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Physiology

Dose-Dependent Effects of Lipopolysaccharide-Induced Neurotoxicity on Memory Processing and Alertness in Wistar Rats

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Abstract | **Original Research Article**

Lipopolysaccharide (LPS), a bacterial endotoxin that has been widely used in experimental neuroscience to model neuroinflammation and its effects on cognitive functions. This study investigates the dose-dependent impact of LPS-induced neurotoxicity on memory processing and alertness in Wistar rats. 20 Wistar rats were randomly divided into four groups: Control, Low-Dose LPS, Medium-Dose LPS, and High-Dose LPS. The animals underwent behavioral assessments using the Barnes Maze, Object Recognition Test (ORT), and Navigational Maze to evaluate memory and alertness. Results indicated that increasing LPS doses led to a progressive decline in memory performance and alertness. The Barnes Maze test showed that spatial learning and memory retention were significantly impaired in the Medium and High-Dose groups compared to controls. The Object Recognition Test revealed that discrimination indices decreased in a dose-dependent manner, indicating deficits in object recognition memory. Similarly, the Navigational Maze test demonstrated reduced exploratory activity and longer escape latencies in LPS-treated rats, suggesting compromised alertness. Biochemical analyses showed elevated levels of nitric oxide (NO) and interleukin-6 (IL-6) in brain tissues of LPS-treated rats, correlating with cognitive impairments. Histopathological examination of the hippocampus revealed neuronal damage, with the highest degree of neurodegeneration observed in the High-Dose LPS group. These findings suggest that LPS-induced neurotoxicity disrupts cognitive functions in a dose-dependent manner, with higher doses exacerbating memory and alertness deficits. The results contribute to understanding the role of neuroinflammation in cognitive decline and provide insights into potential therapeutic strategies for neurodegenerative diseases characterized by chronic inflammation, such as Alzheimer's disease.

Keywords: Lipopolysaccharide, Neurotoxicity, Memory Processing, Nitric Oxide, Interleukin-6.

INTRODUCTION

Memory and alertness are critical cognitive functions that allow organisms to adapt to their environment. Memory encompasses the processes of encoding, storing, and retrieving information, while alertness reflects the ability to remain vigilant and responsive to stimuli (Zlotnik & Vansintjan, 2019). These cognitive functions are dependent on intricate neural networks, neurotransmitter systems, and inflammatory signaling pathways.

Lipopolysaccharides (LPS) are bacterial endotoxins that trigger robust immune responses by activating Toll-like receptor 4 (TLR4), leading to neuroinflammation (Skrzypczak-Wiercioch & Sałat, 2022). Inflammation within the central nervous system (CNS) has been implicated in cognitive dysfunction, neurodegeneration, and conditions such as Alzheimer's disease, Parkinson's disease, and sepsis-associated encephalopathy (McKim *et al.,* 2016). While previous studies have shown that LPS can impair cognitive function, few have systematically examined its dose-dependent effects on memory processing and alertness in Wistar rats. This study aims to fill this gap by assessing behavioral and biochemical responses across different LPS doses.

Neuroinflammation, characterized by microglial activation and increased cytokine release, plays a central role in LPS-induced neurotoxicity (Wen *et al.,* 2016). Pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), interfere with synaptic plasticity and neurotransmission, leading to cognitive deficits (Zhang *et al.,* 2023). Nitric oxide (NO), another key mediator,

has been shown to modulate synaptic activity, but excessive NO production contributes to oxidative stress and neuronal damage (Garthwaite, 2008).

Studies have reported that systemic LPS administration leads to neuroinflammation and cognitive impairments in rodent models (Batista *et al.,* 2019). However, it remains unclear how varying doses of LPS differentially affect memory and alertness. Understanding these dose-dependent effects can help identify critical thresholds for neuroinflammatory damage and guide therapeutic interventions.

This study is significant for several reasons; It provides a systematic analysis of how different doses of LPS impact memory and alertness in an animal model. It explores the relationship between behavioral deficits and neuroinflammatory markers such as IL-6 and NO. It offers insights into potential therapeutic strategies for neurodegenerative diseases linked to chronic inflammation.

MATERIALS AND METHODS

Experimental Animals

Wistar rats were purchased from the animal house of the Faculty of Basic medical sciences, Abuja campus, University of Port Harcourt. The animals were housed in steel cages and kept at room temperature. The rats had no history of drug consumption, that is; they had not been used for any previous investigation. The rats were put on standard rat pellet (feed) and pure drinking water and allowed to get acclimatized for 21 days before the start of the experiment.

