Review

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BDNF-altering cell death mechanisms of brain disorders: pyroptosis and/or ferroptosis? A systematic review

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Abstract: The brain plays a central role in controlling and regulating bodily activities. Given its significance, the brain needs to remain in optimal condition to function properly. Neurodegenerative diseases arise when the mechanisms maintaining brain homeostasis fail, resulting in prolonged and uncontrolled activation of cell death pathways. Increasingly, researchers are focusing on specific types of cell death, such as ferroptosis and pyroptosis, due to their potential as targets for preventing and treating neurodegenerative diseases. Brain-derived neurotrophic factor (BDNF) is a crucial growth factor for neurogenesis, neuronal survival, and maintenance, and is thus implicated in cell death mechanisms. This review aims to elucidate the role of BDNF in the ferroptosis and pyroptosis mechanisms that impact brain health. To achieve this, three databases – PubMed, Scopus, and Web of Science – were searched for relevant studies, yielding 95 articles, of which only 14 were aligned with the study's aim. Ferroptosis (iron-dependent cell death) and pyroptosis (inflammatory cell death) are distinct modes of cell death; however, this review found both mechanisms are associated with oxidative stress. Consequently, the findings suggest that ferroptosis and pyroptosis collectively impact brain health conditions such as depression, cognitive deficiencies, and anxiety, which are notably linked to reduced BDNF levels.

Keywords: ferroptosis; pyroptosis; cell death; brain-derived neurotrophic factor; brain health

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Introduction

Cell death, survival, proliferation, and differentiation are all common processes in our bodies, and each one is critical to our overall health. These processes are also affected by increasing age [1–3]. Cell death is crucial for maintaining homeostasis in the body by removing damaged cells [4]. The first three categories used to categorize cell death were Type I (apoptosis), Type II (autophagy), and Type III (necrosis) [5]. In recent years, numerous novel cell death modalities, such as ferroptosis and pyroptosis, have been discovered and described in terms of corresponding stimuli, molecular mechanisms, and morphologies. Some of these modalities share signal paths that are overlapping yet not identical. Ferroptosis was first proposed in 2012 as a non-apoptotic, iron-dependent method of cell death characterized by the accumulation of lipid reactive oxygen species (ROS) [6]. Recent studies have demonstrated that ferroptosis plays a crucial regulatory role in the onset and progression of many diseases, and it has since become the focus and a hotspot of research on the treatment and prognosis improvement of related disorders [7]. In addition to ferroptosis, pyroptosis has recently gained an increasing amount of interest due to its link to innate immunity and illness. In 2001, coined the term pyroptosis, combining two Greek words, pyro (fire/fever) and ptosis (to-sis, falling), to describe pro-inflammatory

cell death [8]. This process distinguished pyroptosis from other types of cell death, such as apoptosis (non-inflammatory program cell death). Pyroptosis research grew in popularity after the Gasdermins family was discovered. Previous research has linked the mechanisms of pyroptosis and ferroptosis to lower levels of brain-derived neurotrophic factor (BDNF) [9, 10]. This reduction in BDNF adversely affects brain health, contributing to cognitive impairment, depression, and anxiety, as BDNF is a crucial growth factor essential for neurogenesis, neuronal survival, and maintenance [11]. However, previous studies did not explain how BDNF is involved in the two mechanisms. Additionally, crosstalk between ferroptosis and pyroptosis has been reported in cases of COVID-19 [12] and autoimmune diseases [13], but no studies have examined this interaction in the context of brain health. Therefore, the purpose of this review is to elucidate the role of BDNF in the mechanisms of ferroptosis and pyroptosis and to explore the relationship between these mechanisms and brain health.

Materials and methods

This systematic review was conducted according to Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [14]. This review summarizes pre-clinical studies that evaluated ferroptosis and pyroptosis mechanisms in various conditions that affect brain health, especially changes in the level of brain-derived neurotrophic factor (BDNF) level.

Eligibility criteria

Animal (rodent species) and cell culture studies that investigated ferroptosis and pyroptosis mechanisms with various exposure and/or inducers to develop a model that affects brain health, especially memory and cognitive ability, as well as the changes in BDNF level were eligible for inclusion. Only experimental studies, original research, and English articles were included in this review. Studies on humans, books, editorials, reviews, and articles with no author, title, or abstract were excluded from this review.

Data sources and search strategy

Three electronic databases (PubMed, Scopus, and Web of Science) were searched for relevant articles published from inception up to the third week of February 2023. The search string used is as follows: "(BDNF OR brain-derived neurotrophic factor) AND (pyroptosis OR ferroptosis)". The appropriate BOOLEAN operators and MeSH terms were used in developing the keywords for the search strategy. All the results from these three databases were extracted to a citation manager software namely EndNote 20 to remove duplications and to screen the relevant articles.

Study selection and data extraction

Studies identified through a literature search underwent a two-phase selection procedure. In the initial phase, two reviewers (AH and FW) screened the titles and abstracts of identified articles. In the subsequent phase, articles were selected based on pre-specified eligibility criteria. Any disagreements were resolved through consensus between the two reviewers or by consulting a third reviewer (NFJ). During this phase, the following variables were extracted: author names, year of publication, study design, model used (animal and cell), investigated mechanisms (ferroptosis and/or pyroptosis) related to brain health deterioration, and outcome measured (BDNF expression and level). Data extraction was carried out by two reviewers (AH and FW) using a standardized form developed by AH using Google Forms. This form consisted of eight short-answer questions: 1) article title, 2) author names, 3) year of publication, 4) type of investigated mechanism (ferroptosis or pyroptosis), 5) study design, 6) experimental methods, 7) findings, and 8) emerging issues/notes. Subsequently, the extracted information was simplified and summarized in Table 1. Any discrepancies between reviewers were resolved through discussion.

