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Original Research

Investigation of the optimal dose for experimental lipopolysaccharide-induced recognition memory impairment: behavioral and histological studies

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Abstract

Background: Lipopolysaccharide (LPS) administration is one of the most commonly used methods for inducing inflammation in animal models. Several animal studies have investigated the effects of acute and chronic peripheral administration of LPS on cognitive impairment. However, no previous study has compared the effects of different doses of chronically administered LPS on recognition memory performance. **Aim**: Here, we aimed to investigate the optimal dose of chronically administered LPS for the induction of recognition memory impairment in mice. **Materials and methods**: LPS at different doses (0.25, 0.50 and 0.75 mg/kg) was administered to SWR/J mice daily for 7 days. On day 9, the open field, novel object recognition and novel arm discrimination behavioral tests were performed. Additionally, prefrontal cortical histological examination was conducted. **Results**: Compared with the control group, mice injected with 0.75 mg/kg LPS notably showed no object preference (familiar vs. novel), a reduction in the discrimination index, and spatial recognition impairment. Administration of the 0.25 and 0.50 mg/kg doses of LPS showed a preference for the novel object compared with the familiar object, had no significant impact on the discrimination index, and caused spatial recognition impairment. These behavioral results are in line with the histological examination of the prefrontal cortex, which revealed that the 0.75 mg/kg dose produced the most histological damage. **Conclusions**: Our findings suggest that for chronic peripheral administration of LPS, 0.75 mg/kg is the optimal dose for inducing neuroinflammation-associated recognition memory deficits.

Keywords: Lipopolysaccharide; Neuroinflammation; Recognition memory; Prefrontal cortex; Chronic; Alzheimer disease; Peripheral administration; Recognition memory loss; Novel arm discrimination task; Acute

1. Introduction

Neuroinflammation is defined as the immune response within the central nervous system (CNS) and is characterized by stimulation of neuroglial cells and cytokine production [1]. Neuroinflammation occurs naturally as a neuroprotective mechanism to repair damaged neural cells [2]. Moreover, neuroglial cells such as microglia and astrocytes play a pivotal role in neurogenesis and cell survival [3]. However, studies suggest that prolonged brain inflammation has a neurodegenerative effect [4,5]. There is accumulating evidence that uncontrolled neuroinflammation has a substantial pathogenetic role in various neurodegenerative disorders, including Parkinson's disease (PD), multiple sclerosis (MS) and Alzheimer's disease (AD), which are associated with memory deficits and behavioral alterations [6].

Several animal studies have demonstrated a link between neuroinflammation and recognition memory impairment [7–9]. Recognition memory can be referred to as the brain's ability to assess the familiarity for an item in addition to the recollection of specific experienced events [10]. Episodic recognition memory neuroimaging studies reveal retrieval-associated activation of the right prefrontal cortex (PFC) [11,12]. Moreover, another study showed that the PFC plays a key role in both component processes of recognition memory, i.e., familiarity and recollection [13]. Rodent behavioral and electrophysiological studies suggest that the medial prefrontal cortex (mPFC) is critically involved in recognition memory [10,14].

Mouse models have been generally used to investigate basic diseases mechanisms and for preclinical drug evaluation [15]. Lipopolysaccharide (LPS) administration is one of the most commonly used experimental animal models for studying behavioral and neurochemical alterations caused by neuroinflammation [16]. LPS is a stable and potent endotoxin that resists enzymatic breakdown and induces a long-lasting inflammatory response [17]. LPS at different doses and injection frequencies has been used to trigger cognitive impairment [16,18,19]. Moreover, studies have shown that intraperitoneal (i.p.) injection of LPS leads to cognitive impairment in rat and mouse models