Experimental Design

The research design employed for this study was a Randomized Control Trial (RCT), using Wistar rats as a model. The animals were divided into four groups:

1. Control Group (No LPS)
2. Low-Dose LPS Group (0.1 mg/kg)
3. Medium-Dose LPS Group (0.5 mg/kg)
4. High-Dose LPS Group (1.0 mg/kg)

LPS was administered intraperitoneally (IP) once daily for seven days and the rats were exposed to spatial behavior tests after LPS treatment.

Rats from both genders were used to ensure a representative sample. The sample size consisted of groups of rats, each containing five wistar rats. The rats were randomly assigned to one of the four experimental groups. Randomization was conducted to minimize bias and ensure that each rat had an equal chance of being assigned to any group.

The study was conducted in a controlled laboratory environment specifically designed for animal experiments in University of Port Harcourt, Rivers State, Nigeria. The facility adhered to ethical guidelines and provided a standardized setting for administering treatments and conducting behavioral tests.

This table provides a succinct overview of the experimental design, detailing the groups, LPS doses, treatments, and the specific behavioral tests conducted for each group.

Groups	LPS Dose	Treatment	Behavioural Tests
Group 1	None	Control	Navigation Test, Object Recognition, Barnes Test.
Group 2	Low (0.25mg/kg)	LPS + Tests	Navigation Test, Object Recognition, Barnes Test.
Group 3	Medium (0.5mg/kg)	LPS + Tests	Navigation Test, Object Recognition, Barnes Test.
Group 4	High(1.0mg/kg)	LPS + Tests	Navigation Test, Object Recognition, Barnes Test.

Ethical Approval

Ethical approval was obtained from the faculty of basic medical science, Abuja campus, University of Port Harcourt. Rat handling and treatment conform to the guideline of the National Research Council (2011) for care and use of laboratory animals.

Chemicals and Reagents

The chemicals and reagents used for this study were purchased from GGI Intl' Nigeria Ltd. located at GGI Place, Plot 8 GGI Crescent, (Opp. Mikab Filling Station), Port Harcourt, Rivers State, Nigeria. The chemicals and reagents include:

1. **Sodium nitrite ($NaNO_2$)** - Used as a standard for nitric oxide (NO) estimation.
2. **Interleukin-6 (IL-6) -** Used for the determination of IL-6 concentrations in serum and hippocampal tissue.
3. **Lipopolysaccharide (LPS)** - Used to induce neuroinflammation in Wistar rat models.
4. **Griess Reagent** – Employed for the quantification of NO levels in serum and brain tissue.
5. **Phosphate -buffered saline -** Used for sample dilution and washing steps in ELISA assays.
6. **Formalin (10% neutral buffered formalin) -** Used for tissue fixation before histopathological analysis.
7. **Paraffin wax** - Used for embedding brain tissue sections.
8. **Hematoxylin and eosin (H&E) stains** - Used for histological examination of hippocampal tissue.
9. **Primary and secondary antibodies for IL-6 detection** - Used in immunohistochemistry to assess IL-6 expression in hippocampal tissues.

10. **Tissue paper:** Used for cleaning the platform.
11. Ethanol (70%)
12. **Timer:** Used for measuring time\
13. **Scoring system:** Used to record performance.
14. **Barnes Maze Test Materials -** Circular Platform, Escape Holes (20 holes, 5 cm in diameter), Escape box, visual cues.
15. **Object Recognition Test Materials -** Testing arena and objects.
16. **Navigational Maze Materials -** wood maze (10x10 unit sqs) with 15cm high walls.

Nature and Sources of Data

The data collected were behavioral responses of rats in spatial behavior tests. The primary sources of data were the rats in the experimental groups, observed during navigation tests, passive avoidance tests, and the Barnes task. The rats were sacrificed and the hippocampus analysed, the blood was also used to measure the levels of IL-6 and nitric oxide levels.

Biochemical Analysis

Brain tissue homogenates were analyzed for NO levels (Using Griess reagent) and IL-6 levels (Using ELISA).

Histopathology

Hippocampal sections were examined using toluidine blue staining to assess neuronal integrity.

Induction of LPS-Induced Neurotoxicity

LPS, a neurotoxic agent, was administered to the rats via the Intraperitoneal route. The dosage was stratified into low:0.25mg//kg, medium:0.5mg/kg, and high:1.0mg/kg, doses to mimic varying degrees of neuroinflammation. The Intraperitoneal (IP) route was chosen due to its common use in similar studies and its ability to induce a consistent and measurable inflammatory response.

The administration protocol adhered to ethical standards, ensuring the welfare of the animals. The timing of LPS administration was carefully controlled to synchronize with the experimental design.

Blood Collection

Blood samples were collected at specific time points from the tail vein during the experiment to assess systemic markers, including cytokines. The collection process adhered to ethical guidelines and minimize stress on the animals.

Tissue Histology

Tissue samples were collected for histopathological examination to identify structural changes in the brain associated with LPS-induced neurotoxicity.

