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Research article

THE EFFECTS OF PERINATAL PHENCYCLIDINE ADMINISTRATION ON THE DENSITY AND BRANCHING OF ASTROCYTES IN THE BRAIN OF AGED RATS

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Phencyclidine (PCP), an antagonist of the N-methyl-D-aspartate glutamate receptor (NMDA-R), is used for modeling schizophrenia in animals, as NMDA-R hypofunction is vital in its pathophysiology. Recent studies have shown that glial fibrillary acidic protein (GFAP), an astrocyte marker, is altered in the prefrontal cortex and in certain hippocampal regions in individuals with schizophrenia. To our knowledge, no study has examined long-term effects (in aged rats) of perinatal PCP treatment on the density and branching of astrocytes. The aim of this study was to compare the density and branching of astrocytes in the dentate gyrus (DG), CA1 and CA2/3 regions of the hippocampus, and in the medial prefrontal cortex (mPFC) of 18-month-old rats treated perinatally with PCP compared to saline (NaCl) control group. Male Wistar rats (n=6) were treated with PCP (10 mg/kg) on postnatal days (PND) 2, 6, 9 and 12, while the control group (n=6) received NaCl on the same PNDs. All rats were sacrificed at 18 months of age (PND540). Astrocytes were visualized using GFAP immunohistochemistry. Results show that astrocyte density in PCP-treated rats was significantly lower compared to the NaCl group in DG ($p<0.01$) and CA2/3 regions ($p<0.01$) and mPFC ($p<0.05$). There were no changes in the branching of the astrocytes in any of the structures investigated. Our finding of a significant decrease in astrocyte density in the hippocampus and mPFC in aged rats perinatally treated with PCP, indicates that astrocytes may be involved in the morphological and functional impairments in these brain regions, caused by NMDA-R dysfunction.

Keywords: Ageing; Astrocytes; Glial fibrillary acidic protein – GFAP; Phencyclidine; Schizophrenia

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INTRODUCTION

Schizophrenia is a severe mental disorder with a global prevalence of approximately 1 % [1]. The symptomatology of patients with schizophrenia and related spectrum disorders is diverse and can be classified into positive, negative, and cognitive domains [2]. Cognitive deficits in these patients result in significant impairments in daily functioning across all life domains, and the treatment of affected individuals imposes a substantial financial burden on healthcare systems worldwide [3]. The precise pathogenetic mechanisms underlying schizophrenia remain incompletely elucidated, although several hypotheses, most notably the dopaminergic and glutamatergic, have been proposed to explain the pathophysiology of this disorder [4,5].

Animal models of psychiatric disorders, including schizophrenia, are invaluable tools for investigating the pathophysiological mechanisms of human disorders [6]. Phencyclidine (PCP), a dissociative anesthetic, is an antagonist of N-methyl-D-aspartate receptors (NMDA-R) in the brain. It has been shown that the use of PCP in humans leads to the appearance of positive and negative symptoms as well as cognitive dysfunction that are characteristic of schizophrenia. This is why PCP is utilized to model schizophrenia in animal studies. [7]. The perinatal administration of PCP in rats offers significant advantages over acute or subchronic administration in adult animals by encompassing the neurodevelopmental aspects of the disorder [8]. The first two weeks of postnatal development in rats correspond to the second trimester of human gestation [9]. It is during this critical neurodevelopmental period that disruptions are believed to occur in humans, forming the basis of the pathogenesis of schizophrenia, with clinical manifestations of the disorder emerging later in life [10].

Very few studies have investigated the role of astroglial cells in the pathogenesis of schizophrenia, as it was previously believed that they served only as supportive cells for neurons. Today, it is known that astrocytes have numerous functional roles and play a vital part in a wide range of neuropsychiatric disorders. Astrocytes express a wide range of receptors, transporters, enzymes, and ion channels, enabling them to regulate and maintain the homeostasis of ions, neurotransmitters, reactive oxygen species, and pH levels [11]. Astrocytes are closely linked to synapses, where they play an active role in synaptic transmission by synthesizing, buffering, and recycling neurotransmitters, as well as secreting neuromodulators [12]. Additionally, astrocytes communicate directly with blood vessels and, through their end-foot processes, contact the pia mater [13]. In recent years, a particularly relevant theory has emerged, suggesting that inflammation during the neurodevelopmental period predisposes individuals to the development of schizophrenia, with astrocytes potentially playing a vital role in this process. Reactive astrogliosis associated with inflammation leads to a loss of astrocytes' critical role in regulating glutamatergic transmission, which includes the uptake of glutamate and the maintenance of its low extracellular concentration [14]. Excessive accumulation of glutamate has an excitotoxic effect on neurons. It is hypothesized that a combination of insufficient clearance and excessive glutamate release by astrocytes

during their immune activation may result in aberrant extrasynaptic signaling through ionotropic and metabotropic glutamate receptors, ultimately leading to persistent synaptic dysfunction [15]. In studies examining changes in astroglia, results have been inconsistent. Hayzans and associates [16] observed increased astrocyte density following subchronic PCP treatment in rats, measured 7 days after treatment. In post-mortem studies of humans, a reduction in astroglial density has been noted [17]. Furthermore, it has been recently observed that the decline in cognitive flexibility and plasticity in schizophrenia patients during aging is due to the separation of cells from synaptic neuron and astrocyte program (SNAP), which consists of astrocytes and glutamatergic (excitatory) and GABAergic (inhibitory) neurons [18]. To the best of our knowledge, the morphological changes of astroglia in aged rats have not been studied in animal models of schizophrenia. Therefore, we specifically focused on the long-term changes in astroglia in 18-month-old rats perinatally treated with PCP.