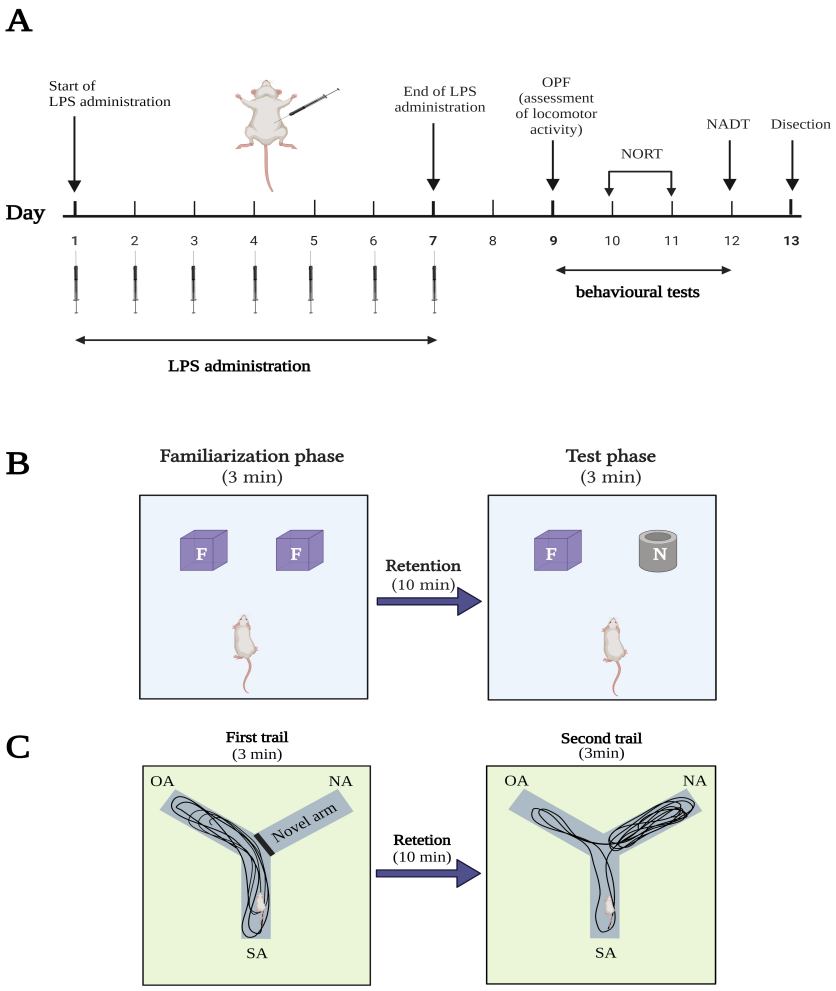

Fig. 1. Timeline and design of the experiments. (A) Total duration of the study was 13 days. On the first 7 days, LPS administration was conducted daily. Behavioral tests were performed from day 9 to 12. Then, mice were sacrificed on day 13 and the brains were removed for histological examination. (B) NORT phases. The familiarization phase is designed to give the mice the chance to explore two identical familiar (F) objects. The test phase is designed to investigate the ability of the mice to recognize the novel (N) object. (C) NADT phases. The first trial is designed to allow free exploration in only 2 arms of the Y maze after blocking the novel arm (NA). The second trial evaluates the ability to recognize and explore the previously blocked arm. LPS, lipopolysaccharide; OFT, open field test; NADT, novel arm discrimination task; NORT, novel object recognition task; F, familiar object; N, novel object; SA, starting arm; NA, novel arm; OA, other arm. Created by Biorender.

[16,20] in various behavioral paradigms, including spatial memory, fear conditioning and recognition memory performance [21–23]. Salmani *et al.* [24] reported that male BALB/c mice treated for 7 days with 0.25 mg/kg LPS, i.p., spent less time exploring the novel object compared with the control group. Another study demonstrated that acute peripheral injection of 0.50 mg/kg LPS decreased preference for the novel object in mice [25]. In addition, Frühauf *et al.* [8] showed that 0.75 mg/kg LPS administered for 7 consecutive days reduced preference for the new object in the novel object recognition task in Swiss mice. However, to the best of our knowledge, there is no study comparing the effects of chronic administration of different LPS doses on PFC neurodegeneration or recognition memory performance. Therefore, in this study, we aimed to determine the optimal dose of chronically administered LPS for the induction of recognition memory impairment in mice.