Risk of bias and calculations

The Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE) 10-item bias risk assessment checklist was used to examine each of the articles that were included. With the use of SYRCLE's Risk of Bias Tool-a screening tool created for risk of bias evaluation in animal research [27], potential biases impacting the chosen publications were assessed. A "yes" (green) score means a low potential for bias, a "no" (red) score means a high chance of bias, and an "unclear" (yellow) score means an unknown risk of bias. Two reviewers (AH and FW) independently assessed the quality of the included studies. Any discrepancies were resolved by discussion or by consulting a third reviewer (NFJ). A risk-of-bias summary table, generated automatically in Review Manager version 5.3, is included as (Figure 1). This

Table 1: Role of BDNF in modulating ferroptosis and pyroptosis mechanisms in brain health.

Author	Study type	Study design & subject characteristics	Important findings
Role of BDNF in ferroptosis mechanisms in brain health			
An et al. [15]	Experimental	Animal study 10 male non-diabetic littermate mice (4 weeks of age) and 20 male diabetic mice (4 weeks of age) were used; and divided into three groups (n=10): i. Non-diabetic group ii. Diabetic group iii. Diabetic group treated with liraglutide (LIRA)	1) Diabetic condition-induced cognitive impairment in mice: increased escape latency and distance (Morris water maze test), decreased spatial memory (Y-Maze) via ferroptosis: significantly decreased ferritin heavy chain (FTH) and ferroportin-1 (FPN1), significantly increased transferrin receptor-1 (TFR1) (increased intracellular free iron), increased iron deposition in the hippocampus (Perls' staining), increased malondialdehyde (MDA) level in mice hippocampus (lipid peroxidation), decreased superoxide dismutase (SOD) and glutathione (GSH) in mice hippocampus (oxidative stress). 2) BDNF protein level reduced; caused decreased synaptophysin (SYN) and postsynaptic density protein (PSD95) (synaptic plasticity impairments) and decreased neuron survival (Nissl staining).
Cao et al. [16]	Experimental	Animal study This study used 3–4 months old 129S1/SvIm mice; divided into two group (n=8–11): i. Control group ii. Chronic unpredictable mild stress (CUMS) group	1) Mice treated with CUMS- induced depression-like symptoms (Sucrose preference test and forced swimming test) and anxiety-like symptoms (open field test) but no significant symptoms of cognitive impairment (Morris water maze test). 2) CUMS promotes depression and anxiety-like symptoms via 1) ferroptosis; increased ferritin heavy chain-1 (FTH1), ferritin light chain-1 (FTL1), and Fth1/Ftl1 ratio (excess iron), increased malondialdehyde level (lipid peroxidation), decreased glutathione level (oxidative stress), and 2) necroptosis; increased RIPK3 and phosphorylated- MLKL. 3) BDNF protein level was reduced after CUMS exposure.
Yang et al. [10]	Experimental	Animal study 30 female C57BL/6 mice (12-month-old); divided into three groups (n=10): i. Control group ii. Sleep deprivation (SD) group iii. Ketogenic diet (KD) group	1) SD-induced cognitive impairment; increased escape latency and reduced time spent in the target quadrant (Morris water maze test) via ferroptosis; increased expression iron regulatory protein-1, leading to increased transferrin receptor-1 and DMT1, decreased ferritin heavy chain-1 and ferroportin-1 (intracellular iron increase), increased iron deposition (Perls' staining), increased malondialdehyde level (lipid peroxidation) and decreased glutathione level, sirtuin-1 and p-Nrf2/Nrf2 (oxidative stress). 2) Low-level BDNF protein accompanied by low expression and protein level of doublecortin (DCX) (reduced neurogenesis).
Wang et al. [17]	Experimental	Animal study Adult male ICR mice 2 months old (25–30 g); divided into four groups (n=10): i. Control group ii. Chronic sleep-deprived (SD) group iii. SD + Medium- chain triglyceride- enriched ketogenic diet (MKD) group iv. SD + long-chain triglyceride- enriched ketogenic diet (LKD) group	1) SD-induced cognitive impairment; increased escape latency and reduced time spent in the target quadrant (Morris water maze test) via ferroptosis; increased transferrin receptor-1, decreased ferritin heavy chain-1 and ferroportin-1 (increased iron content), increased malondialdehyde level (lipid peroxidation), decreased super oxidative dismutase, glutathione level, sirtuin-1 and Nrf2 (oxidative stress). 2) Low-level BDNF protein; caused decreased protein levels of synaptophysin (SYN) and postsynaptic density protein 95 (PSD95) (synapse impairment).
Role of BDNF in pyroptosis mechanisms in brain health			
Chai et al. [18]	Experimental	Animal and cell study 1) Male C57BL/6 mice (8 weeks old, 20–22 g); randomly divided into five groups (n=10):	1) CORT/LPS-induced depression (Sucrose preference test and forced swim test) through pyroptosis; via activation of P2X7/NF-kB signaling pathway, then lead to activate

Table 1: (continued)