Guidelines from the National Research Council on the handling of laboratory animals (National Research Council, 2011) AND Principles and techniques of histopathological examination as outlined by Bancroft *et al.,* (2004) were followed.

METHODS OF DATA ANALYSIS

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS). Descriptive statistics, including means and standard deviations, were calculated for each group. To assess the effects of LPS treatment on spatial behavior, inferential statistics, such as Analysis of Variance (ANOVA) or t-tests, were employed. Post-hoc tests may be used for further comparisons if necessary.

RESULTS AND DISCUSSION

RESULTS

The experimental groups in this study were categorized as follows:

Group 1: Negative Control, Group 2: 0.25mg/kg of LPS (low dose), Group 3: 0.5mg/kg of LPS (Medium dose), Group 4: 1mg/kg of LPS (High dose).

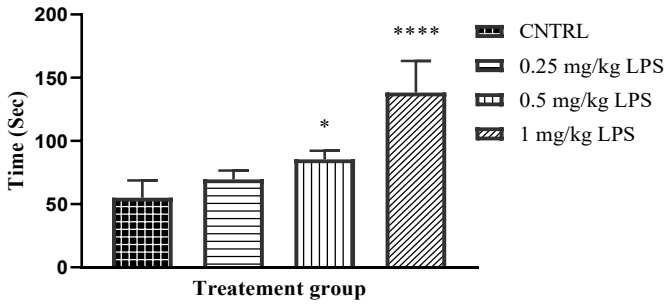


Figure 1: Effect of IP administration of different doses of LPS on spatial memory using barnes maze test on wister rats. Results are presented as mean ± SEM. N=5

The columns represent the mean values and error bars indicate the Standard deviation (SD) of our independent experiments. * Indicates statistical significance of LPS treatments versus the Negative controls * significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001

Behavioral Findings

Barnes Maze: Escape latencies increased significantly with higher LPS doses.

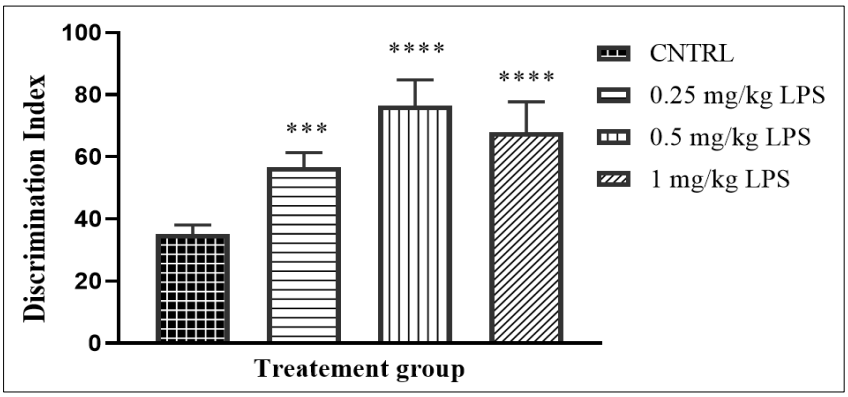


Figure 2: Effect of IP administration of different doses of LPS on behavioural test using object recognition (CIRCLE) test on wister rats. Results are presented as mean ± SEM. N=5

The columns represent the mean values and error bars indicate the Standard deviation (SD) of our independent experiments. * Indicates statistical significance of LPS treatments versus the Negative controls * significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001

Object Recognition Test: The discrimination index declined in a dose-dependent manner.

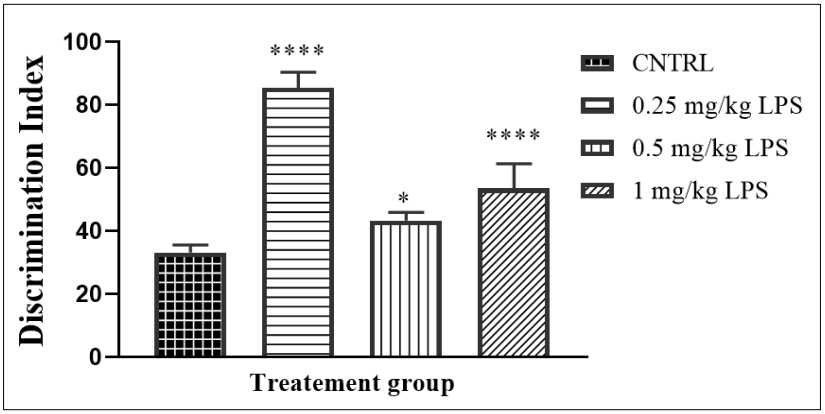


Figure 3: Effect of IP administration of different doses of LPS on behavioural test using object recognition (CROSS) test on wister rats. Results are presented as mean ± SEM. N=5