2. Materials and methods

2.1 Animals

In this study, 24 adult male Swiss mice (SWR/J) weighing 18–25 g were obtained from the animal facility of the King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. Mice were housed three per cage under controlled temperature (23 $\pm$ 2 °C) and humidity (65%), with a standard 12/12 h light/dark cycle and *ad libitum* access to water and standard food.

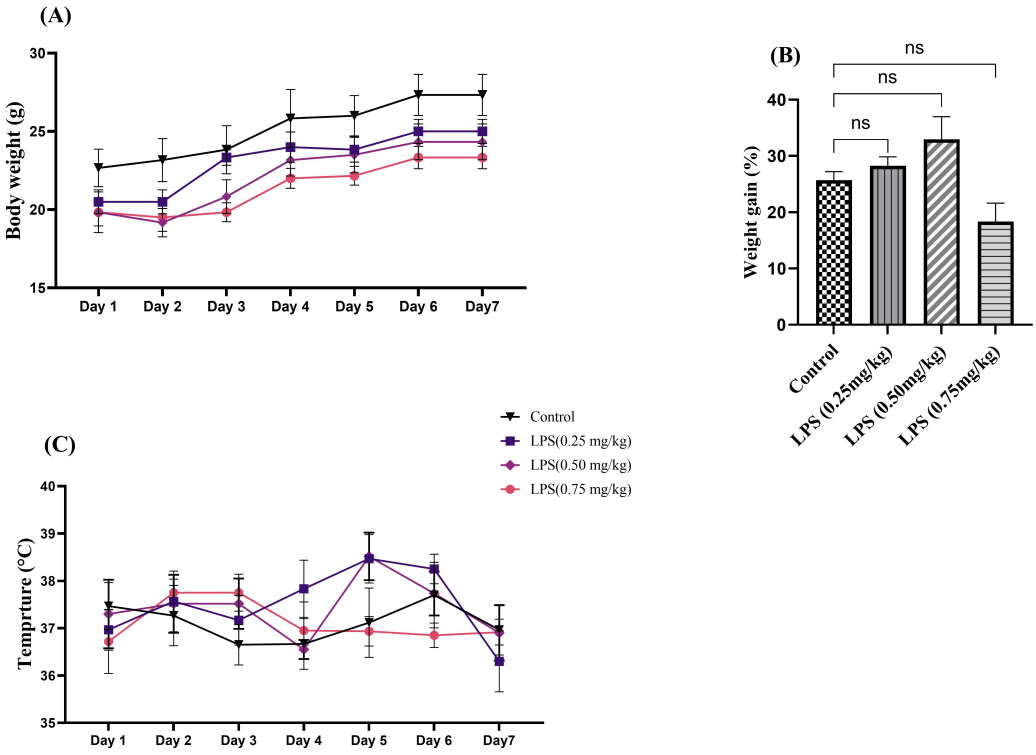

Fig. 2. Effect of lipopolysaccharide (LPS) on (A) body weight (g) (B) body weight gaining (%) and (C) body temperature. Body weight were measured daily during the 7 days of LPS administration (A). The total body weight gain (%) at the end of LPS administration is calculated in (B). Temperature was measured daily during the 7 days of LPS administration (C). Mice body weight and temperature showed no significant difference compared to the control group. Data are presented as mean ± SEM. Two-way ANOVA followed by the Tukey's test was used for (A,C). One-way ANOVA followed by Tukey's test was used for (B). SEM, standard error of the mean; ANOVA, analysis of variance; ns, non-significant.

2.2 Treatment preparation

LPS (*E. coli* O111: B4) was obtained from Invivogen, France. A stock solution of 5 mg/mL LPS was prepared by dissolving 5 mg of powdered LPS in 1 mL of endotoxin-free water, and divided into 100 μL aliquots, which were stored at –20 °C. LPS injections of different doses (0.25, 0.50, 0.75 mg/kg) were prepared according to Ramírez *et al*. [26] and given i.p.. Briefly, all injections were freshly prepared daily in the morning and diluted from the stock solution to the desired concentration (0.1 mL/10 g body weight, i.p.).

