulted in memory improvement and clinical symptoms stabilization [13, 14]. Moreover, *C. acnes* during metabolism processes produces Propionic acid (PPA) [15]. PPA has shown major effects on physiology of nervous system, such as stimulating of gap junction gating and gene expression, intracellular pH/calcium gating, immune function, neurotransmitter synthesis and release, specific G protein coupled receptors (GPCR), mitochondrial function, and lipid metabolism [16]. In rats, intraventricular administration of PPA caused behavioral, neuropathological, and biochemical abnormalities [17]. Our bioinformatics study showed that PPA-interacted genes are mainly AD hallmarks [18]. In addition, in another study, we illustrated a relationship between *C. acnes* infection and cognitive decline in Wistar rats after ICV inoculation of bacteria [19]. We showed that accumulation of *C. acnes* in brain could active innate immune system through inducing TLR4/NFkb/inflamasome signaling pathway, that finally increase inflammation in brain. Its result was associated with increasing Aβ and cell dead through apoptosis and pyroptosis [19]. In the current study, we hypothesize that neural degeneration might occur through tau hyperphosphorylation in axon and dissociation of MAP2 from cell bodies and dendrites. Therefore, we evaluated the influence of *C. acnes* ATCC 6919 infection on neural structure in hippocampus of Wister rats.

Materials and methods

Preparation of *C. acnes* suspensions

The strain *C. acnes* ATCC 6919 (belongs to the phylotype IA-1 and ribotype 1 subgroup) was obtained from the Pasteur Institute of Iran, and was stored as a freeze-dried culture. Before commencing the study, the strain underwent revival and maintenance in accordance with established microbiological approaches. The *C. acnes* ATCC 6919 strain was sub-cultured on blood agar plates (Merck, Germany) containing 5% defibrinated sheep blood before being transferred to the solution. A single colony was carefully chosen from the plate and inoculated into a liquid culture medium, Reinforced Clostridial Medium (RCM) broth, to facilitate targeted bacterial growth. Subsequently, the bacterial culture within the RCM broth was subjected to anaerobic incubation at 37 °C for a duration of 72 h, accompanied by gentle agitation at 120 rpm, until it reached the mid-log phase. This process was executed within an anaerobic incubator (Anoxomat MARK II system) utilizing a gas blend comprising 80% N_2, 10% CO_2, and 10% H_2. McFarland standard was used as a reference to adjust the turbidity of bacterial suspensions. Serial dilution was prepared as 1.4×10^8 colony-forming units (CFU)/ml of bacteria.

Experimental design and groups

A group of healthy adult male Wistar rats, who were four months old and weighed between 220 and 250 g, were obtained from the Institute for Cognitive Science Studies. The rats were kept in a controlled environment at 22±2 °C, 60±5% humidity, and a 12-hour dark/light cycle. They had access to food and water at all times. All experimental methods meet the National Institute of Health Care and Use of Laboratory Animals Guideline and conformity with the regional guidelines. This study was approved by Medical Ethics Committee at Shahid Beheshti University of Medical Sciences, Tehran, Iran (Approved no: IR.SBMU.AEC.1401.022). In this study, 24 rats were randomly divided into the following 3 groups (8 rats in each group): (1) Control group, after incisions in the scalp to expose the skull, burr holes were drilled at the right lateral cerebral ventricle and punctured by the needle without injecting any solution. (2) Sham group, intracerebroventricular (ICV) injection of 2 µL normal saline, (3) *C. acnes* treated group, ICV injection of 2 µL bacteria with a concentration of 1.4×10^8 CFU/ml ($2.8\times10\text{^}5$ CFU) into the right lateral cerebral ventricle. In the previous study, we identified the cognition impairment in behavioral tests, and between 3 doses of *C. acnes*, 1.4×10^8 CFU/ml showed the higher impairment [19]. In order to identify any structural changes in the neuronal cells that were related to bacterial infection in the hippocampus, an immunohistochemistry analysis was conducted to evaluate the expression of Aβ, β-tubulin, and MAP2. Additionally, western blot was conducted to evaluate Tau and p-Tau to identify changes in axons. The workflow of this study is presented in Fig. 1.

Surgery and intracerebroventricular (ICV) injection

The animals were first anesthetized with a mixture of 100 mg/kg Ketamine hydrochloride and 25 mg/kg Xylazine (Sigma) after 12 h of fasting and 4 h of water