The hippocampus is a key region of the limbic system, essential for regulating cognition and memory functions. It is characterized by an exceptionally high concentration of NMDA receptors, which facilitates the high degree of plasticity necessary for declarative learning and memory – the two mental functions that are significantly impaired in individuals with schizophrenia [19]. The hippocampal formation comprises the dentate gyrus (*gyrus dentatus*) and the hippocampus proper (Ammon's horn), which is subdivided into three fields: CA1, CA2, and CA3. The CA2 and CA3 regions are often considered together as CA2/3 due to the ambiguous anatomical boundary between them and the relatively small size of the CA2 region. Damage to the prefrontal cortex in humans and primates is associated with many of the negative and cognitive symptoms observed in schizophrenia [20]. It has been demonstrated that cognitive performance and memory processes are linked to the remodeling of pyramidal dendritic spines in both the hippocampus and prefrontal cortex, with the loss of these contributing to cognitive dysfunction [21]. As far as we know, no studies have investigated the long-term effects of perinatal PCP treatment on astroglial morphology. The objective of this study was to assess changes in the density and branching of astrocytes using immunohistochemical staining of glial fibrillary acidic protein (GFAP), a marker of astrocytic cytoskeleton, in the dentate gyrus, CA1 and CA2/3 regions of the hippocampus, and the medial prefrontal cortex (mPFC) of 18-month-old (PND540) rats that were perinatally treated with phencyclidine.

Working hypothesis**:** The density and branching of GFAP-positive astrocytes in the brain of 18-month-old rats perinatally treated with phencyclidine differs from that in age-matched control animals perinatally treated with physiological saline.

MATERIALS AND METHODS

For this experiment, 12 male Wistar rats were used, sourced from the breeding facility at the Institute for Biomedical Research Galenika in Belgrade. Male rats were selected specifically due to the observed sexual dimorphism in response to PCP [22].

All experimental procedures adhered to the NIH Guide for the Care and Use of Laboratory Animals, with efforts to minimize animal suffering. The study received ethical approval from the Faculty of Medicine Ethics Committee, University of Belgrade, under decision number 1322/IX – 48, dated September 29, 2022.

Twelve hours after birth, the animals were combined and then randomly assigned to lactating females to eliminate litter-specific differences and to examine the effects of PCP in a genetically heterogeneous group [23]. The subjects were randomly divided into an experimental group (n=6) and a control group (n=6). They were treated on postnatal days (PND) 2, 6, 9, and 12, with the experimental group receiving phencyclidine (10 mg/kg) and the control group receiving saline solution (NaCl 0.9 %) [24-26]. Phencyclidine hydrochloride (Sigma, St. Louis, MO) was dissolved in physiological saline (NaCl 0.9 %) and administered subcutaneously in the interscapular region. The control animals received NaCl in the same volume as the PCP solution, using the same administration method [27]. The pups were housed in their respective litters until postnatal day 30 (PND30), after which the males were separated from the females. The animals were maintained in cages (2–3 animals per cage) under controlled environmental conditions throughout the study, with a 12-hour light/dark cycle (lights on at 07:00).

The animals were sacrificed at 18 months of age (PND540). The rats were first anaesthetised with 3.6 % chloral hydrate and then perfused with 4 % paraformaldehyde (250 ml). Following perfusion and decapitation, brain structures were isolated and fixed in paraformaldehyde solution for 24 hours, further transferred to sucrose solutions with increasing concentrations (10 %, 20 %, 30 %), and kept for 24 hours in each. The brains were sectioned using a cryostat (Leica Instruments, Germany) into 40 μm thick slices. The sections were stored submerged in an antifreeze solution at -20 °C until staining.

Immediately before immunohistochemical staining, sections of the medial prefrontal cortex (approximately Bregma 3.2mm) and hippocampus (approximately Bregma – 4.1mm) were chosen for analysis. A 3 % hydrogen peroxide (H_2O_2) solution was applied to block endogenous peroxidase activity in the tissue. Following this, the samples were incubated for 1 hour at room temperature in normal goat serum (NGS) to block non-specific sites. The sections were then incubated for 24 hours at room temperature with a primary anti-GFAP rabbit polyclonal antibody (Agilent Cat# Z0334, RRID: AB_10013382), followed by a 1-hour incubation with a complementary secondary antibody (Vectastain Vector, UK). For visualization, the commercial Vectastain ABC Kit (HRP) (Vector Laboratories, UK) was used, which includes a secondary antibody conjugated with biotin and an avidin-biotin complex linked to horseradish peroxidase (HRP). The sections were then incubated with 3,3'-diaminobenzidine (DAB), a chromogenic substrate for HRP, which undergoes a reaction producing a brown reaction product at the site of the target antigen. Between each steps, samples were washed three times for 5 minutes in PBS (phosphate-buffered saline, pH=7.3). The sections were transferred to SuperFrost Plus slides (Menzel Braunschweig, Germany).

Following this, they were counterstained with Mayer's hematoxylin, dehydrated through increasing ethanol concentrations (70 %, 96 %, 100 %), and cleared in xylene. Finally, the sections were mounted with a cover slip using DPX (Sigma-Aldrich, Germany).

Stained sections of the hippocampus and medial prefrontal cortex were photographed using a Leica DM 4000 B LED microscope with a Leica DFC295 digital camera, employing the Leica Application Suite (LAS, 4.4.0) software system at 10x magnification. A total of 12 histological slides with a total of 24 sections (6 sections of the hippocampus and 6 sections of the mPFC from both groups of animals) were photographed (Figure 1).