Author	Study type	Study design & subject characteristics	Important findings
		i. Control group ii. Corticosterone (CORT)/lipopolysaccharide (LPS)- injection group iii. CORT/LPS + fluoxetine treatment group iv. CORT/LPS + 20 mg/kg salidroside (Sal) group v. CORT/LPS + 40 mg/kg Sal group 2) PC12 cells that were cultured in DMEM with 10 % FBS; randomly divided into six groups: i. Control group ii. 200 μM CORT group iii. 10 μM Nigericin (Nig) group iv. 200 μM CORT + 50 μM Sal group v. 10 μ Nig + 50 μM Sal group vi. 200 μM CORT + 50 μM Sal + 10 μM Nig group	NLRP3 inflammasomes, cleaved caspase-1, cleaved gasdermin-D and release of inflammatory cytokines such as Interleukin-1β and Interleukin-18. 2) The protein level of BDNF was also reduced in the group that was treated with CORT.
Muhammad et al. [19]	Experimental	Animal study The study used 56 male Wistar rats (30–33 days old; 105 g–120 g); randomly divided into five groups: i. Control group, n=10 (1 mL/day; p.o saline) ii. Chronic unpredictable stress (CUS) + saline group, n=13 iii. CUS + Escitalopram (Escita) group, n=10 iv. CUS + dapagliflozin (Dapa) group, n=10 v. CUS + BQ788 + Dapa group, n=13	1) Chronic unpredictable stress (CUS) procedure-induced depression-like symptoms (Sucrose preference test and forced swim test) via pyroptosis; increased NF-kB, mRNA expression of NLRP3, caspase-1 activity, Interleukin-1β and Interleukin-18. 2) CUS reduced BDNF protein level; causing decreased synapsin-1 content (synapse impairment).
Taha et al. [20]	Experimental	Animal study 48 male Albino rats (180–230 g) divided into four groups (n=12): i. Control group ii. Cerium oxide nanoparticles (CeNPs) group (35 mg/kg CeNPs solution via gastric tube- daily) iii. Doxorubicin (DOX) group (DOX at dose 2 mg/kg/week at 0, 7, 14 and 21 days of the experiment via intraperitoneal) iv. DOX + CeNPs (2 mg/kg/week DOX via i.p. + 35 mg/kg CeNPs orally)	1) DOX-induced cognitive impairment; increased escape latency and decreased time spent at the target quadrant (Morris water maze) via 1) apoptosis; increased the expression of cytochrome c protein and caspase-3, 2) pyroptosis; increased NLRP3 gene expression and caspase-1. 2) DOX also increased malondialdehyde (lipid peroxidation), decreased glutathione, superoxide dismutase, and catalase (oxidative stress). 3) The protein expression of BDNF and its receptor, tropomyosin receptor kinase B (TrkB) significantly decreased. The protein expression of neurotransmitters, such as serotonin and dopamine decreased.
Tan et al. 2020 [21]	Experimental	Animal and cell study 1) 96 Male Sprague-Dawley rats (280–300 g) were divided into 6 groups, n=12 (cerebral ischemia-reperfusion (CIR) injury established by middle cerebral artery occlusion (MCAO) model): i. Control group (treat with normal saline) ii. Lexiscan (Lex) group iii. Hydroxysafflor (HSYA) (10 mg/kg) group iv. HYSA (20 mg/kg) group v. Lex-HYSA (10 mg/kg) group iv. Lex-HYSA (20 mg/kg) group 2) Cells used in this study were PC12 cells (the grouping was the same as the animal groups)	1) MCAO model-induced pyroptosis; increased the expression of NLRP3, caspase-1, gasdermin-D, interleukin-1β and interleukin-18. Decreased BDNF protein level. 2) HYSA ameliorates pyroptosis by elevated BDNF protein level.
Tan et al. [22]	Experimental	Animal study Male Sprague-Dawley (387–463 g); randomly divided into three groups (n=15): i. Sham group ii. Control/Cardiac arrest CA) group iii. CA + Beta-hydroxybutyrate (HB) group	1) Cardiac arrest (CA)-induced neurological injury and cognitive dysfunction; reduced spatial memory (Y-maze) via o) Oxidative stress; increased average optical density (AOD) of 8-OHdG and increased ROS level (ROS assay kit) l) Lipid peroxidation; increased AOD of 4- HNE, a) Apoptosis; increased caspase-3, 4) pyroptosis; increased caspase-1 and gasdermin-D. 2) Cardiac arrest downregulates BDNF protein levels. Beta-HB ameliorates neurological injury after CA by upregulating BDNF protein levels.

Table 1: (continued)

Author	Study type	Study design & subject characteristics	Important findings
Tang et al. [23]	Experimental	Animal study 20 months old adult male C57/BL were divided into five groups, n=10: i. Sham group ii. Abdominal exploratory laparotomy (AEL) iii. AEL + low dose VRT-043198 (1 mg/kg, 0.5 mL) iv. AEL + medium dose VRT-043198 (10 mg/kg, 0 5 mL) v. AEL + high dose VRT-043198 (100 mg/kg, 0 5 mL)	1) Abdominal exploratory laparotomy was performed to stimulate perioperative neurocognitive disorders (PND) – induced cognitive impairment; increased escape latency and decreased time spent at the target quadrant (Morris water maze) via pyroptosis; increased caspase-1, interleukin-1β and interleukin-18. 2) PND- reduced BDNF protein level. 3) VRT-043198 alleviated abdominal exploratory laparotomy-induced cognitive dysfunction by suppressing pyroptosis via inhibition of caspase-1 activity and upregulated BDNF protein levels.
Wang et al. [9]	Experimental	Animal study Male C57BL/6 mice (5–6 weeks old, 20–25 g); (n=8 per group): i. Sham group ii. Amyloid-beta (1–42) oligomers (AβO) group iii. AβO + Astragaloside (AS)-IV 10 mg/kg/day, i.g. iv. AβO + AS-IV 20 mg/kg/day i.g. v. AβO + AS-IV 40 mg/kg/day i.g. vi. AβO + donepezil (5 mg/kg/day i.g.) vii. AβO + AS-IV 20 mg/kg/day i.g. + GW9662 (1 mg/kg/day i.p.)	1) Amyloid-beta infusion (AβO) produced Alzheimer's disease (AD) – like phenotype- induced pyroptosis; upregulating NLRP3, caspase-1, and interleukin-1β. 2) AβO reduced BDNF protein level; caused reduced postsynaptic density protein (PSD95) and synaptophysin (SYN) (synapse impairment). 3) AS-IV inhibited pyroptosis induced by AβO via promoting BDNF promoting expression.
Xiong et al. [24]	Experimental	Animal study Adult male C57BL/6 mice (10 weeks old, weighing 20–25 g); randomly divided into four groups, (n= 9–15): i. Control group ii. Control + modafinil (MD) group iii. Sleep deprivation (SD) group iv. SD + MD group	1) SD-induced cognitive impairment; decreased time spent in the target quadrant (Morris water maze) via pyroptosis; increased level of NLRP3, gasdermin-D, and caspase-1 protein expressions and reduced BDNF protein level. 2) MD inhibited pyroptosis and increased BDNF protein level.
Zhao et al. [25]	Experimental	Cells and animal study 1) Cell culture Primary hippocampal neurons and astrocytes were isolated from the Sprague- Dawley rat hippocampus; cells were divided into four groups: i. Control Group-culture in normal condition ii. Oxygen-glucose deprivation/reoxygenation (OGD/R) iii. OGD/R + negative control (NC) group iv. OGD/R + zinc finger E-Box binding homeobox 2 (ZEB2) group 2) Animal Sprague- Dawley rats (weighing 240–270 g); divided into three groups, (n=6): i. Sham group ii. Middle cerebral artery occlusion (MCAO) group iii. MCAO + ZEB2 group	1) MCAO model-induced cognitive impairment; decreased time spent in target quadrant (Morris water maze) via pyroptosis; increased NLRP 3, caspase-1, gasdermin-D, interleukin-1β and interleukin-18. BDNF protein level was also reduced in this model. 2) ZEB2 decreased inflammation and pyroptosis by astrogliosis and increased BDNF protein level.
Dong et al. [26]	Experimental	Animal study Sprague-Dawley rats aged 12 months (350–500 g) induced focal cortical infarction by distal middle cerebral artery occlusion (MCAO). Rats were divided into three groups, n=12: i. Sham group ii. MCAO + vehicle group iii. MCAO + VX-765 therapy group	1) MCAO model-induced cognitive impairment; increased escape latency and decreased time spent in the target quadrant (Morris water maze) via pyroptosis; increased NLRP 3, caspase-1, gasdermin-D, interleukin-1β, and interleukin-18. 2) MCAO model-reduced BDNF protein level; caused decreased postsynaptic density protein (PSD95) and synaptophysin (SYN) (synapse impairment). 3) VX-765 ameliorates cognitive impairment by inhibiting pyroptosis and increasing BDNF protein by improving synaptic plasticity.