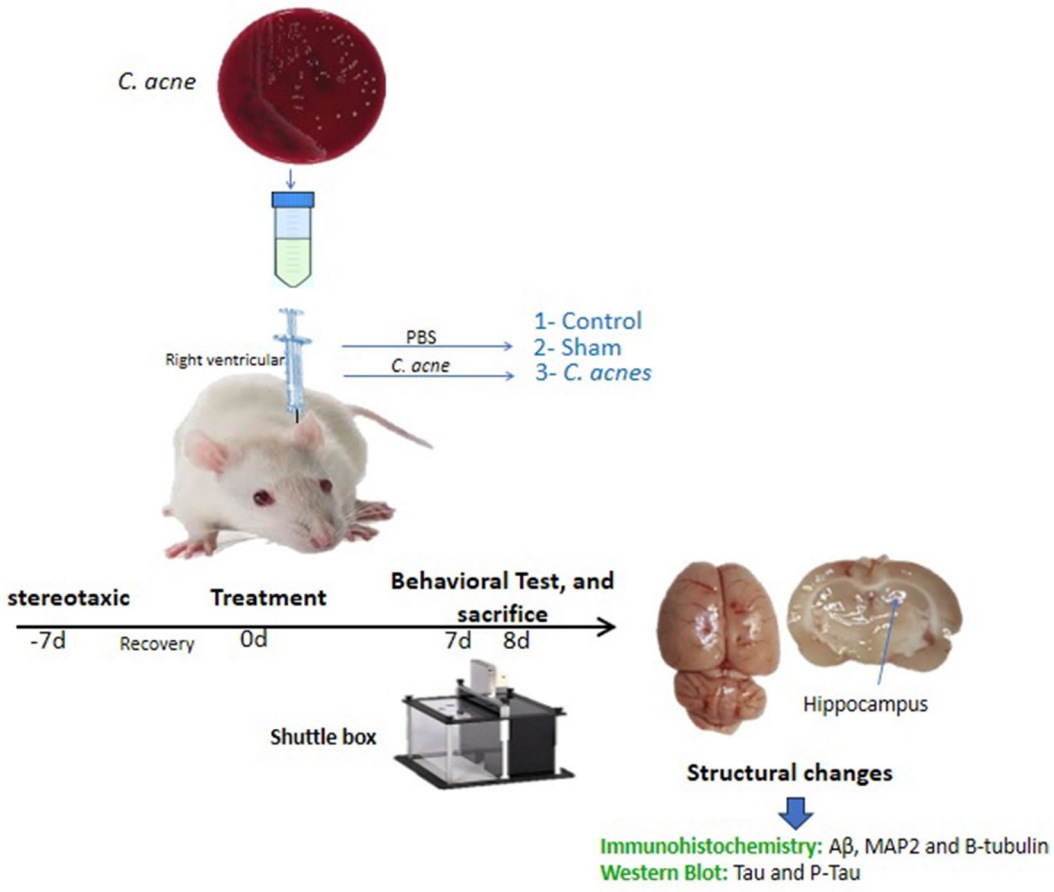


Fig. 1 Work flow of study contain 3 parts, preparation of *C. acnes*, injection ICV to animals and behavioral assess, and finally molecular study including IHC and western blot techniques

deprivation. They were then positioned in a stereotaxic instrument, and a cannula (22 Gauge) was inserted into the right ICV region as per Paxinos and Watson's atlas (Paxinos, 2007). The cannula was positioned at ML: 1.4 mm right of the midline, DV: 2.4 mm ventral, and AP: 0.8 mm posterior to bregma to the superior surface of the skull, and was fixed with jeweler's acrylic cement. The tip of the cannula was 1 mm above the microinjection site. After the surgery, the rats were allowed to recover from anesthesia and placed in a clean room. After 7 days, PBS and different doses of *C. acnes* solutions was injected using a set of polyethylene tubes (Single lumen PE50 tubing), a microsyringe (ILS, Stuetzerbach, Germany), and a dental needle (27 Gauge). The drug flow was established by the movement of an air bubble inside the polyethylene tube that attached the microsyringe and then to the dental needle. The solution was injected gently for over 1 min, and the needle was kept in position for an additional 60 s to prevent the backflow of the solution. After 7 days of *C. acnes* or PBS inoculation, some animals in each group were sacrificed for molecular studies, and the rest were assayed through behavioral test to evaluate the bacterial infection effect on memory performance.

Passive avoidance learning (PAL) test

The step-through passive avoidance apparatus was applied for measuring avoidance memory retention in rats [20]. The apparatus consists of two compartments, including a lighted chamber and a dark chamber with same size (20 cm x 20 cm x 30 cm) and separated by a guillotine door that blocks light. The chamber floors consist of stainless steel rods (3 mm diameter) that are spaced 10 mm apart. The bottom of the dark chamber is linked to an apparatus that generates electric shocks [21]. The test involved three steps, including a habituation step, an acquisition training step and a retrieval test. In the first step, animals were located for 10 min within the apparatus with the opened door and gate due to habituation. The acquisition trial was performed on the second day. In this step, the rats were allowed to acclimate in the light chamber for a short while. After one minute, the door to the chamber was raised, allowing the rats to move into the dark compartment. Once inside,

the door was closed, and a three-second delay occurred before an electrical shock (1 mA, 50 Hz) was delivered for three seconds. After 20 s, we moved the rats back to their cages. Two minutes later, we placed the rats in the light compartment. The acquisition trial was considered successful if the rat did not enter the dark compartment within 120 s. However, if the rat entered the dark compartment before the 120 s were up, it would receive the same shock again. Each rat was limited to a maximum of three foot shocks. On the third day, the retention trial was performed according to the same procedure as the acquisition trial but without electrical shock. In both the acquisition and retrieval trials, entry latencies time (sec) into the dark compartment were recorded for each Rat. The time spent in the dark compartment (TDC) was also noted in retrieval trial. Animals that failed to enter the dark compartment during the acquisition trial were excluded from the study.

Immunohistochemistry (IHC)

The brains of rats that were deeply anesthetized were removed from their skulls. They were then fixed with 4% paraformaldehyde and embedded in paraffin. We used three brain sections per animal. To prepare the brain tissue sections for staining, they were deparaffinized with fresh xylene and rehydrated using a series of graded ethanol. To efficiently expose epitopes to the antibodies, phosphate-buffered saline (PBS, pH=7.4) was used for antigen retrieval. The slides were then treated with 3% H_2O_2 for 10 min at 37℃ to reduce endogenous peroxidase activity. To avoid non-specific protein binding, the slides were blocked using 10% normal goat serum at room temperature for 1 h. The primary antibodies and their corresponding secondary antibodies (conjugated to HRP), including $A\beta_{1\text{-}42}$, (1:500, sc-28365 - Santa Cruz); MAP2, (1:500, Sc-74421- Santa Cruz); and β-tubulin, NBP1-62416 (1:400) were then incubated with the slides. Finally, the sections were subjected to staining procedures utilizing 3, 3′-diaminobenzidine (DAB, Zhongshan Biotech Co., Ltd, Beijing, China) for 5 min followed by a re-staining process with hematoxylin for 2 min. In addition, to visualize nuclei, cell nuclei in the sections were stained with DAPI dye at a dilution of 1:500 (Sigma-Aldrich). The stained sections were assayed using Labomed TCM 400 fluorescence microscope coupled with a digital camera. For each brain section, three images were taken from the hippocampus region. Investigation of protein expression was carried out using ImageJ Fiji software. For Aβ plaques, a threshold was applied to convert the images to binary, and the "Analyze Particles" function was used to count the plaques. For MAP2-positive neurons, the cell counter plugin was employed to manually count the neurons with visible MAP2 staining.