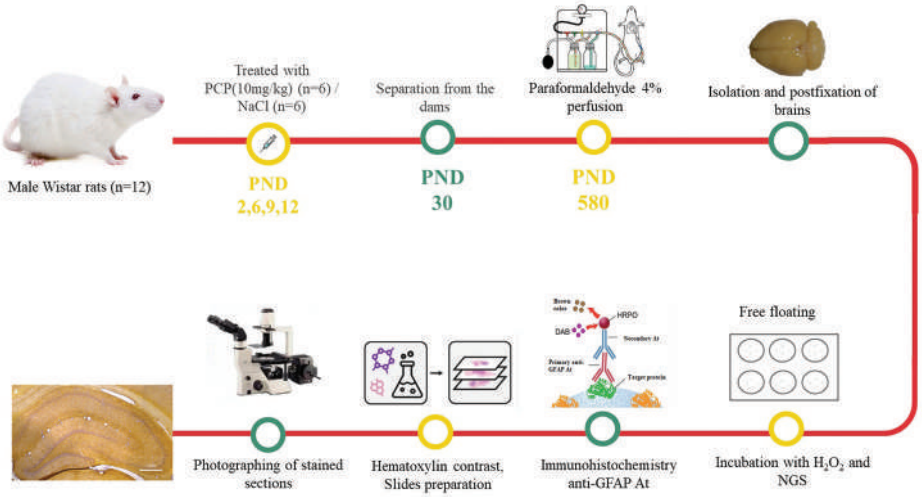

Figure 1. Experimental protocol, scheme; **PND** – Postnatal day; **PCP** – phencyclidine; **NGS** – Normal goat serum; Primary Anti-GFAP rabbit polyclonal antibody (Agilent Cat# Z0334, RRID: AB_10013382).

The surface area of all regions of interest (ROI) was measured on the scanned slides, and then GFAP immunoreactive astrocytes were manually counted by two independent researchers. Only GFAP immunoreactive astrocytes showing their cell bodies were identified as single cells for counting. The density of astrocytes was expressed as the number of astrocytes per 1 mm^2 of ROI [24]. Results are presented as mean ± standard deviation. The morphology of the astrocytes was observed and analyzed using a light microscope at 40x magnification by two independent researchers. Additionally, using the ImageJ program (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, Maryland, USA), the area fraction (%) of GFAP-immunoreactivity was measured [28]. First, the threshold is set in the color threshold window through RGB mode (red, green and blue) with white as the threshold color. Then, the intensity of red, green and blue was adjusted according to the recommendation for DAB (brown) color selection [29]. Then, in the analysis window, the area fraction (%) was measured for each region of interest separately [30]. Within

the examined brain regions, sections at the same distance from Bregma were compared (hippocampus – 4.1 mm; mPFC 3.2 mm).

Statistical analysis was conducted using the SPSS statistical software (IBM SPSS Statistics for Windows, v23.0). The Kolmogorov-Smirnov test was used to determine the distribution of the data. The Student's T-test for two independent samples was used to compare the density of immunoreactive astrocytes between the experimental and control groups. Branching of astrocytes was also assessed by an objective method, in addition to the subjective analysis on a light microscope, through the Pearson correlation coefficient between the density of astrocytes and the area share (%) of GFAP immunoreactivity. The confidence interval was set at 95 % (α=0.05), and statistical significance was determined at $p<0.05$. Tables and graphs were created using Excel (Excel 2019, Microsoft).

RESULTS

Our study found no significant differences in body weight or age between the two experimental groups. The density of GFAP-immunoreactive astrocytes was significantly reduced in the CA2/3 region ($p<0.01$) and dentate gyrus ($p<0.01$) of the hippocampus in PCP-perinatally treated rats compared to controls (Figure 2).

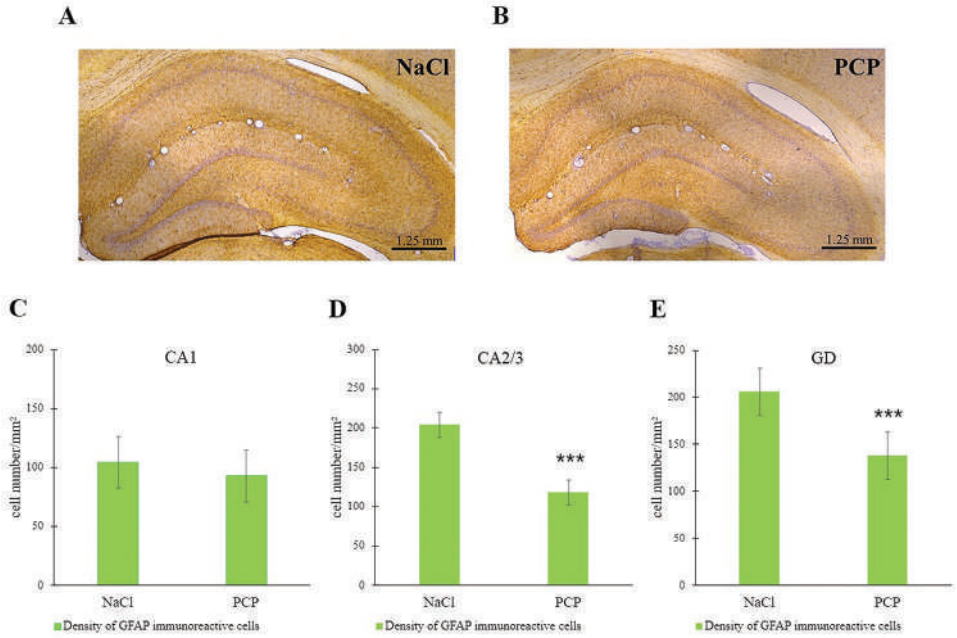

Figure 2. Representative photographs of the hippocampus after GFAP immunohistochemical staining in NaCl-treated rats (**A**) and PCP-treated rats (**B**); density of GFAP-immunoreactive astrocytes in CA1 (**C**), CA2/3 (**D**) and GD (**E**) hippocampal regions of brains of NaCl treated rats (n=6) and PCP treated rats (n=6). The data on graphs C, D, and E are presented as the mean ± S.D. ***p<0.01 compared to the NaCl treated group.

In PCP-treated rats, a significant decrease in the density of GFAP-immunoreactive astrocytes compared to controls ($p<0.05$) was also observed in the medial prefrontal cortex. (Figure 3).