The columns represent the mean values and error bars indicate the Standard deviation (SD) of our independent experiments. * Indicates statistical significance of LPS treatments versus the Negative controls * significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001

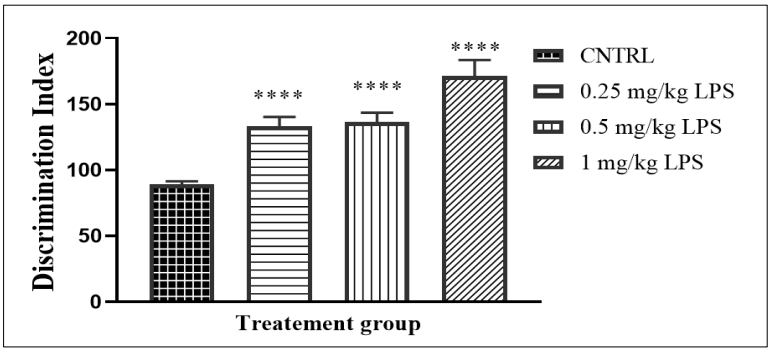


Figure 4: Effect of IP administration of different doses of LPS on behavioural test using object recognition (SQUARE) test on wister rats. Results are presented as mean ± SEM. N=5.

The columns represent the mean values and error bars indicate the Standard deviation (SD) of our independent experiments. * Indicates statistical significance of LPS treatments versus the Negative controls * significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001

Group 1: Negative Control, Group 2: 0.25mg/kg of LPS (low dose), Group 3: 0.5mg/kg of LPS

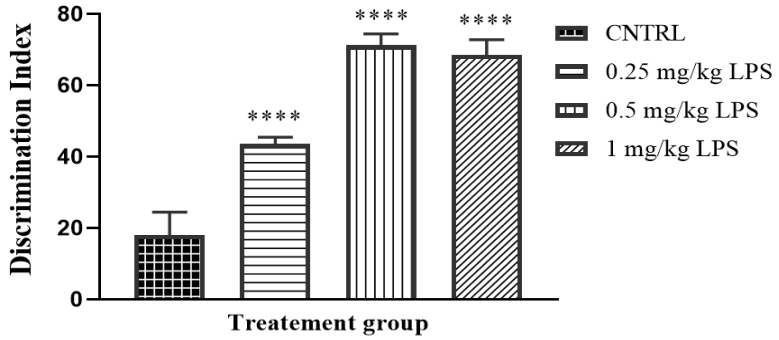


Figure 5: Effect of IP administration of different doses of LPS on behavioural test using object recognition(STAR) test on wister rats. Results are presented as mean ± SEM. N=5

The columns represent the mean values and error bars indicate the Standard deviation (SD) of our independent experiments. * Indicates statistical significance of LPS treatments versus the Negative controls * significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001

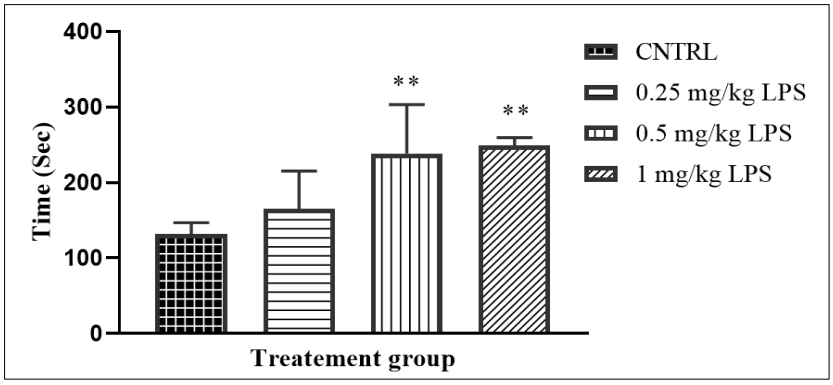


Figure 6: Effect of IP administration of different doses of LPS on spatial memory using navigational maze test on wister rats (Week 1). Results are presented as mean ± SEM. N=5.

The columns represent the mean values and error bars indicate the Standard deviation (SD) of our independent experiments. * Indicates statistical significance of LPS treatments versus the Negative controls * significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001

Navigational Maze: High-dose LPS rats showed reduced alertness and increased escape latencies.