Western blot

Brain tissues were immediately removed, and both hippocampi were dissected and lysed. Proteins were separated by gradient SDS polyacrylamide gel electrophoresis (15%) and after which they were transferred to an Immobilon-P membrane by electroblotting according to the manufacturer's instruction (Invitrogen). Primary antibodies (purchased from Santa Cruz Biotechnology, United States) against Phospho-Tau (p-Tau) (PHF-13, sc32275) and Tau (sc32274), were incubated with the membranes overnight at 4 °C. After this step, the membranes were treated with Anti-Rabbit secondary antibody (1:3000, BA1054-2-bosterbio) for 2 h at room temperature. To ensure consistency in sample loading and protein transfer, and to standardize the levels of p-Tau and tau proteins, GAPDH was utilized.

Statistical analysis

GraphPad PRISM software (version 10) and Excel were utilized for statistical analysis. The data is presented as mean ± SD. For the statistical analysis of parametric data, one-way ANOVA was performed to compare several groups, and the Tukey test was used for comparing two groups. To analyze the non-parametric data, the Kruskal-Wallis test was used to compare multiple groups and Dunn's multiple comparisons test was used to compare two groups. A significance level of $P<0.05$ was considered statistically significant.

Results

C. acnes inoculated rats showed impairment in learning-memory performance

We used the PAL test to evaluate cognitive dysfunction and behavioral outcomes following bacterial infection in rats. In the PAL test, step-through latency (STL) in both the acquisition and retention trials was determined for control and sham rats and *C. acnes* treated groups. There were no significant differences in the STL between the groups during the acquisition trial. This indicates that the exploratory behavior of all three groups of rats was similar in the absence of electrical shock, as shown in Fig. 2A. However, during the retention trial, *C. acnes* infected animals showed a significant reduction in the transfer latency time into the dark chamber compared to the sham and control groups (Fig. 2B). This manifested a decrease in the learning-memory performance of the infected group. Infection by *C. acnes* also affected TDC, and animals spent more time in the dark compartment in comparison with the sham and control groups (Fig. 2 C). The infected group showed significantly higher TDC than the other groups ($p<0.05$).

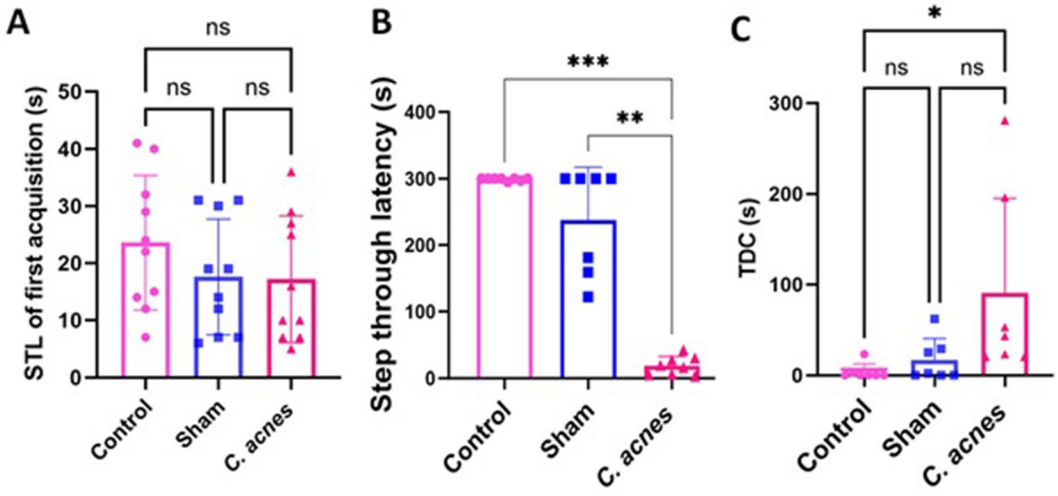


Fig. 2 Passive avoidance learning (PAL). (**A**) Step-through latency (STL) in the first acquisition trial. There were no significant differences in the STL between the groups during the acquisition trial. (**B**) STL in the retention trial. A significant reduction was observed in the transfer latency time of the *C.acnes* infected animals compared to the sham and control groups. (**C**) Time spent in the dark compartment (TDC). *C.acnes* infected groups exhibited a longer TDC compared to the other groups. The data distribution did not follow a normal pattern; hence, the Kruskal-Wallis test was used to analyze the three groups. To compare the differences between the two groups, Dunn's multiple comparisons test was applied. The results were obtained based on the mean ± SD, $n = 8$, ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$, ns = non-significant

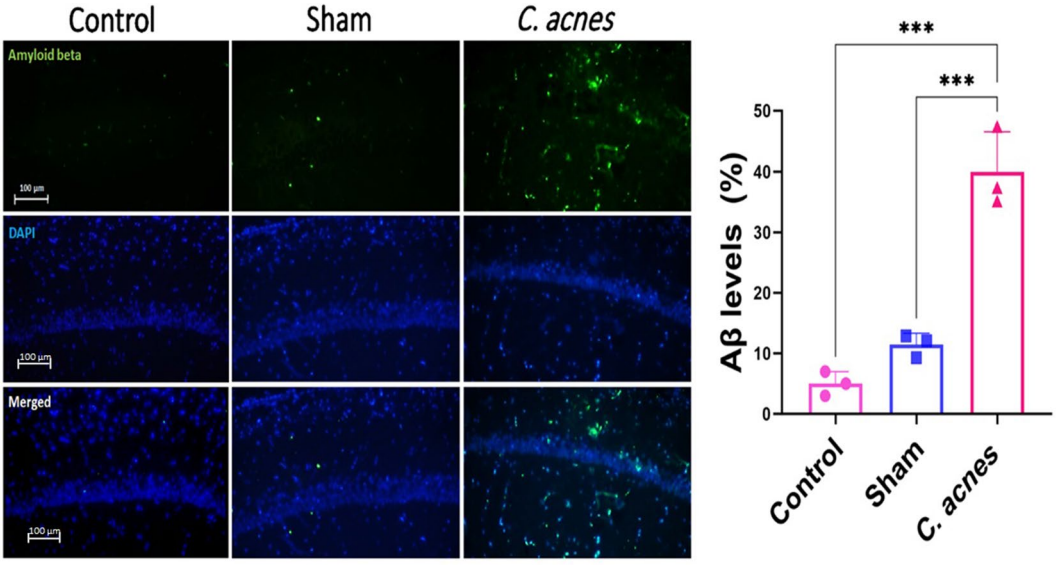


Fig. 3 Immunohistochemistry staining and quantification of $A\beta_{1\text{-}42}$ in the hippocampus in different groups, showing the significant increase in $A\beta_{1\text{-}42}$ deposition in *C. acnes* infected animals (100 µm). The samples were counterstained with DAPI (blue). All data are indicated as the mean ± SD ($n = 8$ for each group), ***$p < 0.001$