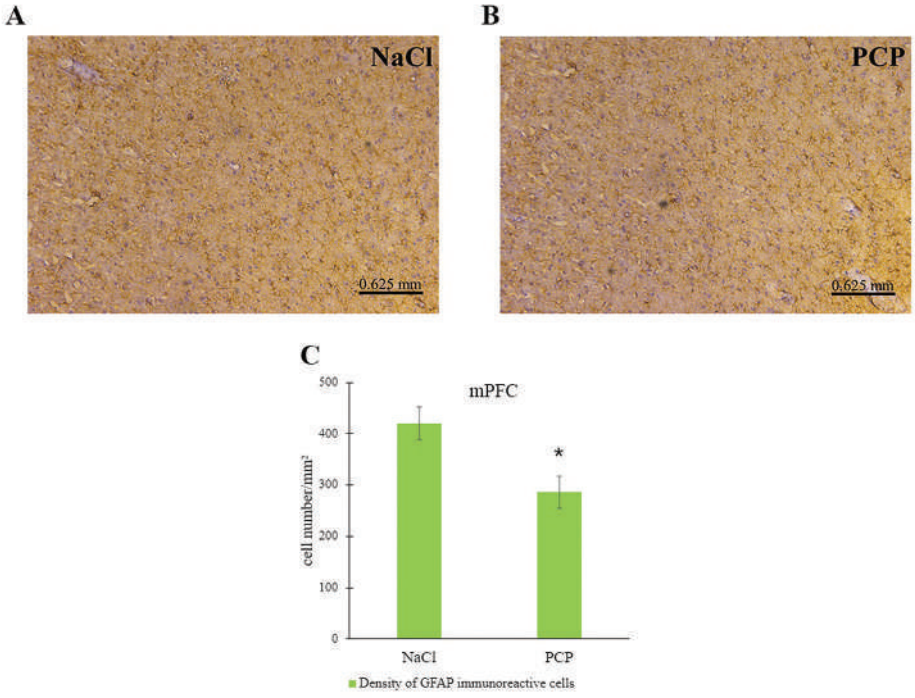

Figure 3. Representative photographs of the medial prefrontal cortex after GFAP immunohistochemical staining in NaCl-treated rats (**A**) and PCP-treated rats (**B**); density of GFAP-immunoreactive astrocytes in mPFC (**C**) in NaCl treated rats (n=6) and PCP treated rats (n=6). The data are presented as the mean ± S.D. ***** $p<0.05$ compared to the NaCl treated group.

The assessment of branching of astrocytes analyzed by two independent investigators, didn't reveal any difference between experimental and control groups. The finding of a strong positive correlation ($r>0.64$; $p<0.05$) between astrocyte density and the area fraction (%) of GFAP-immunoreactivity in all regions in both groups, further supported that astrocyte branching remained unchanged (Figures 4 and 5).