2.3 Experimental design

Mice were randomly divided into the following four groups (six animals per group): (I) i.p. saline vehicle (0.9%) (control) group; (II) i.p. LPS (0.25 mg/kg) group; (III) i.p. LPS (0.50 mg/kg) group; and i.p. LPS (0.75 mg/kg) group. All treatments were administered for 7 consecutive days between 11 AM and 12 PM. Behavioral tasks were conducted from day 9 to 12. On day 13, the mice were sacrificed, and the brain tissues were collected for further analyses (Fig. 1A).

2.4 Assessment of body weight and temperature

Mice weight and temperature were recorded daily immediately before the injection during the 7 days of LPS administration. Temperature was measured using a DT-8826 (SCC Inc., Gampaha, Sri Lanka) non-contact infrared thermometer following the device procedure and protocol.

2.5 Assessment of locomotor activity by open field task (OFT)

Locomotor activity was quantified for 10 min with the open field test. Each mouse was placed gently into a rectangular field (45 × 45 × 34 cm) and allowed to explore the arena freely. Velocity and total distance moved (TDM) were calculated with the EthoVision XT8A system (Noldus Information Technology, Wageningen, The Netherlands) [27].

2.6. Assessment of recognition memory performance

2.6.1 Novel object recognition task (NORT)

The NORT is a widely applied method for assessing short-term recognition memory based on the exploratory behavior of rodents [28]. Here, it was used to examine the effect of LPS on short-term visual recognition. The proto-

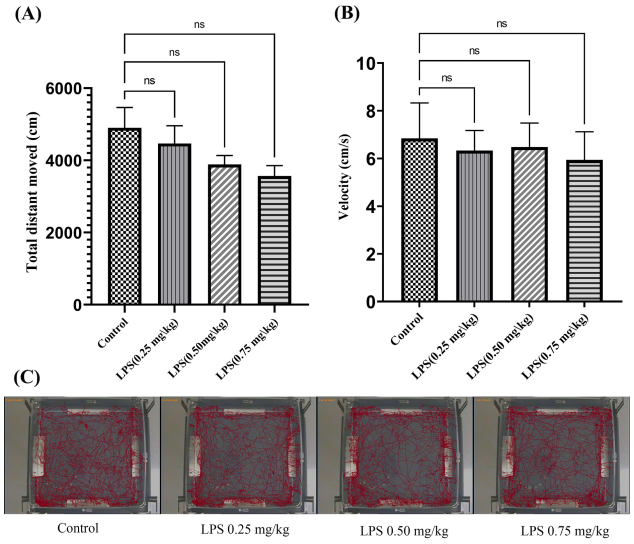

Fig. 3. Locomotor activity assessment in the open field test (OFT). (A) No significant differences were observed in the TDM among all groups. (B) There were no significant differences in velocity among the groups. (C) Representative track sheets for the open field test. Data are presented as mean ± SEM. One-way ANOVA followed by Tukey's multiple comparisons test was used. LPS, lipopolysaccharide; TDM, total distance moved; ns, non-significant; SEM, standard error of the mean; ANOVA, analysis of variance.

col was performed as described previously with slight modifications. The task was carried out over a period of 2 days: day 1 for habituation, and day 2 for testing. During the habituation session, mice were allowed to move freely and habituated to the arena for 10 min. After 24 h, animals underwent the familiarization phase in which each mouse were exposed to two identical objects for 3 min (Fig. 1B). After a retention interval of 10 min, in their home cage, the mice were returned to the arena for the test phase, in which one of the familiar objects was replaced with a cleanable and unmovable novel object that differed in shape, color and texture from the familiar one (Fig. 1B). After each trial, the arena was cleaned with 10% ethanol to eliminate odor cues. In both the familiarization and test phases of the test, the frequency of sniffing of each object was calculated (to ensure that all mice had a similar chance of investigating both objects) as follows: Frequency of sniffing (%) = (novel or familiar object frequency of sniffing/total frequency of sniffing of the two objects) × 100. Exploratory behavior was defined as pointing the nose to the object at a distance of less than 2 cm and touching the object with the nose. Turning around or sitting on the object was not considered exploratory behavior. The time spent exploring each object during the test phase was recorded automatically using the EthoVision XT8A video tracking system. As an index of memory, the discrimination index (DI) was calculated as follows: DI = (time spent exploring the novel object-time spent exploring the familiar object/total exploration time).