Histological assessments

Rats infected with C. Acnes undergo Aβ deposition in the brain

$A\beta_{1\text{-}42}$ oligomers are one of the prominent neuropathological markers of AD. Following 7 days after exposure to *C. acnes*, IHC analysis of rat brains using an anti-$A\beta_{1\text{-}42}$ antibody showed an accumulation of $A\beta_{1\text{-}42}$ in the infected hippocampus (Fig. 3). Bacterial treated rats showed remarkable amounts of $A\beta_{1\text{-}42}$ deposition, which are significantly higher than control, and sham groups. These results suggest that infection of brain with *C. acnes* in high doses enhances the amyloidogenic processing of Aβ protein precursor (APP) and Aβ deposition is occurred in the form of plaque-like structures.

C. acnes infection reduced expression of neuronal markers of wellbeing: β-tubulin and MAP2 in the hippocampus region

For visualizing neurons and quantifying the total number of cells in the hippocampus tissue, β-tubulin-positive and microtubule-associated protein 2 (MAP2)-positive

cells were investigated by IHC analysis. β-tubulin, a neuronal cytoskeleton protein, functions in many processes, such as structural support and is required to ensure cell viability [22]. MAP2 also is the predominant regulator of microtubules within dendrites of postmitotic neurons and is a marker of neuronal health [23]. In our study, we detected a significant decrease of β-tubulin, in animals infected with *C. acnes* compared to the sham ($P<0.001$) and control ($P<0.0001$) groups in the hippocampal regions (Fig. 4A). The same result was also observed with the IHC analysis of MAP2. In this case, as well, we observed a significant decrease in MAP2 expression after infection with *C. acnes* compared to the sham ($P<0.01$) and control ($P<0.001$) groups (Fig. 4B).

Phosphorylated tau levels in the *C. Acnes* infected rats were increased

Another key feature of AD neuropathology is hyperphosphorylated tau aggregation affecting the synaptic plasticity. Therefore, we analyzed the phosphorylation status of tau and total tau ratios at control, sham and *C. acnes* treated groups with western blot analysis. As shown in Fig. 5, after 7 days of infection of rats' brains with C. *acnes*, the level of p-Tau at hippocampus region was significantly increased compared to the sham ($P<0.01$) and control ($P<0.001$) groups (Fig. 5).

Effect of C. Acnes in disintegrating microtubules of axons and dendrites

The loss of microtubules from axons and dendrites is a significant factor in the degeneration of the nervous system in AD [24]. Our study shows that infection of the brain with *C. acnes* leads to the accumulation of Aβ plaques and neurofibrillary tangles, resulting in the degeneration of dendrites and axons in hippocampal neurons. The hyperphosphorylation of tau proteins can lead to the disassembly of microtubules in axons and dendrites by sequestering both normal tau and MAP2 [25]. Similarly, hyperphosphorylation of MAP2, a phosphoprotein, reduces its ability to promote microtubule assembly, contributing to neural degeneration and cognitive decline [25]. Overall, the infection of rat brains with *C. acnes* exhibited AD-like pathology and caused the dissociation of neural microtubules (Fig. 6).

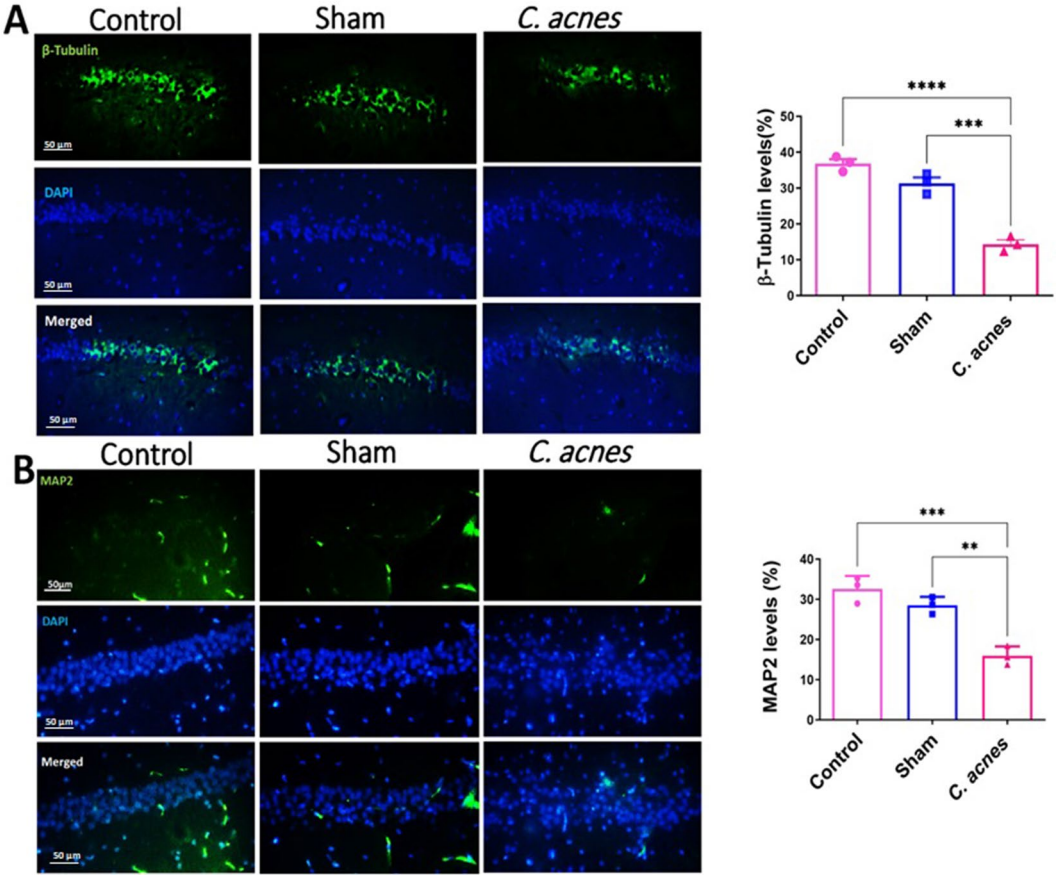


Fig. 4 Immunohistochemistry staining and quantification of β-tubulin (**A**) and MAP2 (**B**) in the hippocampus of infected animals compared to control and sham groups (50 µm). *C. acnes* infection decreases β-tubulin and MAP2 expression. The samples were counterstained with DAPI (blue). All data are indicated as the mean ± SD ($n=3$ for each group), ****$p<0.0001$, ***$p<0.001$ and **$p<0.01$