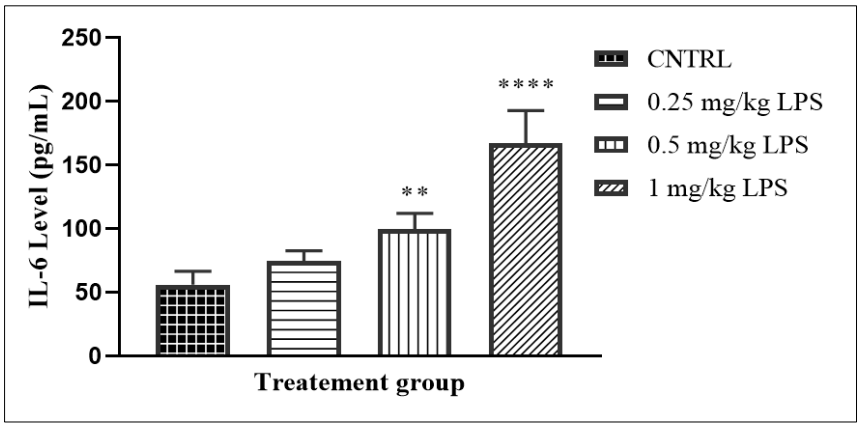


Figure 7: Effect of IP administration of different doses of LPS on interleukein-6 levels on wister rats. Results are presented as mean ± SEM. N=5

The columns represent the mean values and error bars indicate the Standard deviation (SD) of our independent experiments. * Indicates statistical significance of LPS treatments versus the Negative controls * significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001

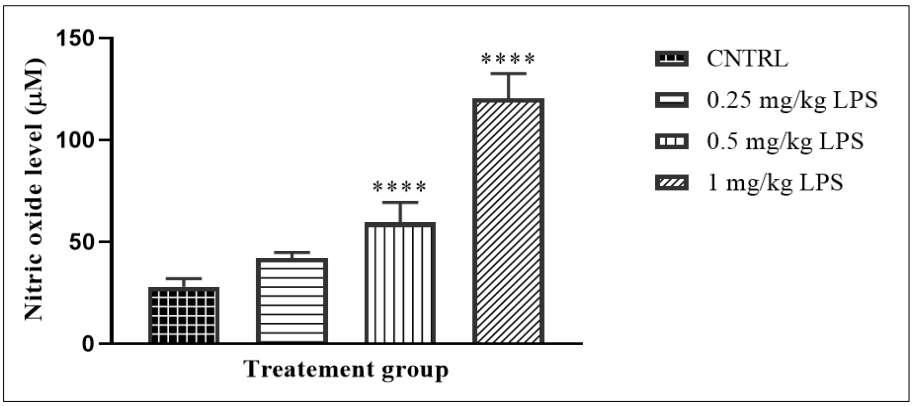


Figure 8: Effect of IP administration of different doses of LPS nitric oxide levels on wister rats. Results are presented as mean ± SEM. N=5

The columns represent the mean values and error bars indicate the Standard deviation (SD) of our independent experiments. * Indicates statistical significance of LPS treatments versus the Negative controls * significant at P < 0.05, ** significant at 0.01, *** significant at 0.001 and **** significant at 0.0001

Biochemical and Histological Findings

NO and IL-6 Levels: Elevated in LPS-treated rats, with the highest increase in the High-Dose group.

HIPPOCAMPUS (TOLUIDINE BLUE STAIN)

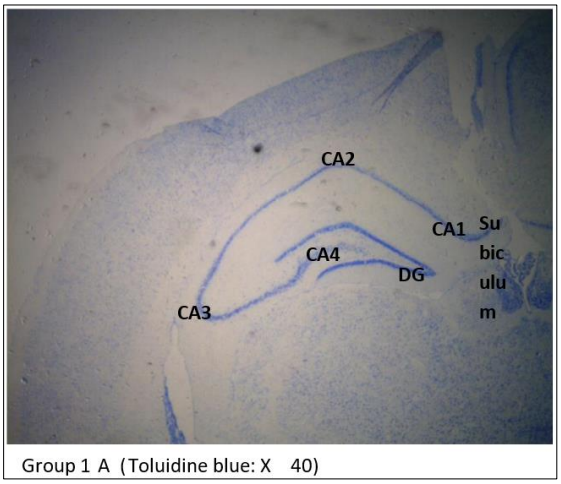
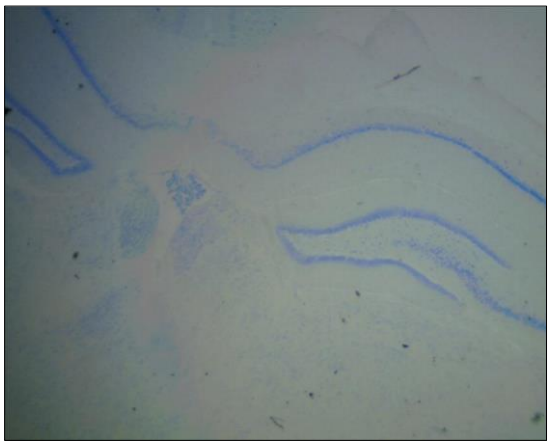