BDNF, brain-derived neurotrophic factor; RIPK3, receptor-interacting protein kinase 3; MLKL, mixed lineage kinase domain-like pseudokinase; DMT1, divalent metal transporter 1; Nrf2, nuclear factor erythroid-2, related factor 2; P2X7, purine receptor P2X7, NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, nucleotide-binding domain, leucine-rich- containing family, pyrin-domain- containing 3; DMEM, dulbecco's modified eagle medium.

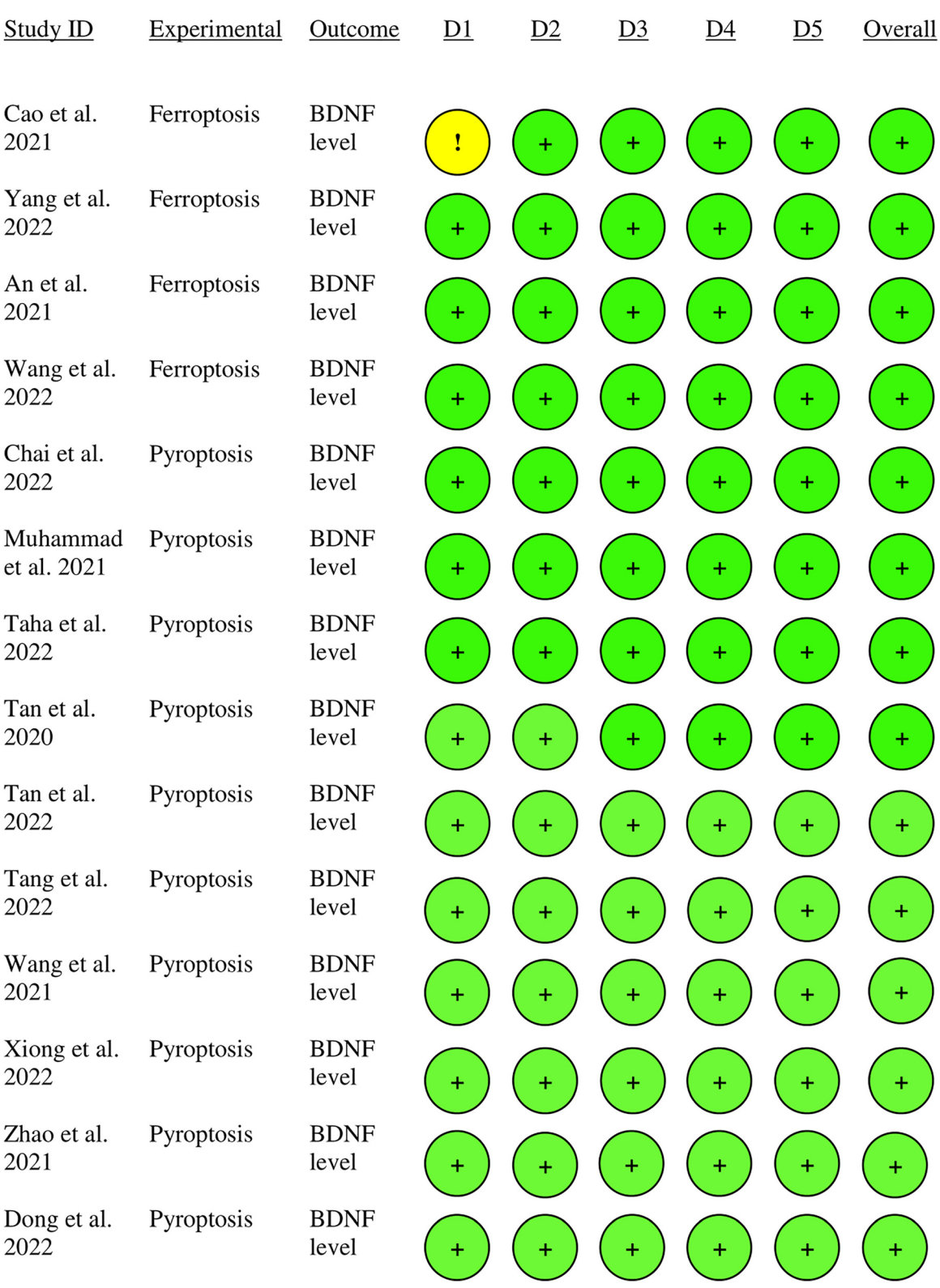

Figure 1: Risk of bias in individual studies. The (+) indicates "low risk" of bias and the (!) indicates "some concerns" of bias. This risk of bias summary table was created automatically by review manager, version 5.3. This risk of bias summary table consists of 5 domains; 1) randomization process (D1), 2) deviations from intended interventions (D2), 3) missing outcome data (D3), 4) measurement of the outcome data (D4), and 5) selection of the reported result (D5).