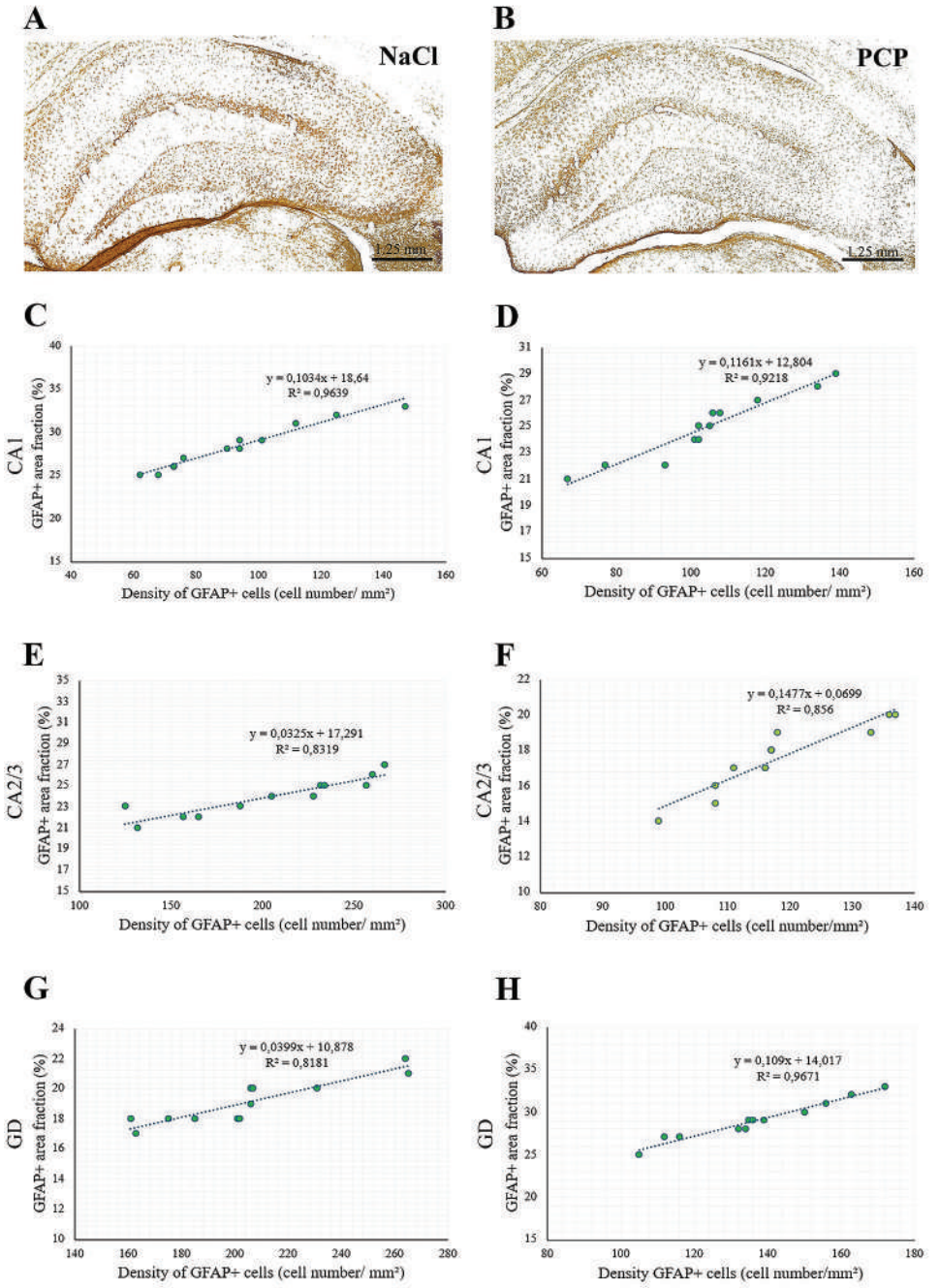

Figure 4. Representative images of DAB staining in the hippocampus, obtained in the ImageJ program in NaCl treated rats (**A**) and PCP treated rats (**B**). The correlation between the density of GFAP-immunoreactive astrocytes and area fraction (%) of GFAP-immunoreactive product in CA1 region (**C**), CA2/3 region (**E**), and GD region (**G**) of NaCl treated rats, and in CA1 region (**D**), CA2/3 region (**F**), and GD region (**H**) of PCP treated rats.

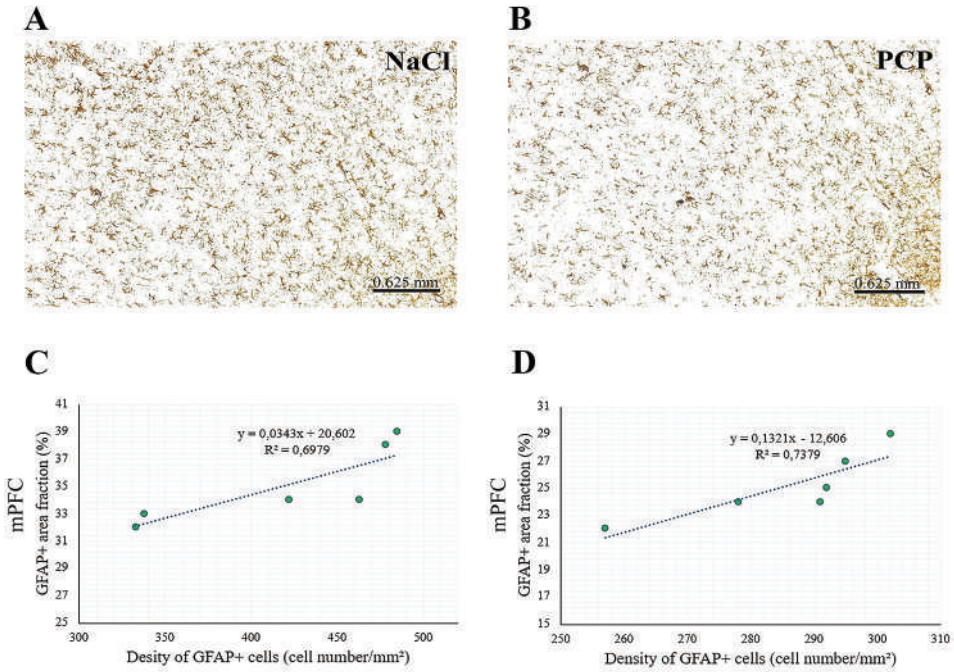

Figure 5. Representative images of DAB staining in the medial prefrontal cortex, obtained in the ImageJ program in NaCl treated rats (**A**) and PCP treated rats (**B**). The correlation between the density of GFAP-immunoreactive astrocytes and area fraction (%) of GFAP-immunoreactive product in mPFC of NaCl treated rats (**C**) and PCP treated rats (**D**).

DISCUSSION

The findings of this study have revealed a significant reduction in astrocyte density within CA2/3 region of the hippocampus, the dentate gyrus, and the medial prefrontal cortex in aged rats that received perinatal PCP treatment. A reduction in astrocyte density was also observed in the CA1 region of the hippocampus; however, this finding did not reach statistical significance. The astrocytes' morphology remained unchanged in all investigated structures. These observations suggest that alterations in astrocytes may play a role in the neurobiological disruptions initiated by perinatal administration of the glutamate NMDA receptor antagonist phencyclidine.

Due to its role as a crucial excitatory neurotransmitter and neurotoxic potential, glutamate concentration in brain tissue is tightly regulated by a series of enzymes predominantly localized in astrocytes, essential for regulating glutamatergic transmission [31]. The changes in astrocytes have been frequently found in animal models of schizophrenia and schizophrenia patients. Zhu et al. noticed that PCP induces a neuroinflammatory response in a murine model of schizophrenia, characterized by astrogliosis and the release of the pro-inflammatory cytokine IL-16 through the GSK3β signaling

pathway, as an acute reaction to disrupted glutamatergic transmission [32]. Roberts et al., [33] conducted a post-mortem study in which they immunohistochemically stained astrocytes marker GFAP in the hippocampus of schizophrenia patients who had not received neuroleptic therapy. They concluded that astrocytosis was absent in these patients, except in those also diagnosed with depression. However, other studies have reported reduced GFAP expression in specific brain regions of schizophrenia patients. A study of Webster et al. found decreased number of astrocytes adjacent to blood vessels in the prefrontal cortex and hippocampus of schizophrenia and major depression patients. [34]. Additionally, a systematic review noted that out of 33 studies evaluating GFAP expression, 21 found no schizophrenia-associated changes, while 6 reported decreased expression [35]. These discrepancies may arise from differences in study methodologies, patient populations, or specific brain regions examined.