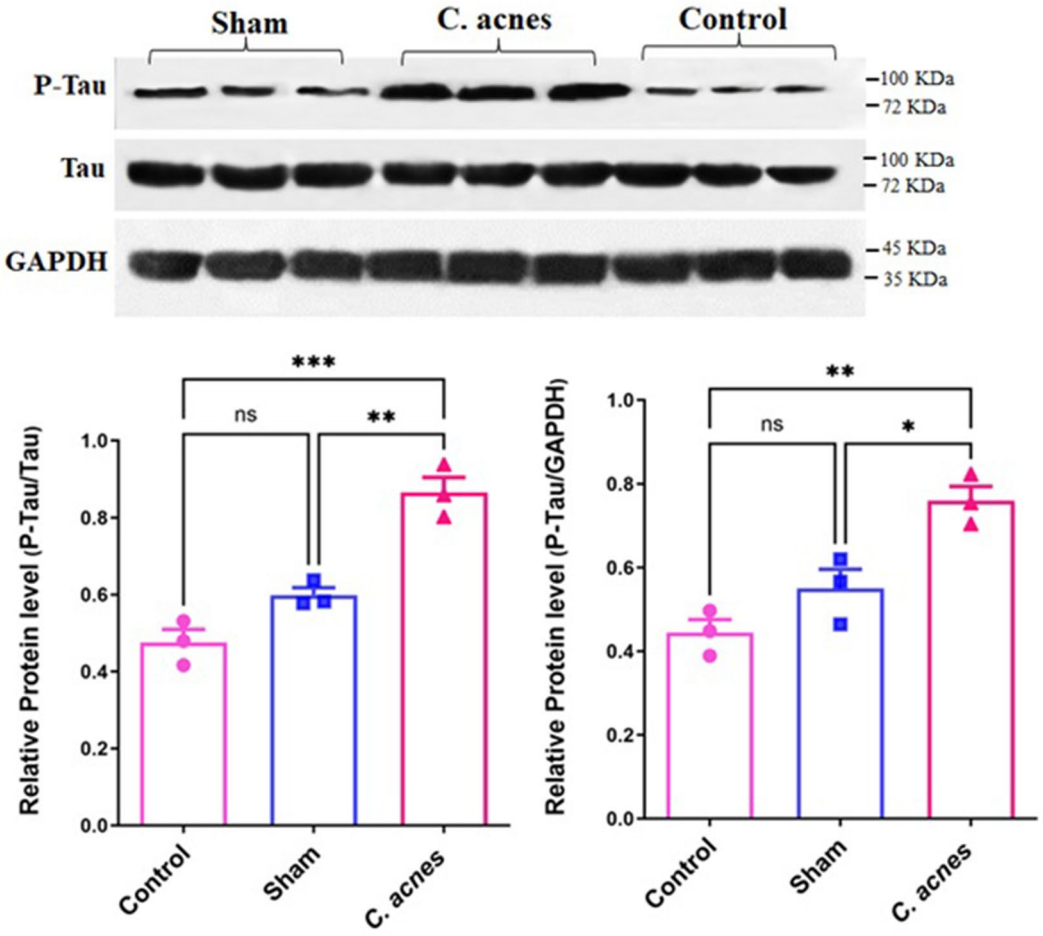


Fig. 5 Western blot analysis of p-Tau expression and quantification in the hippocampus of infected animals compared to control and sham groups. *C. acnes* infection leads to hyperphosphorylated tau deposition. All data are indicated as the mean ± SD ($n = 3$ for each group). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$

Discussion

Accumulating evidence suggests a connection between neurodegenerative disorders and alterations in microbial communities throughout the body, including the skin microbiota. According to previous studies, the multi-pathogen infections in the postmortem brain tissue of patients with various neurodegenerative disorders have been reported. *C. acnes* is one of the bacterial species detected in the brain of ALS [26], PD [27], HD [28], and AD [29, 30] patients. Nevertheless, there aren't clinical or preclinical studies to confirm the direct or indirect effect of *C. acnes* in the onset or progression of these diseases. In the present work, we focused and investigated the relationship between infection of rats' brains by *C. acnes* ATCC 6919 and the appearance of AD-like pathology by investigating memory retention, Aβ deposition, p-Tau aggregation, and neuronal cell survival in the hippocampus tissues. We opted to use the ATCC 6919 because it is a standardized, well-characterized, and extensively studied strain, facilitating comparison with other studies using the same strain. Moreover, as a whole-genome sequenced strain, ATCC 6919 provides access to its complete genetic information, facilitating the interpretation of our results and understanding the molecular mechanisms involved in the host-microbe interaction. The ATCC6919 strain is also known to be susceptible to antibiotics, enabling us to control bacterial growth and maintain a stable experimental environment [31]. In this study, we used local administration of *C. acnes* (1.4×10^8 CFU/ml) into the rats' hippocampus using stereotaxic injection. Seven days post infection, PAL results manifested that *C. acnes* infection declines cognitive performance. Infection with *C. acnes* did not affect the PAL acquisition but significantly reduced PAL retention that was conducted 24 h after the training. Moreover, infected animals spend more time in the dark compartment, indicating cognitive decline and memory deficits. Our recent study showed that *C. acnes* infection could promote neuroinflammation and expression of brain AD markers, and they contribute to cognitive