Review Manager tool encompasses five domains: 1) randomization process, 2) deviations from intended interventions, 3) missing outcome data, 4) measurement of the outcome data, and 5) selection of the reported result. These domains are utilized to evaluate the risk of bias in the articles included in this review.

Results

Study selection and data extraction

The electronic search performed yielded 95 articles in total with 18 articles from PubMed, 60 articles from Scopus, and 17 from Web of Science. After removing 33 duplicates, 62 articles were screened based on title and abstract. Of the 62 articles, 29 were screened further because 32 were unrelated articles and 1 was not an English article. The full text of 29 articles was screened and 15 articles were excluded for the following reasons: Twelve review articles, one chapter in a book, and two editorial articles. Finally, only 14 articles were included in this systematic review. The study selection process is summarized using a flowchart as shown in (Figure 2). Included study characteristics are summarized in (Table 1).

Study characteristics

All animal studies that used rodent species regardless of age and gender, also cell cultured studies that related to brain health were included. Animal and cells model related to brain health problems developed with various types of procedures and induction types including chronic unpredictable mild stress (CUMS) procedure, middle cerebral artery occlusion (MCAO) procedure, abdominal exploratory laparotomy, oxygen-glucose deprivation, sleep deprivation, diabetic models, and induced neurotoxic substances [lipopolysaccharide (LPS), doxorubicin (DOX) and amyloid beta oligomers (AβO)]. Only pyroptosis and ferroptosis studies with notable changes in BDNF level were included regardless of induction and procedures period.

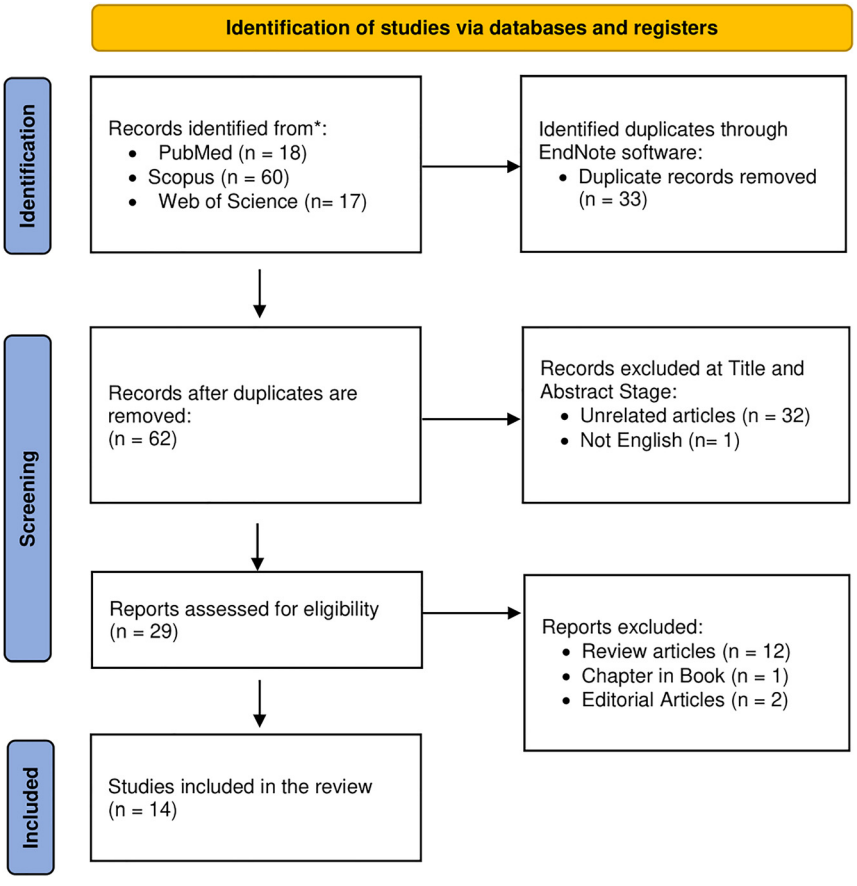

Figure 2: The study selection process is summarized using the PRISMA flowchart.

Results of individual studies

Ferroptosis

Four studies on ferroptosis were included in this systematic review. The findings indicate that ferroptosis is associated with several changes in iron metabolism in the hippocampus, including decreased ferritin heavy chain-1 [10, 15, 17], increased ferritin heavy chain-1 [16], increased ferritin light chain-1 [16], reduced ferroportin-1 [10, 15, 17], and increased transferrin receptor-1 [10, 15, 17]. These alterations contribute to iron accumulation in the hippocampus. All four studies demonstrated that ferroptosis mechanisms lead to cognitive deficiencies (n=3) [10, 15, 17], anxiety, and depression (n=1) [16] in mouse models. Additionally, a significant decrease in hippocampal BDNF levels was detected via western blot analysis. These pathological changes are not only due to ferroptosis but also involve redox imbalances. Specifically, the studies noted lower levels of superoxide dismutase (SOD) [15, 17] and glutathione (GSH) [10, 15–17], as well as higher levels of malondialdehyde (MDA), an indicator of lipid peroxidation [10, 15–17].