However, involvement of astrocytes in schizophrenia pathogenesis is further suggested by the evidence that neuronal dysfunction, rather than neurodegeneration, may underlie hippocampal disruptions associated with cognitive decline in schizophrenia patients [36]. Post-mortem studies have identified significant disturbances in the relationship between astrocytes and glutamatergic synapses in the hippocampal CA3 region, potentially attributable to astrocyte dysfunction [37]. Consistently, positron emission tomography (PET) studies have revealed hypometabolism in the hippocampus of individuals with schizophrenia. Given that astrocytes supply energy to neurons, this hypometabolism may reflect astrocytic dysfunction [38]

In our study, we evaluated astrocyte morphology, particularly their branching patterns, using two approaches: light microscopy analysis and determining the correlation between cell density and the area fraction (%) of the GFAP immunoreactivity. Subjective assessment using light microscopy did not reveal any changes in astrocyte morphology, including their branching extent. Additionally, a positive correlation was observed between the density of astrocytes and the area fraction (%) of the GFAP-immunoreactivity. These results suggest that the reduced area fraction (%) of the GFAP immunoreactivity is primarily a direct consequence of the decreased number of cells per ROI. In the study by Zhu et al., 3-month-old C57BL/6 mice were acutely treated with phencyclidine (PCP) for 7 days at a dosage of 20 mg/kg, resulting in significant glial cell activation, characterized by increased cell density and enhanced branching [34]. Similarly, other rodent models of schizophrenia, such as the maternal immune activation model, have demonstrated astrocytes activation in the frontal cortex on PND 60 [39]. However, none of these studies have examined the astrocyte density and morphology in aged rats. The contrasting outcomes between acute and perinatal PCP exposures may indicate that acute exposure to PCP activates astrocytes and alters their branching patterns, while perinatal PCP treatment leads to a long-term, age-related reduction in the total number of astrocytes without affecting their activation status. Observed decrease in astrocyte numbers may impair their role in maintaining molecular homeostasis within neural tissue, potentially increasing the vulnerability of other cells [40].

Our findings indicate alterations in astrocyte density within the mPFC and hippocampus of aged rats that received perinatal PCP treatment. These brain regions are critically implicated in the pathophysiology of schizophrenia. The mPFC plays a vital role in behavioral regulation, and the hippocampus is integral to cognitive processes [41]. Alterations in astrocyte density within these structures may influence neuronal function and synaptic connectivity, potentially contributing to the behavioral and cognitive deficits characteristic of schizophrenia. It has been demonstrated that in individuals whose cortical neurons exhibited stronger expression of genes encoding synaptic components, cortical astrocytes also showed increased expression of distinct synaptic-related genes and genes involved in cholesterol synthesis, an essential astrocyte-supplied component of synaptic membranes. This coordinated relationship is referred to as the SNAP (synaptic neuron and astrocyte program). In schizophrenia and aging, two conditions associated with reduced cognitive flexibility and plasticity, astrocytes, glutamatergic neurons, and GABAergic neurons all demonstrated a corresponding decline in SNAP expression [18]. Furthermore, a recent finding has demonstrated that ageing leads to significant changes in dopamine receptor expression across various brain regions [42]. Astrocytes express dopamine receptors and can influence dopaminergic signaling. Alterations in dopamine receptors interactions within astrocytes may contribute to the abnormalities observed in schizophrenia [43].

To better understand the association between schizophrenia and accelerated aging, it is necessary to investigate alterations across various brain cell types and neurotransmitter systems within the PCP-induced animal model. Particularly, examining the interactions between astrocytes and these systems can provide deeper insights into the disorder's mechanisms. Such research may uncover novel therapeutic targets aimed at restoring normal astrocytic function and neurotransmitter balance, potentially improving treatment outcomes for individuals with schizophrenia.

Our study acknowledges several limitations that warrant consideration. Firstly, to comprehensively understand the impact of aging on astrocytes following perinatal phencyclidine (PCP) treatment, it is essential to evaluate these changes at multiple developmental stages. This includes immediate effects observed on PND 13, as well as subsequent assessments on PND 35, corresponding to sexual maturity, and PND 70, representing young adulthood. Such longitudinal analysis would provide insights into the progression and potential persistence of astrocytic alterations over time. Furthermore, our current research primarily focuses on astrocytes; however, it is crucial to extend investigations to other neural cell types. Specifically, examining the effects of perinatal PCP treatment on neurons, interneurons, oligodendrocytes, and microglia across various brain regions would offer a more holistic understanding of the neuropathological consequences. Finally, the study was conducted with a limited number of animals, which may affect the generalizability of the findings.

CONCLUSION

Our study demonstrates that perinatal administration of phencyclidine exerts a lasting impact on astroglial cell numbers in the hippocampus and medial prefrontal cortex of aged rats, without affecting astrocyte branching. These findings highlight the potential involvement of astrocytes in the morphological and functional brain alterations associated with schizophrenia. Investigating the effects of perinatal phencyclidine exposure on astroglia within the central nervous system may offer valuable insights into the disorder's pathogenesis and its association with accelerated aging. Understanding the role of astrocytes in schizophrenia could aid in identifying novel therapeutic targets for this complex condition.