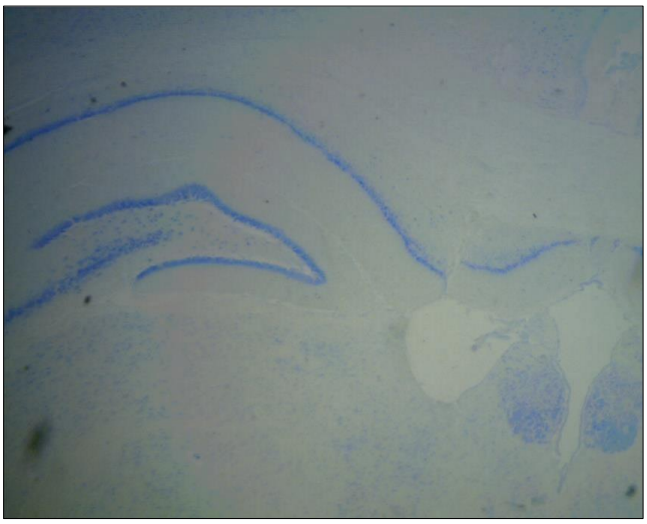
Figure 9: Photomicrograph (Toluidine X40) of the normal Hippocampus showing cornu ammonis CA1 and CA2 with small pyramidal cells, and CA3 and CA4 large pyramidal cell, the dentate gyrus (DG) and the subicular cortex (subiculum).



Group 2A (Toluidine blue: X40)

Figure 10: Photomicrograph (Toluidine X40) of the Hippocampus showing mild neuronal cell loss

Diagnosis: Mild pyramidal Cell Loss



Group 3A (Toluidine blue: X40)

Figure 11: Photomicrograph (Toluidine blue X40) of the Hippocampus showing minimal neuronal cell loss

Diagnosis: Minimal Pyramidal Cell Loss

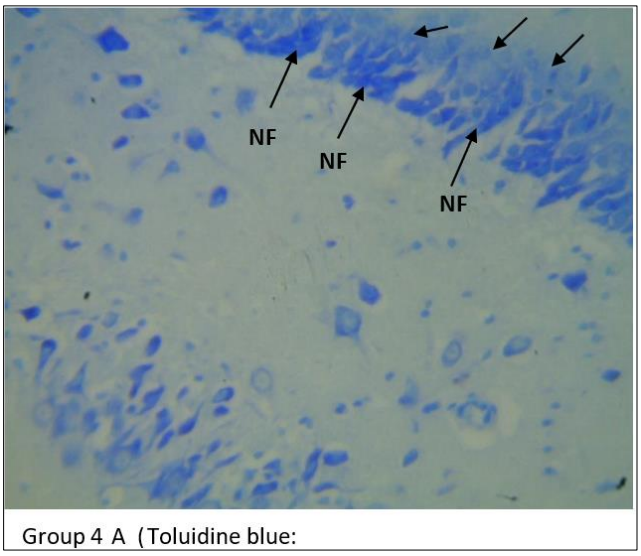


Figure 12: Photomicrograph (Toluidine blue X400) of the Hippocampus showing minimal neuronal darkening and multiple necrotic foci (NF) with associated chromatolysis

Diagnosis: Minimal Neuronal Darkening

Histopathology: Neuronal loss was most pronounced in the High-Dose group.

DISCUSSION

Barnes Maze Test

The Barnes Maze results revealed intriguing patterns in spatial behavior across the different groups. Group 4 showed significantly higher mean times to find the target (T1, T2, T3), suggesting reduced spatial memory compared to the control group (Group 1).

The significant increase in mean times suggests a potential impact of prolonged LPS exposure on memory consolidation or anxiogenic effects. This finding is in keeping with that of Cho *et al.,* (2018) and Zhao *et al.,* (2019) which revealed that spatial behaviours are affected by neuroinflammato rs such as LPS.

In conclusion, LPS administration significantly increased the time taken to complete the Barnes maze, indicating impaired spatial memory. This effect was dose-dependent, with higher LPS doses leading to more pronounced impairments.

Navigational Maze Test

The Navigational Maze results show variations in mean times to navigate the maze. Notably, Groups 3 and 4 exhibited significantly higher mean times compared to the control group (Group 1), indicating potential spatial learning deficits. The initial deficits/degree of impairment in Group 4 align with the Barnes Maze results, supporting the idea of spatial learning challenges. LPS-treated rats exhibited longer completion times in the navigation test, suggesting deficits in spatial learning and memory.

The findings are in line with that of (Engler-Chiurazzi *et al.,* 2023), intermittent systemic exposure to lipopolysaccharide-induced inflammation disrupts hippocampal long-term potentiation and also impairs the mice cognition.

In summary, these results highlight the complex nature of LPS-induced cognitive impairments and the potential for both short-term deficits and long-term adaptive responses. Further research is required to understand fully the underlying mechanisms and to develop effective ways for mitigating the negative effects of LPS on cognitive function.