Pyroptosis

This systematic review includes 10 studies on pyroptosis. The findings from these studies indicate that pyroptosis in the hippocampus is characterized by increased levels of NLRP3 [9, 18–21, 24–26], caspase-1 [9, 18–26], gasdermin-D [18, 21, 22, 24–26], interleukin-1β [9, 18, 19, 21, 23, 25, 26], and interleukin-18 [18, 19, 21, 23, 25, 26]. According to Chai et al. and Muhammad et al., pyroptosis contributes to depression [18, 19]. Chai et al. also found a decrease in BDNF expression and protein levels in the hippocampus using immunohistochemistry and western blot analysis [18]. Similarly, Muhammad et al. [18] reported reduced BDNF protein levels in the hippocampus using enzyme-linked immunoassay (ELISA) [18]. In addition to depression, Tan et al. reported that pyroptosis leads to nerve injury and a significant reduction in hippocampal BDNF expression, as observed through immunofluorescence analysis [21]. Zhao et al. found that pyroptosis also results in neuronal loss and decreased BDNF protein expression in the hippocampus, detected by immunofluorescence analysis [25]. Most studies conclude that pyroptosis leads to cognitive deficits [9, 20, 22–24, 26], with decreased hippocampal BDNF protein levels confirmed by western blot analysis [22–24, 26]. Additionally, Taha et al. and Wang et al. reported reduced BDNF protein levels in the hippocampus through ELISA and immunohistochemistry analysis, respectively [9, 20]. Overall, the pyroptosis mechanism leads to brain health deterioration, resulting in depression (n=2), neuronal loss (n=2), and cognitive deficiency (n=6). These impairments are all associated with decreased levels of BDNF protein and expression in the hippocampus, as detected by various analyses.

Risk and bias assessments

All studies in this review were assessed for the risk of bias by using the SYRCLE tool (Figure 1). Thirteen studies that clearly stated randomization in grouping subjects were attributed to “Low risk” of bias while one study that unclearly stated randomization in grouping subjects was attributed to “Some concerns” of bias for the Domain 1 or D1 (randomization process). All studies in this systematic review do not show any deviations from the intended interventions, and because of that, all studies included in this review were attributed “Low risk” of bias for D2. All studies in this review present sufficient data whether in tables, graphs, or illustrations, and explained the outcome based on the data presented, therefore all studies in this review were attributed “Low risk” of bias in D3 (missing outcome data). Other than that, all studies in this review used appropriate analysis to measure the outcome as explained in the methodology, because of that all studies are attributed a “Low risk” of bias for D4. For the last domain which is D5, all studies in this review are attributed as “Low risk” in the selection of the reported result. Overall, the risk of bias for all included studies was considered as low.

Discussion

Stress [16], sleep deprivation [10, 17], and diabetes [15] all had different effects on ferroptosis mechanisms. All of these effects can lead to neurodegeneration caused by ferroptosis and have an impact on brain health. Hepcidin levels in type 2 diabetes mellitus (T2DM) decreased, causing changes in ferroportin regulation, which resulted in iron accumulation in the brain [15]. Iron accumulation can also be caused by the activation of iron regulatory proteins (IRPs) [10, 15, 17]. The binding of iron regulatory proteins (IRPs) with iron-responsive elements (IREs) results in the activation of transferrin receptor 1 (TfR1) and reduced iron storage which is ferritin, this process will lead to iron accumulation [28].

In some cases, ferritin levels are high to indicate excess iron in the intracellular [16]. Both conditions; 1) decreased

clearance of iron due to reduced ferritin and 2) increased ferritin levels, indicate excess iron levels in the intracellular and lead to neuron damage [29]. Iron accumulation is more than just a process that contributes to ferroptosis mechanisms. In ferroptosis, an increase in reactive oxygen species (ROS) also contributes to the neurodegenerative process.

In ferroptosis mechanisms, reactive oxygen species (ROS) increase. This is because iron accumulation in ferroptosis mechanisms initiates the Fenton reaction, which increases ROS [10, 15–17]. The Fenton reaction is a reaction that occurs between iron and hydrogen peroxide. This reaction generates hydroxide and hydroxyl radicals leading to an increase in ROS [30]. Increased ROS levels will cause lipid degradation via the lipid peroxidation process [31]. This process contributes to neuronal loss in ferroptosis mechanisms by increasing malondialdehyde (MDA) levels [10, 15–17], a known marker of lipid peroxidation. This process is referred to as non-enzymatic lipid peroxidation [32].

Additionally, there is an enzymatic lipid peroxidation mechanism involving high expression of acyl-coenzyme A (CoA) synthetase long-chain family member 4 (ASCL4) [15, 17, 32]. Polyunsaturated fatty acids (PUFAs) are more prone to oxidation, so increased PUFA synthesis resulted in increased susceptibility to lipid peroxidation [33]. ASCL4 was involved in the PUFA synthesis mechanism by adding coenzyme A (CoA) to PUFAs. Then, lysophosphatidylcholine acyltransferase 3 (LPCAT3) transfers PUFAs-CoA into phospholipids, which are then oxidized by arachidonate lipoxygenase (ALOX) enzyme resulting in increased MDA lipid peroxidation in ferroptosis [34, 35]. MDA lipid peroxidation is a hallmark of ferroptosis mechanisms, and it can alter the structure and function of cell membranes, resulting in cell dysfunction and extensive tissue damage [36].

Lipid peroxidation worsens when the system x_c^- cysteine/glutamate antiporter and Sirt1/Nrf2 signaling pathway do not function properly in the ferroptosis mechanism [10, 15–17] XCT/SLC7A11 is a light-chain subunit and CD98hc/SLC3A2 is a heavy-chain subunit, both composed of system x_c^- cysteine/glutamate antiporter [37]. This antiporter is responsible for the uptake of extracellular cysteine and the transport of intracellular glutamate as an exchange. Cysteine is required for the synthesis of glutathione (GSH) and glutathione peroxidase 4 (GPX4), both of which function to reduce ROS and lipid peroxidation [38]. Sirt1/Nrf2 signaling can also reduce ROS. Sirtuin 1 (Sirt1) can regulate the transcription of nuclear erythroid factor 2-related factor 2 (Nrf2), which leads to the activation of antioxidant genes such as superoxide dismutase (SOD) and the reduction of ROS [39].