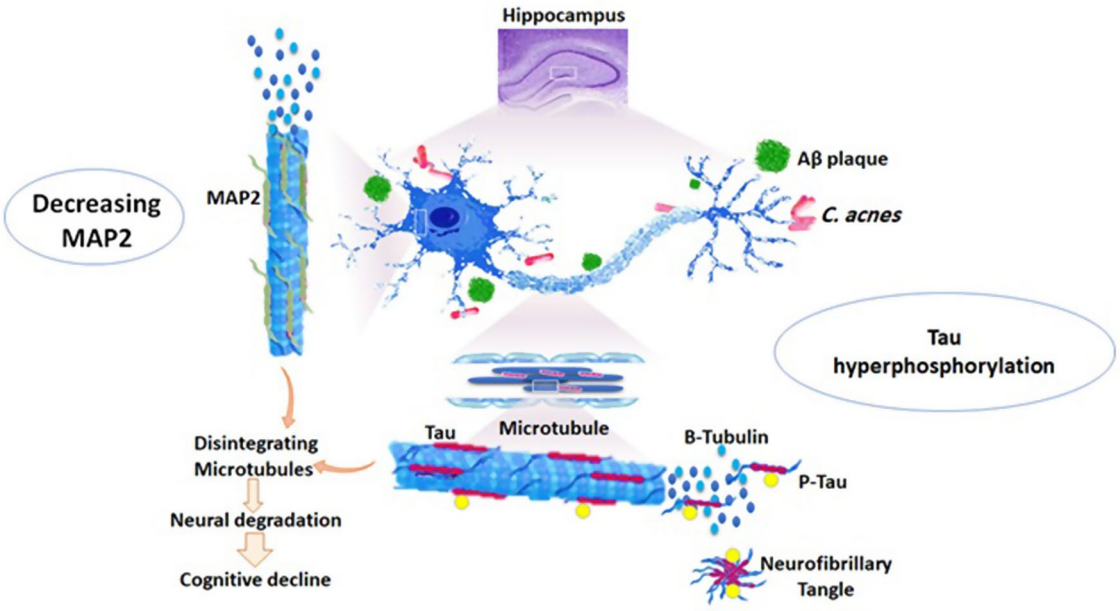


Fig. 6 The two main effect of *C. acnes* on dissociating microtubules through hyperphosphorylation of tau and MAP2, two crucial proteins in assembling microtubule in axons and dendrites

decline [19]. In agreement with our hypothesis, recently, *C. acnes* was suggested as a causative of AD and was founded in three out of four biopsies from the brain of postmortem Alzheimer's patients [29]. Moreover, it is well known that *C. acnes* can activate non-specifically the innate immune system by producing proinflammatory cytokine-inducing factors and chemotactic. *C. acnes* can stimulate complement pathways and secrets proteases, hyaluronidases and neuraminidases, which leads to epithelial permeabilization and inflammatory infiltration [29]. Therefore, following neuroinflammation, accumulation of Aβ peptides and neurofibrillary tangles can occur [32, 33]. In line with the our results, previous studies have demonstrated that various microbial components, such as lipopolysaccharide (LPS), a major endotoxin found in Gram-negative bacteria, can elicit inflammatory responses and augment the severity of postoperative cognitive dysfunction [34]. A recent study also demonstrated that systemic infection of mice by *Listeria monocytogenes*, a neuroinvasive bacterial pathogen, induces brain leukocytosis, resulting in a progressive decline in cognitive impairment. They found that $CD8^+$ T-lymphocytes, including $CD8^+$ tissue-resident memory T cells, are involved in the etiology of this impairment [35]. Our study ratified that exposure of the rats' brain to high dose of *C.acnes* stimulate the appearance of pathological hallmarks of AD in the hippocampus tissue, including Aβ deposition and hyperphosphorylation of tau. In contrast, the deposits observed in uninfected rats, including control and sham groups were mostly low, exhibiting a baseline level in uninfected animals. Moreover, according to previous studies infection the brain with other bacteria, such as *Chlamydia pneumoniae*may induces activation of astrocytes and amyloidosis [36]. One recent study, also confirmed the occurrence of neuroinflammation, cortical Aβ plaque deposition, elevated whole brain levels of p-Tau, and some behavioral deficits in a LPS-induced rat sepsis model [37]. Recently, *Porphyromonas gingivalis*, a periodontal bacterium, has been associated with AD pathogenesis due to its ability to worsen brain pathology in AD-transgenic mice, stimulate memory decline, and cause age-dependent neuroinflammation in middle-aged wild-type animals [38]. In another study, exposing mammalian neuronal and glial cells to LPS and the *Borrelia burgdorferi spirochetes* indicated Aβ deposition and increased the levels of AβPP and hyperphosphorylated tau [39]. Therefore, obtained results from our work and previous studies support the new concept that Aβ probably is an antimicrobial peptide and microbial infection enhances the synthesis of this antimicrobial agent [4]. There are different cellular and molecular mechanisms for the clearance of Aβ from the brain, but mainly growing evidence supports a key role for microglia, as brain macrophages, in the physiological engulfing of cerebral Aβ [40]. However, due to an imbalance between Aβ production and removal, as well as because of the aged and diseased microglia and loss of their functionality, misfolded Aβ begins gradually accumulating and depositing in plaques [41]. Moreover, persistent microglia activation and production of pro-inflammatory cytokines, such as IFNγ and TNFα, might impair their capacity to ingest all the Aβ and inhibit the production of Aβ-degrading

proteases [42]. On the other hand, pathogens or pattern recognition receptors (PRRs) such as the CD36, TLRs, and RAGE are activated in the presence of Aβ and stimulate a robust inflammatory response resulting in the overproduction of pro-inflammatory cytokines and free radicals following loss of neurons in the brain [43]. However, increasing evidence suggests that immune signaling not only results from protein aggregation in the brain, but also initiates the formation of aggregates during the early stages of the disease process [33]. Therefore, according to the mentioned concepts, we estimate that bacterial infection stimulates AD-like pathology through two mechanisms: (1) direct activation of the immune system resulting in Aβ accumulation; (2) stimulating the antimicrobial peptide Aβ production and following neuroinflammatory cascade responses and consequently neuronal death.