2.6.2 Novel arm discrimination task (NADT)

The NADT was used to examine spatial recognition memory [16]. In brief, the three arms were randomly marked as starting arm (SA), novel arm (NA), and the other arm (OA). The protocol consisted of two 3 min training and test trials. In the first trial, mice were placed in the SA, and the NA was blocked. Mice were freely allowed to move only between the SA and the OA (Fig. 1C). After 10 min, the second trial was implemented in which the NA was opened and the mice were allowed again to explore all three arms (Fig. 1C). The duration in the NA arm was calculated as an indicator of spatial recognition memory performance. Mice with intact spatial recognition memory were expected to spend more time exploring the NA.

2.7 Histological studies of PFC

Hematoxylin–Eosin (H&E) Staining

On the last day of the study, mice were anesthetized by isoflurane inhalation. Brains were removed and post-fixed for 48 h in 10% paraformaldehyde fixative. For histological study, sagittal sections were cut and processed as paraffin sections of 2–5 μm thickness, and then mounted on slides and stained with an (abcam) H&E staining kit (ab245880, Cambridge, MA, USA) according to the manufacturer's protocol.

2.8 Statistical analysis

All data are expressed as mean ± standard error of the mean and were statistically analyzed using GraphPad Prism 8.3.8 (GraphPad Software Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by post hoc Tukey's test was used for comparing differences between the groups for weight gain (%), TDM, velocity, DI, and duration in the NA. For frequency of sniffing, body weight and body temperature, two-way ANOVA followed by post hoc Tukey's test was used. The differences between the groups were considered statistically significant when the p-value was <0.05.

3. Results

3.1 Chronic LPS administration did not affect mice body weight and body temperature

During the 7 days of LPS administration, two-way repeated measures ANOVA of body weight showed no significant difference for days × groups [$F(18, 120) = 0.8168$, $p = 0.6775$] (Fig. 2A). Weight gain (%) showed no statistically significant difference between the LPS and control groups (LPS 0.25 mg/kg, $p = 0.9159$; LPS 0.50 mg/kg, $p = 0.2939$; and LPS 0.75 mg/kg, $p = 0.2858$) (Fig. 2B). During the 7 days of LPS administration, two-way repeated measures ANOVA of body temperature showed no significant difference for days × groups [$F(18, 120) = 1.069$, $p = 0.3919$] (Fig. 2C).