A decrease in brain-derived neurotrophic factors (BDNF) levels, in addition to iron accumulation, increased ROS levels, and lipid peroxidation, may also contribute to neurodegenerations caused by ferroptosis mechanisms. Reduced BDNF levels resulted in synapse impairment [15–17] since these studies report on the reduced protein levels of synaptophysin (SYN) and postsynaptic density protein 95 (PSD95). SYN is a glycoprotein that is present in the membrane of neuron synaptic vesicles [40] and PSD95 is a protein complex that is attached to the postsynaptic membrane [41], so the decreased level of both proteins indicated that synapse signaling is affected. Other than that, reduced BDNF protein levels also caused reduced in neurogenesis [10], as this study reports the doublecortin (DCX) expression and protein level decreased. DCX is a marker for young neurons [42].

Other than ferroptosis, pyroptosis is also a mode of cell death that can cause neurodegenerations. There are various implications of pyroptosis mechanism activation such as depression [18], stress [19], brain ischemia [21, 25, 26], sleep deprivation [24], perioperative neurocognitive disorders [23], Alzheimer's disease [9], chemo fog [20], and cardiac arrest [22]. Based on the findings from all these studies, there was a significantly increased level of NLRP3 inflammasomes which affect brain health. Several mechanisms contribute to NLRP3 inflammasome overactivation-induced pyroptosis. The P2X7 receptor, a subgroup of the P2X family found on cell membranes, is abundant in many neurons and immune cells [43], while nuclear factor kappa B (NF/kB) is a key transcriptional activator of the NLRP3 inflammasomes [19, 20]. ATP activation of the P2X7 receptor results in the activation of nuclear factor kappa B (NF-B), which results in the activation of NLRP3 inflammasome-induced pyroptosis mechanisms [18].

Aside from that, oxidative stress can activate NLRP3 inflammasomes [21, 22]. The activation of the NLRP3 inflammasome may be upstream of the pyroptosis mechanism, but gasdermin-D (GSDMD) may be the true executor of pyroptosis-induced neuronal loss. The gasdermins, a class of pore-forming proteins, have recently been linked to the immune response [44]. Gasdermin-D (GSDMD) particularly, is one of the six identified paralogous genes of the gasdermin family [18, 44]. The activation of NLRP3 inflammasomes will result in the activation of caspase-1, which will then activate GSDMD-induced pore formation in neuron cells, resulting in neurodegeneration [18, 21, 22, 25]. Because of this function, GSDMD is known as the pyroptosis executor.

Besides activation of NLRP3 inflammasomes and GSDMD, BDNF regulation may also be linked to pyroptosis mechanisms. This is because all the studies included in this review reported that BDNF levels were found to be decreased as NLRP3 inflammasome expression increased. Other than that, Tropomyosin receptor kinase B (TrkB) protein level also decreased as reported by [20]. TrkB is a receptor for BDNF and its signaling maintains neuronal survival, synapse plasticity, and neuronal growth [45]. Reduced BDNF caused reduced synaptophysin (SYN) and postsynaptic density protein 95 (PSD 95) protein levels, indicating synapse impairment [9, 26]. Reduced synaptophysin (SYN) also leads to a reduction in the secretion of neurotransmitters protein levels such as serotonin and dopamine [20].

Furthermore, the synapsin-1 protein level also decreased as reported by [19], synapsin-1 also important for synapse maintenance since this protein is associated with the cytoplasmic surface of synaptic vesicles [46]. Based on these findings, the signaling of BDNF and TrkB receptors may be involved in the pyroptosis mechanism pathway by causing synapse impairment and leading to neurodegenerations.

Ferroptosis and pyroptosis are two distinct modes of cell death. Based on the articles included in this review, ferroptosis is an iron-dependent form of cell death initiated by iron accumulation in the brain. This process leads to oxidative stress and increased lipid peroxidation at the cell membrane, ultimately resulting in cell death. In contrast, pyroptosis is a pro-inflammatory form of cell death that begins with activating the inflammasome, subsequently activating caspase-1. Activated caspase-1 then activates pro-inflammatory cytokines (interleukin-1β and interleukin-18), and the cleavage of gasdermin-D (GSDMD). The activation of GSDMD causes pore formation in the cell membrane, allowing the release of activated pro-inflammatory cytokines through these pores, ultimately leading to cell death.

Although ferroptosis and pyroptosis are different types of cell death, studies included in this review suggest the possibility that ferroptosis and pyroptosis may occur simultaneously or sequentially, contributing to brain disorders, such as in cases of COVID-19 [12] and autoimmune diseases [13]. There is a possibility that both mechanisms are involved together in some of the studies included in this review, particularly in conditions such as stress and sleep deprivation. Stress has been shown to cause depression-like symptoms, as reported by two studies [16, 19]. These studies indicate the involvement of different mechanisms: one study reports the involvement of ferroptosis [16], while the other implicates pyroptosis [19]. Sleep deprivation has been linked to cognitive impairment through both ferroptosis mechanisms [10, 17] and pyroptosis mechanisms [24]. However, none of the studies included in this review have investigated the concurrent occurrence of both ferroptosis and pyroptosis and their combined impact on brain health.