Other hypothesis related to the cognitive decline may contribute with structural changes in neurons. In parallel, the tau protein that stabilizes the shape and normal function of neuronal axons is hyper-phosphorylated during neuroinflammation processes and aggregates into neurofibrillary tangles leading to maul the structure of neuronal cells, disruption axonal outgrowth, synaptic degeneration, and consequently hippocampal neuronal death [44, 45]. In this study, we observed that p-Tau deposition was significantly increased in the hippocampus of bacterial infected animals. When tau proteins in the brain are abnormally hyperphosphorylated, they become detached from the microtubules they support, which can lead to the destabilization of those microtubules [46]. Moreover, the anomalous aggregation of tau protein hinders its ability to bind with microtubules, consequently affecting the dynamics of the microtubules [46]. In our study, we also found that MAP2 and β-Tubulin, two main neuronal markers, were significantly decreased when rats' hippocampus was subjected to C. *acnes.* This can be due to the aggregation of Aβ and neurofibrillary tangles that cause the degeneration of neurons. Tubulin, as the primary building block of microtubules, plays a critical role in cellular processes, like cell proliferation, neuronal migration, and laminar organization of cortical neurons for cortical development [47]. MAP2 is a central component of cross-bridges among microtubules in dendrites of post-mitotic neurons, which stabilizes microtubules and contributes to synaptic plasticity [48]. Similar to tau protein, the binding affinity of MAP2 to microtubules is altered by its phosphorylation state, and high levels of phosphorylation reduce the association of MAP2 with tubulin [46]. The reduced expression of these proteins leads to neuronal destruction. Indeed, MAP2, in altered expression, converts into granules, leading to neurotoxicity and neuronal death [49]. The loss of MAP2 and β-Tubulin has been widely linked to AD pathology [50]. Consequently, our results show that infection of the brain with *C. acnes* significantly disintegrates microtubules of axons and dendrites and disrupts neural cell structures. The limitation of this study was using *C. acnes* that is a very heterogenous bacterial species with many phylotypes and ribotypes, with very different virulence, surface properties and metabolic activity. In host-microbiota interaction studies using more representative strains can provide valuable insights into knowing disease etiology. For future investigations, we suggest isolate *C. acnes* strains from the brain of mild cognitive impairment (MCI) and AD patients to further explore the potential role of this bacterium in Alzheimer's disease development. This approach will enable us to compare different strains and understand the impact of *C. acnes* on brain health more comprehensively. One additional limitation of our study is the lack of assessment regarding the survival and metabolic state of *C. acnes* in the rat hippocampus post-infection. The detection of viable *C. acnes* in the brain tissues would provide evidence of bacterial survival and potential adaptation to the host environment. Moreover, to obtain more precise quantitative data on Aβ and MAP2 levels, Western blot or ELISA analysis on protein extracts is suggested.

Conclusion

The results of this study propose evidence that *C. acnes* infection of the brain of rats can play a major role in AD-like pathogenesis, which can be suggested as a causative agent for the occurrence of AD-like symptoms. Accumulating Aβ and p-Tau with a significant decrease in β-tubulin and MAP2 levels may indicate destabilization of the neuronal cytoskeleton network, leading to impairment of synaptic structure and function and, consequently, cell death. Further research is necessary to investigate the molecular mechanisms involved in bacteria-induced Aβ and p-Tau aggregations over a longer exposure time. This perspective provides a new landscape for designing novel approaches for the prevention or treatment of AD.

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Author contributions
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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval
All methods were performed in accordance with the relevant guidelines and regulations)IR.SBMU.AEC.1401.022(.

Consent for publication
Not applicable.