Object Recognition Test

Group's 3 and 4 showed significantly higher mean values in recognizing the cycle and square compared to the control. Group's 2 demonstrated significantly lower recognition of the star, suggesting a potential selective memory impairment.

LPS administration significantly decreased the discrimination index in the object recognition test, indicating impaired object recognition memory. This effect was consistent across all LPS-treated groups. Group-specific responses emphasize the need for nuanced interpretations based on the nature of the memory task. This result is similar to the study conducted by Valero et al. (2014), whose result strongly suggest that a single systemic administration of LPS aggravates long-term memory impairment in mice.

Analysis of Interleukin-6 (IL-6) Levels

IL-6 levels increase dose-dependently, with the High Dose group exhibiting the highest concentration. Statistical analysis (ANOVA) indicates significant differences between groups 3-4 ($p < 0.05$).

There is a clear dose-response relationship, with IL-6 levels increasing proportionally with the dose of LPS. This suggests that higher doses of LPS result in a more pronounced inflammatory response. Within each group, there is a general trend of increased IL-6 levels over time. This indicates that the inflammatory response induced by LPS persists and may even intensify over the observation period. The control group shows a minimal increase in IL-6 levels. This can be considered within the normal variation and may be attributed to factors unrelated to LPS administration. The transition to high doses of LPS results in a substantial increase in IL-6 levels. This suggests a potential threshold effect where a certain level of LPS is required to trigger a significant neuroinflammatory response. Elevated IL-6 levels are associated with various neurological conditions. The observed increases in IL-6 support the hypothesis that LPS-induced neurotoxicity contributes to neuroinflammation, potentially impacting cognitive functions.

The above findings are similar to that of X. Li *et al.,* (2009) who carried out a similar survey and found that LPS dramatically induced astrocytes to secrete IL-6 in a dose-dependent manner.

In conclusion, the IL-6 data strongly indicates a dose-dependent and time-dependent neuroinflammatory response to LPS administration, providing valuable insights into the implications of neurotoxicity on the immune environment in the brain. These findings contribute to our understanding of the molecular mechanisms underlying neuroinflammation in the context of LPS exposure.

Analysis of Nitric Oxide Levels (µM)

The result reveals similar dose-dependent trend in nitric oxide levels, with the High Dose group having the highest concentration. Statistical analysis indicates significant differences between group 3 and 4 ($p < 0.05$).

The study revealed a discernible dose-dependent response in Nitric Oxide levels. As the LPS dose increased from low to high, there was a progressive elevation in Nitric Oxide concentrations. This implies that the administration of LPS correlates with an increased production of Nitric Oxide in a dose-dependent manner. Statistical analysis demonstrated significant differences in Nitric Oxide levels between the various dosage groups. The high dose groups exhibited notably higher levels compared to the control and low dose groups. This discrepancy underscores the importance of dosage in influencing Nitric Oxide modulation. Nitric Oxide serves as a key signaling molecule in various physiological processes, including neurotransmission. The observed fluctuations in Nitric Oxide levels bear significance in the context of neural function. Elevated levels may be indicative of heightened cellular activity, inflammation, or response to neurotoxic stimuli.

The findings align with existing literature that associates LPS administration with increased Nitric Oxide production. Studies by Yang et al. (2012) have reported similar dose-dependent relationships between LPS exposure and Nitric Oxide synthesis. This consistency strengthens the validity of the current study's results and underscores the reproducibility of these patterns across different experimental setups. However, it is essential to note discrepancies in specific thresholds and magnitudes of Nitric Oxide response, which could be attributed to variations in experimental conditions,

species differences, or methodological approaches (Yang *et al.,* 2012).

The Nitric Oxide findings provide valuable insights into the nuanced dynamics of LPS-induced neurotoxicity. The dose-dependent response and significant differences between groups emphasize the need for a comprehensive understanding of Nitric Oxide modulation in neuroinflammatory processes. These results contribute to the growing body of literature on neuroimmune interactions and provide a foundation for further investigations into the intricate mechanisms of LPS-induced neurotoxicity.

Normal Hippocampal Structure (Figures 15)

Figures 15 shows a normal hippocampus, with magnification at X40 providing an extensive view of the cornu ammonis regions CA1 through CA4, along with the subicular cortex and the dentate gyrus. These regions form the main parts, both functional and structural parts of the hippocampus. Specifically, the pyramidal cells in CA3 and CA4 are larger than those in CA1 and CA2.Pyramidal cells are essential neurons within the hippocampus, known for their role in synaptic transmission and memory processing (Graves *et al.,* 2012).