Acknowledgments

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Author contributions

SI was responsible for processing animal tissue, performing immunohistochemistry, conducting statistical processing of the results, and writing the paper. NPuškaš developed and implemented the laboratory protocol, critically evaluated the manuscript, and conducted immunohistochemistry. BP performed immunohistochemistry and conducted statistical processing of the results. TS developed and implemented the laboratory protocol and performed immunohistochemistry. TN implemented the laboratory protocol and acquired data. MVB and MŽ both implemented the laboratory protocol. NPetronijević was involved in the research conceptualization, study design, and critically evaluated the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of conflicting 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. Saha S, Chant D, Welham J, McGrath J: A systematic review of the prevalence of schizophrenia. PLoS Med 2005, 2(5):413–433.
2. Moskowitz A, Heim G: Eugen Bleuler's Dementia Praecox or the Group of Schizophrenias (1911): A centenary appreciation and reconsideration. Schizophr Bull 2011, 37(3):471–479.
3. Green M.F, Kern RS, Braff DL, Mintz J: Neurocognitive deficits and functional outcome in schizophrenia: Are we measuring the "right stuff"? Schizophr Bull 2000, 26(1):119–136.
4. Martel JC, Gatti McArthur S: Dopamine receptor subtypes, physiology and pharmacology: new ligands and concepts in schizophrenia. Front Pharmacol 2020, 11(July):1–17.
5. Uno Y, Coyle JT: Glutamate hypothesis in schizophrenia. Psychiatry Clin Neurosci 2019, 73(5):204–215.
6. Wang P, Li M, Zhao A, Ma J: Application of animal experimental models in the research of schizoph. Am J Med Genet Part B Neuropsychiatr Genet 2021, 186(4):209–227.
7. Mouri A, Noda Y, Enomoto T, Nabeshima T: Phencyclidine animal models of schizophrenia: Approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. Neurochem Int. 2007, 51(2-4 spec. iss.):173–184.
8. Carmichael O, Lockhart S: Neurotrophins and Brain Imaging Behav Neurosci 2012, (November 2011):289–320.
9. Clancy B, Darlington RB, Finlay BL: Translating developmental time across mammalian species. Neuroscience 2001, 105(1):7–17.
10. Marenco S, Weinberger DR: The neurodevelopmental hypothesis of schizophrenia: Following a trail of evidence from cradle to grave. Dev Psychopathol 2000, 12(3):501–527.
11. Verkhratsky A, Nedergaard M: Physiology of astroglia. Physiol Rev 2018, 98(1):239–389.
12. Mahmoud S, Gharagozloo M, Simard C, Gris D: Astrocytes maintain glutamate homeostasis in the cns by controlling the balance between glutamate uptake and release. Cells 2019, 8(2):1–27.
13. Mestres-Ventura P, Del Rosario Hernández G: The neuroglia of the rat optic nerve. Part I. Golgi-Hortega and Golgi-EM studies. Eur J Anat 2022, 26(2):167–177.
14. Bechtholt-Gompf AJ, Walther HV, Adams MA, Carlezon WA, Ngür D, Cohen BM: Blockade of astrocytic glutamate uptake in rats induces signs of anhedonia and impaired spatial memory. Neuropsychopharmacology 2010, 35(10):2049–2059.
15. Haroon E, Miller AH, Sanacora G: Inflammation, glutamate, and glia: a trio of trouble in mood disorders. Neuropsychopharmacology 2017, 42(1):193–215.
16. Hajszan T, Leranth C, Roth RH: Subchronic phencyclidine treatment decreases the number of dendritic spine synapses in the rat prefrontal cortex. Biol Psychiatry 2006, 60(6):639–644.
17. Stark AK, Uylings HBM, Sanz-Arigita E, Pakkenberg B: Glial cell loss in the anterior cingulate cortex, a subregion of the prefrontal cortex, in subjects with schizophrenia. Am J Psychiatry 2004, 161(5):882–888.
18. Ling E, Nemesh J, Goldman M, Kamitaki N, Reed N, Handsaker RE, Genovese G, Vogelgsang JS, Gerges S, Kashin S, Ghosh S, Esposito JM, Morris K, Meyer D, Lutservitz A, Mullally CD, Wysoker A, Spina L, Neumann A, Hogan M, Ichihara K, Berretta S, McCarroll SA: A concerted neuron-astrocyte program declines in ageing and schizophrenia. Nature 2024, 627(8004):604-611.

19. Javitt DC: When doors of perception close: bottom-up models of disrupted cognition in schizophrenia. Annu Rev Clin Psychol 2009, 5:249–275.
20. Ridley RM, Clark BA, Durnford LJ, Baker H: Stimulus-bound perseveration after frontal ablations in marmosets. Neuroscience 1993, 52(3):595–604.
21. Hof PR, Morrison JH: The aging brain : morphomolecular senescence of cortical circuits. Trends Neurosci 2004, 27(10):607-613.
22. McDougall SA, Moran AE, Baum TJ, Apodaca MG, Real V: Effects of ketamine on the unconditioned and conditioned locomotor activity of preadolescent and adolescent rats: impact of age, sex, and drug dose. Psychopharmacology (Berl) 2017, 234(18):2683-2696.
23. Stojković T, Radonjić NV, Velimirović M, Jevtić G, Popović V, Doknić M, Petronijević ND: Risperidone reverses phencyclidine induced decrease in glutathione levels and alterations of antioxidant defense in rat brain. Prog Neuro-Psychopharmacology Biol Psychiatry 2012, 39(1):192–199.
24. Nikolić T, Bogosavljević MV, Stojković T, Kanazir S, Lončarević-Vasiljković N, Radonjić NV, Popić J, Petronijević N: Effects of antipsychotics on the hypothalamus-pituitary-adrenal axis in a phencyclidine animal model of schizophrenia. Cells 2024, 13(17):1425.
25. Grayson B, Barnes SA, Markou A, Piercy C, Podda G, Neill JC: Postnatal Phencyclidine (PCP) as a neurodevelopmental animal model of schizophrenia pathophysiology and symptomatology: a review. Curr Top Behav Neurosci 2016, 29:403-428.
26. Jones CA, Watson DJ, Fone KC: Animal models of schizophrenia. Br J Pharmacol 2011, 164(4):1162-1194.
27. Adams SM, De Rivero Vaccari JC, Corriveau RA: Pronounced cell death in the absence of NMDA receptors in the developing somatosensory thalamus. J Neurosci 2004, 24(42):9441–9450.
28. Cobb JA, O'Neill K, Milner J, Mahajan GJ, Lawrence TJ, May WL, Miguel-Hidalgo J, Rajkowska G, Stockmeier CA: Density of GFAP-immunoreactive astrocytes is decreased in left hippocampi in major depressive disorder. Neuroscience 2016, 316:209-220.
29. Luijerink L, Rodriguez M, Machaalani R: Quantifying GFAP immunohistochemistry in the brain – Introduction of the Reactivity score (R-score) and how it compares to other methodologies. J Neurosci Methods 2024, 402:110025.
30. Roksandić D, Radovanović A, Danilović Luković J, Marković D, Kovačević Filipović M, Čolić M: Stereological and immunohistochemical study of the spleen in hypothyroid juvenile rats. Acta Vet Brno 2015, 65(2):246–259.
31. Maciejewski PK, Rothman DL: Proposed cycles for functional glutamate trafficking in synaptic neurotransmission. Neurochem Int 2008, 52(4–5):809–825.
32. Zhu S, Wang H, Shi R, Zhang R, Wang J, Kong L, Sun Y, He J, Kong J, Wang JF, Li XM: Chronic phencyclidine induces inflammatory responses and activates GSK3β in mice. Neurochem Res 2014, 39(12):2385–2393.
33. Roberts GW, Colter N, Lofthouse R, Bogerts B, Zech M, Crow TJ: Gliosis in schizophrenia: A survey. Biol Psychiatry 1986, 21(11):1043–1050.
34. Webster MJ, Knable MB, Johnston-Wilson N, Nagata K, Inagaki M, Yolken RH: Immunohistochemical localization of phosphorylated glial fibrillary acidic protein in the prefrontal cortex and hippocampus from patients with schizophrenia, bipolar disorder, and depression. Brain Behav Immun 2001, 15(4):388-400.