Competing interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Li H, et al. Amyloid, tau, pathogen infection and antimicrobial protection in Alzheimer's disease–conformist, nonconformist, and realistic prospects for AD pathogenesis. Translational Neurodegeneration. 2018;7:1–16.
2. Li F, Hearn M, Bennett LE. The role of microbial infection in the pathogenesis of Alzheimer's disease and the opportunity for protection by anti-microbial peptides. Crit Rev Microbiol. 2021;47(2):240–53.
3. Moné Y et al. Evidence for the Existence of a Bacterial Etiology for Alzheimers Disease and for a Temporal-Spatial Development of a Pathogenic Microbiome in the Brain. bioRxiv, 2022: p. 2022.08. 28.505614.
4. Vojtechova I, et al. Infectious origin of Alzheimer's disease: amyloid beta as a component of brain antimicrobial immunity. PLoS Pathog. 2022;18(11):e1010929.
5. Landry RL. M.E. Embers 2022 Does Dementia have a Microbial cause? Neuro-Sci 3 2 262–83.
6. Costa MJF, et al. Relationship of Porphyromonas gingivalis and Alzheimer's disease: a systematic review of pre-clinical studies. Clin Oral Invest. 2021;25:797–806.
7. Parra-Torres V, et al. Periodontal bacteria in the brain—implication for Alzheimer's disease: a systematic review. Oral Dis. 2023;29(1):21–8.
8. Perry A, Lambert P. Propionibacterium acnes: infection beyond the skin. Expert Rev anti-infective Therapy. 2011;9(12):1149–56.
9. Lu X, et al. Transcellular traversal of the blood-brain barrier by the pathogenic Propionibacterium acnes. J Cell Biochem. 2019;120(5):8457–65.
10. Zaffiri L, et al. Brain abscess induced by Propionibacterium acnes in a patient with severe chronic sinusitis. New Microbiol. 2013;36(3):325–9.
11. Burnham JP, et al. De novo meningitis caused by Propionibacterium acnes in a patient with metastatic melanoma. J Clin Microbiol. 2014;52(4):1290–3.
12. Emery DC et al. 16S rRNA next generation sequencing analysis shows bacteria in Alzheimer's post-mortem brain. Front Aging Neurosci, 2017: p. 195.
13. Kornhuber H. Propionibacterium acnes in the cortex of patients with Alzheimer's disease. Eur Arch Psychiatry Clin NeuroSci. 1996;246:108–9.
14. Piekut T, et al. Infectious agents and Alzheimer's disease. J Integr Neurosci. 2022;21(2):73.
15. Bukhari SI, et al. Efficacy of Novavit in ameliorating the neurotoxicity of propionic acid. Translational Neurosci. 2020;11(1):134–46.
16. MacFabe DF. Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. Microb Ecol Health Disease. 2012;23(1):19260.
17. Al-Orf N, et al. Therapeutic effects of probiotics on neurotoxicity induced by clindamycin and propionic acid in juvenile hamsters. Metab Brain Dis. 2018;33(6):1811–20.
18. Aliashrafi M, et al. Association of microbiota-derived propionic acid and Alzheimer's disease; bioinformatics analysis. J Diabetes Metabolic Disorders. 2020;19(2):783–804.
19. Aliashrafi M, et al. Intracerebroventricular Cutibacterium acnes generates manifestations of Alzheimer's Disease-like Pathology in the Rat Hippocampus. Neuroscience. 2024;540:103–16.
20. Labban S, et al. Effects of melatonin and resveratrol on recognition memory and passive avoidance performance in a mouse model of Alzheimer's disease. Behav Brain Res. 2021;402:113100.
21. Noorbakhshnia M, et al. Omega-3 fatty acids prevent LPS-induced passive avoidance learning and memory and CaMKII-α gene expression impairments in hippocampus of rat. Pharmacol Rep. 2015;67:370–5.
22. Bera A, Gupta ML Jr. Microtubules in microorganisms: how Tubulin Isotypes Contribute to Diverse Cytoskeletal functions. Front Cell Dev Biology. 2022;10:913809.
23. DeGiosio RA, et al. More than a marker: potential pathogenic functions of MAP2. Front Mol Neurosci. 2022;15:974890.
24. Jean DC, Baas PW. It cuts two ways: microtubule loss during Alzheimer disease. EMBO J. 2013;32(22):2900–2.
25. Alonso AdC, et al. Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. Proc Natl Acad Sci. 1997;94(1):298–303.
26. Alonso R, Pisa D, Carrasco L. Searching for bacteria in neural tissue from amyotrophic lateral sclerosis. Front NeuroSci. 2019;13:171.
27. Leheste JR, et al. P. acnes-driven disease pathology: current knowledge and future directions. Front Cell Infect Microbiol. 2017;7:81.
28. Alonso R, Pisa D, Carrasco L. Brain microbiota in Huntington's disease patients. Front Microbiol, 2019: p. 2622.
29. Emery DC, et al. 16S rRNA next generation sequencing analysis shows bacteria in Alzheimer's post-mortem brain. Front Aging Neurosci. 2017;9:195.
30. Kornhuber H. Propionibacterium acnes in the cortex of patients with Alzheimer's disease. Eur Arch Psychiatry Clin NeuroSci. 1996;246(2):108–9.
31. Zhang N, et al. Antimicrobial susceptibility, biotypes and phylotypes of clinical cutibacterium (formerly propionibacterium) acnes strains isolated from acne patients: an observational study. Dermatology Therapy. 2019;9:735–46.
32. Webers A, Heneka MT, Gleeson PA. The role of innate immune responses and neuroinflammation in amyloid accumulation and progression of Alzheimer's disease. Immunol Cell Biol. 2020;98(1):28–41.
33. Zhang W, et al. Role of neuroinflammation in neurodegeneration development. Signal Transduct Target Therapy. 2023;8(1):267.
34. Fidalgo AR, et al. Systemic inflammation enhances surgery-induced cognitive dysfunction in mice. Neurosci Lett. 2011;498(1):63–6.
35. Cassidy BR, et al. Progressive cognitive impairment after recovery from neuroinvasive and non-neuroinvasive Listeria monocytogenes infection. Front Immunol. 2023;14:1146690.
36. Little CS, et al. Chlamydia pneumoniae induces Alzheimer-like amyloid plaques in brains of BALB/c mice. Neurobiol Aging. 2004;25(4):419–29.
37. Kirk RA, et al. Lipopolysaccharide exposure in a rat sepsis model results in hippocampal amyloid-β plaque and phosphorylated tau deposition and corresponding behavioral deficits. Geroscience. 2019;41:467–81.
38. Díaz-Zúñiga J, et al. Alzheimer's disease-like pathology triggered by Porphyromonas gingivalis in wild type rats is serotype dependent. Front Immunol. 2020;11:588036.
39. Miklossy J, et al. Beta-amyloid deposition and Alzheimer's type changes induced by Borrelia spirochetes. Neurobiol Aging. 2006;27(2):228–36.
40. Zuroff L, et al. Clearance of cerebral Aβ in Alzheimer's disease: reassessing the role of microglia and monocytes. Cell Mol Life Sci. 2017;74:2167–201.
41. Sehar U, et al. Amyloid beta in aging and Alzheimer's disease. Int J Mol Sci. 2022;23(21):12924.
42. Merighi S, et al. Microglia and Alzheimer's disease. Int J Mol Sci. 2022;23(21):12990.
43. Solito E, Sastre M. Microglia function in Alzheimer's disease. Front Pharmacol. 2012;3:14.
44. Fulop T, et al. Can an infection hypothesis explain the beta amyloid hypothesis of Alzheimer's disease? Front Aging Neurosci. 2018;10:224.

45. Otero-Garcia M, et al. Molecular signatures underlying neurofibrillary tangle susceptibility in Alzheimer's disease. Neuron. 2022;110(18):2929–48. e8.
46. Zhang J, Dong X-P. Dysfunction of microtubule-associated proteins of MAP2/tau family in prion disease. Prion. 2012;6(4):334–8.
47. Romaniello R, et al. Tubulin genes and malformations of cortical development. Eur J Med Genet. 2018;61(12):744–54.
48. Harada A, et al. MAP2 is required for dendrite elongation, PKA anchoring in dendrites, and proper PKA signal transduction. J Cell Biol. 2002;158(3):541–9.
49. Impellizzeri D, et al. Açai Berry mitigates Vascular Dementia-Induced neuropathological alterations modulating Nrf-2/Beclin1 pathways. Cells. 2022;11(16):2616.
50. Santiago-Mujika E, et al. Tubulin and tubulin posttranslational modifications in Alzheimer's disease and vascular dementia. Front Aging Neurosci. 2021;13:730107.

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