The diagnosis here indicates a normal hippocampus, showing that all cellular layers, especially in the CA3 region, display the expected morphology with no indication of cell loss or chromatolysis. These normal findings in the structure of the hippocampus are in line with Per Andersen et al. (2007) studies that details the organized and structural layering of pyramidal cells and their role in cognitive function and processes. Also, the presence of vesicular nuclei is a distinctive characteristic of healthy neurons, as they signify cellular metabolism and active transcription necessary for proper neuronal function (Witter, 2010).

Mild/Minimal Neuronal Cell Loss (Figures 10,11)

Figures 10 and 11 presents the examples of mild to moderate neuronal cell loss. Mild neuronal loss is illustrated by Figure 10 X40, while Figure 11 X40 depicts regions of moderate pyramidal cell loss with diffuse chromatolysis. Dissolution of the Nissl body, scientifically known as chromatolysis, normally implies a cellular reaction to injury and is frequently observed in states in which neurons are under stress or damaged (Cowan *et al.,* 2004). This coupled with a loss of pyramidal cells could show early signs/symptoms of degenerative processes inside the hippocampus.

The unpleasant effect of the mild to moderate cell loss are important for a number of reasons, one of which is because pyramidal cells are intrinsic to the organization and capacity of the hippocampus. Loss of these cells affects the ability of the hippocampus to produce and stabilize new memories; something that is observed in Alzheimer's diseases, mild cognitive impairments (MCI) and other related diseases. The observed chromatolysis may also indicate cellular stress extending to the molecular level that might result from metabolic demands or excitotoxicity through overstimulation of neurons that results in neuron death (Krieglstein, 1997).

Minimal Neuronal Darkening and Necrosis (Figure 12)

Figure 12 shows little neuronal darkening and multipe necrotic foci. Neuronal darkening and necrosis signify very high level of cellular stress or injury and can be best described by cellular changes that feature condensed nucleus and shrunken cytoplasm commonly before the cell death (A. W. Brown *et al.,* 1979). Hippocampal necrosis may be due to ischemic conditions, in which insufficient blood circulation leads to a deficiency of oxygen and glucose, or due to excitotoxicity which is typical of acute neurological diseases.

The finding showed in this figure tally with studies that record the securing of necrotic foci and chromatolysis due to oxidative stress, a phenomenon well observed in hippocampal tissue subsequent to hypoxic or ischemic events (Adameova *et al.,* 2022). In general, necrosis, even in the form of small foci, may interrupt the cellular cohesion required for effective signal relay through the networks of the hippocampus and hinder the allocation of new memories and spatial orientation.

IMPLICATIONS

In reviewing the sequence of these figures, the range of hippocampal changes can be interpreted as a spectrum from normal to various degrees of injury or dysplasia. The figures collectively illustrate a gradient of hippocampal pathology, from normal cellular structure and organization to mild and moderate cell loss, neuronal darkening, chromatolysis, and necrosis. These findings are relevant for understanding hippocampal responses to injury and the cellular changes that precede neurodegeneration.

The diagnosis of "normal hippocampus" in Figures 9, contrasts sharply with findings of cell loss, darkening, and necrosis in Figures 10 - 12. The findings provide insight into how the hippocampus may respond to different disease or stress by indicating how cellular responses differ depending on the degree of dysfunction or damage. Understanding how neurological conditions like Alzheimer's and epilepsy go from healthy tissue to dysplasia or necrosis helps us to identify the stages in which an intervention may be the most successful. For example, though severe chromatolysis or necrosis shows irreparable damage, modest neuronal loss or darkening may be recoverable with using measures which are therapeutic.

CONCLUSION

Dose-Dependent Cognitive Impairments: LPS administration led to memory and alertness deficits in a dose-dependent manner. High doses caused the most significant impairments, consistent with previous studies on neuroinflammation-induced cognitive dysfunction (Zhang *et al.,* 2023).

Neuroinflammatory Mechanisms: Increased NO and IL-6 levels indicate a strong inflammatory response. IL-6 has been shown to modulate synaptic plasticity, while excessive NO production can lead to oxidative stress and neuronal apoptosis (Gruol, 2015).

Histological Correlates: Histopathological analysis confirmed neuronal degeneration in the hippocampus, particularly in high-dose LPS rats. This aligns with findings that neuroinflammation contributes to hippocampal dysfunction (McKim *et al.,* 2016).

Clinical Implications: These findings have implications for neurodegenerative diseases associated with chronic inflammation, such as Alzheimer's disease. Understanding dose-dependent effects may aid in developing therapeutic strategies targeting neuroinflammation.

In summary, study demonstrates that LPS-induced neurotoxicity impairs memory processing and alertness in a dose-dependent manner. The findings highlight the role of neuroinflammation in cognitive dysfunction and suggest that controlling inflammatory responses may mitigate cognitive decline. Future research should explore potential neuroprotective interventions.

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