35. Trépanier MO, Hopperton KE, Mizrahi R, Mechawar N, Bazinet RP: Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. Mol Psychiatry 2016, 21(8):1009-1026.
36. Medoff DR, Holcomb HH, Lahti AC, Tamminga CA: Probing the human hippocampus using rCBF: Contrasts in schizophrenia. Hippocampus 2001, 11(5):543–550.
37. Kolomeets NS, Uranova N: Ultrastructural abnormalities of astrocytes in the hippocampus in schizophrenia and duration of illness: A post-mortem morphometric study. World J Biol Psychiatry 2010, 11(2 part 2):282–292.
38. Townsend L, Pillinger T, Selvaggi P, Veronese M, Turkheimer F, Howes O: Brain glucose metabolism in schizophrenia: a systematic review and meta-analysis of 18FDG-PET studies in schizophrenia. Psychol Med 2023, 53(11):4880-4897.
39. de Souza DF, Wartchow KM, Lunardi PS, Brolese G, Tortorelli LS, Batassini C, Biasibetti R, Gonçalves CA: Changes in astroglial markers in a maternal immune activation model of schizophrenia in wistar rats are dependent on sex. Front Cell Neurosci 2015, 9:489.
40. Lieberman JA, Girgis RR, Brucato G, Moore H, Provenzano F, Kegeles L, Javitt D, Kantrowitz J, Wall MM, Corcoran CM, Schobel SA, Small SA: Hippocampal dysfunction in the pathophysiology of schizophrenia: a selective review and hypothesis for early detection and intervention. Mol Psychiatry 2018, 23(8):1764–1772.
41. Sweatt JD: Hippocampal function in cognition. Psychopharmacology (Berl) 2004, 174(1):99–110.
42. Kawahata I, Finkelstein DI, Fukunaga K: Dopamine D1–D5 receptors in brain nuclei: implications for health and disease. Receptors 2024, 3(2):155-181.
43. Guidolin D, Tortorella C, Marcoli M, Cervetto C, De Caro R, Maura G, Agnati LF: Modulation of neuron and astrocyte dopamine receptors via receptor–receptor interactions. Pharmaceuticals 2023, 16(10):1427.

EFEKTI PERINATALNE PRIMENE FENCIKLIDINA NA GUSTINU I GRANANJE ASTROCITA U MOZGU STARIH PACOVA

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Tretman fenciklidinom (PCP), antagonistom N-metil-D-aspartatnih glutamatnih receptora (NMDA-R), prihvaćen je animalni model shizofrenije s obzirom da je hipofunkcija NMDA-R kritična komponenta u patofiziologiji ove bolesti. Nedavne studije su pokazale da je ekspresija glijalnog fibrilarnog kiselog proteina (GFAP), markera astrocita, izmenjena u prefrontalnom korteksu i određenim regionima hipokampusa kod osoba sa shizofrenijom. Prema našim saznanjima, nijedna studija nije ispitivala dugoročne efekte (kod starih pacova) perinatalnog PCP tretmana na gustinu i grananje astrocita, koji su važni za regulisanje glutamatergične transmisije. Cilj studije bio je da se uporede gustina i grananje astrocita u dentatnom girusu (DG), CA1 i CA2/3 regionima hipokampusa i u medijalnom prefrontalnom korteksu (mPFC) kod pacova starih

18 meseci, a perinatalno tretiranih PCP-om u poređenju sa kontrolnom grupom tretiranom fiziološkim rastvorom (NaCl). Mužjaci Wistar pacova (n=6) su tretirani PCP (10 mg/kg) 2, 6, 9. i 12. postnatalnog dana (PND), dok je kontrolna grupa (n=6) tretirana NaCl-om. Svi pacovi su žrtvovani u starosti od 18 meseci (PND540). Astrociti su vizuelizovani korišćenjem GFAP imunohistohemije. Rezultati pokazuju da je gustina astrocita kod pacova tretiranih PCP bila značajno niža u poređenju sa grupom tretiranom NaCl-om u DG ($p<0,01$), CA2/3 regionima ($p<0,01$) i u mPFC ($p<0,05$). Nije bilo promena u grananju samih astrocita ni u jednom regionu od interesa. Naš nalaz značajnog smanjenja gustine astrocita u hipokampusu i mPFC mozgova starih pacova, perinatalno tretiranih sa PCP ukazuje da bi astrociti mogli biti uključeni u morfološke i funkcionalne promene u ovim regionima prouzrokovane disfunkcijom NMDA-R.