Additionally, both ferroptosis and pyroptosis lead to a decrease in BDNF levels, resulting in neurodegeneration, particularly in the hippocampus. This observation strengthens the suggestion that ferroptosis and pyroptosis may occur simultaneously or sequentially, contributing to brain disorders. Ferroptosis, an iron-dependent cell death mechanism, increases ROS in the intracellular environment (oxidative stress), which leads to increased MDA lipid peroxidation. Increased ROS can also stimulate the activation of pyroptosis mechanisms [20, 22], suggesting a potential interaction between these pathways. The hippocampus, which is crucial for learning and memory, is particularly vulnerable to such oxidative stress and inflammation. The reduction in BDNF, a key neurotrophic factor in the hippocampus, exacerbates synaptic dysfunction and neuronal loss. However, more research is needed to elucidate the specific pathways through which ferroptosis and pyroptosis lower BDNF levels and contribute to hippocampal neurodegeneration. All the possible pathways are summarized and visualized in Figure 3.

Strength and limitations

The strength of this systematic review comprises the uniformity of outcomes reported by the included studies indicating that BDNF level and expression may play a role in ferroptosis and pyroptosis. However, the precise mechanism and pathways involved remain unknown.

Conclusions

In conclusion, BDNF may play a role in ferroptosis and pyroptosis mechanisms, but more research is needed to determine its precise role. It should be noted that BDNF itself has different forms; proBDNF and mature BDNF, and each one activates distinct cell signaling pathways, resulting in two different fates for neurons; death or survival. The findings of the selected articles in this review only reported that BDNF levels were decreased in these two modes of cell death mechanisms; however, it is still unclear how BDNF is involved in mediating these mechanisms. Both mechanisms are thought to be linked and could regulate neurodegeneration, whether simultaneously or sequentially. Further investigation into the relationship between ferroptosis and pyroptosis is highly anticipated.

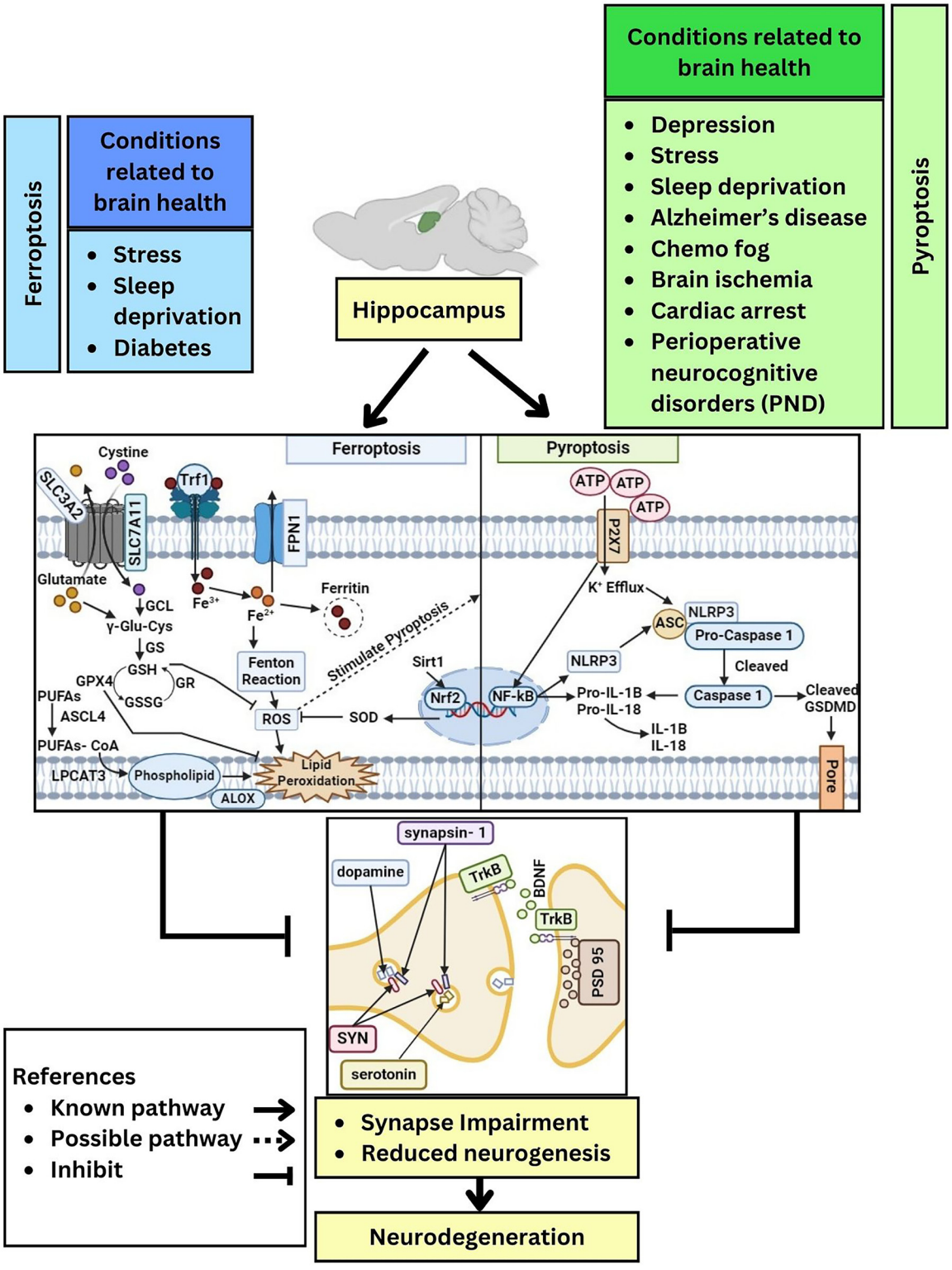

Figure 3: A diagram that summarizes the level of BDNF in ferroptosis and pyroptosis mechanisms. Image created with BioRender.com. This Figure illustrates the relationship between ferroptosis and pyroptosis mechanisms and their impact on various brain health conditions. The conditions related to brain health include depression, stress, sleep deprivation, Alzheimer's disease, chemo fog, brain ischemia, cardiac arrest, and perioperative neurocognitive disorders (PDN). Both ferroptosis and pyroptosis contribute to synapse impairment and reduced neurogenesis in the hippocampus, ultimately leading to neurodegeneration.

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