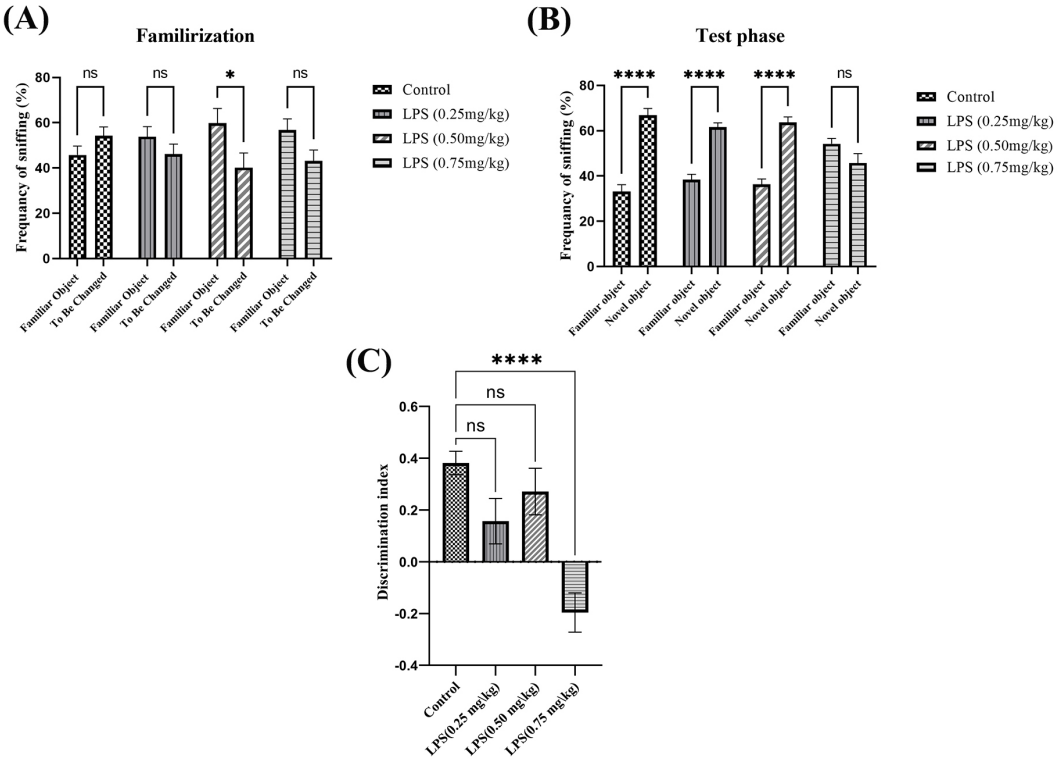

Fig. 4. Frequency of sniffing (%) (A, B) and discrimination index (DI) (C). (A) Among all groups, no significant differences were found during the familiarization stage in the frequency of sniffing (%) of the two identical objects, except for the LPS 0.50 mg/kg group. (B) In the test phase, a significant difference was found in the frequency of sniffing (%) of each object (familiar vs. novel) among the Control, LPS 0.25 mg/kg and LPS 0.50 mg/kg groups, but not the LPS 0.75 mg/kg group. (C) There was no significant difference in the DI in the LPS 0.25 and 0.50 mg/kg groups compared with the control group. However, there was a significant decrease in the DI in the LPS 0.75 mg/kg group compared with the control group. (D) Representative track sheets in the NORT test. Data are presented as mean ± SEM. Two-way ANOVA followed by Tukey's multiple comparisons test was used for (A) and (B), and one-way ANOVA followed by Tukey's multiple comparisons test was used for (C). *$p < 0.05$, ****$p < 0.0001$. LPS, lipopolysaccharide; NORT, novel object recognition test; ns, non-significant; SEM, standard error of the mean; ANOVA, analysis of variance.

3.2 Chronic LPS administration did not affect mice locomotor activity

LPS-administered mice traveled a shorter distance compared with the control group, but the difference was not significant ($p = 0.1413$) (Fig. 3A). Furthermore, there was no significant difference in velocity ($p = 0.6451$) (Fig. 3B). These data were automatically calculated by EthoVision Tracking system (Fig. 3C).

3.3 Chronic LPS administration induce recognition memory impairment

3.3.1 NORT

During the familiarization phase, there was no significant difference in the frequency of sniffing (%) of each object (familiar vs. novel) among the groups, except the LPS 0.5 mg/kg group (control, $p = 0.9660$; LPS 0.25 mg/kg, $p < 0.9999$; LPS 0.50 mg/kg, $p = 0.0338$; LPS 0.75 mg/kg, $p = 0.2426$) (Fig. 4). In the test phase, sniffing was significantly greater for the novel object than for the familiar object among the control and lower LPS dose (0.25 and 0.50 mg/kg) groups (control, $p < 0.0001$; LPS 0.25 mg/kg, $p < 0.9999$; LPS 0.50 mg/kg, $p < 0.0001$). Notably, the LPS 0.75 mg/kg group showed no significant difference in frequency of sniffing (%) between the two objects (familiar vs. novel, $p = 0.1346$) (Fig. 4A,B). Compared with the control group, the DI was significantly decreased in the LPS 0.75 mg/kg group ($p < 0.0001$), while the two other groups showed a non-significant decrease in DI (LPS 0.25 mg/kg, $p = 0.1550$; LPS 0.50 mg/kg, $p = 0.9665$) (Fig. 4C). These data were automatically calculated by EthoVision Tracking system (Fig. 4D)

3.3.2 NADT

During the second trial in the NADT, mice in the control group, but not the LPS groups, spent most of their time in the NA (LPS 0.25 mg/kg, $p = 0.0004$; LPS 0.50 mg/kg, $p = 0.0012$; LPS 0.75 mg/kg, $p = 0.0003$) (Fig. 5).

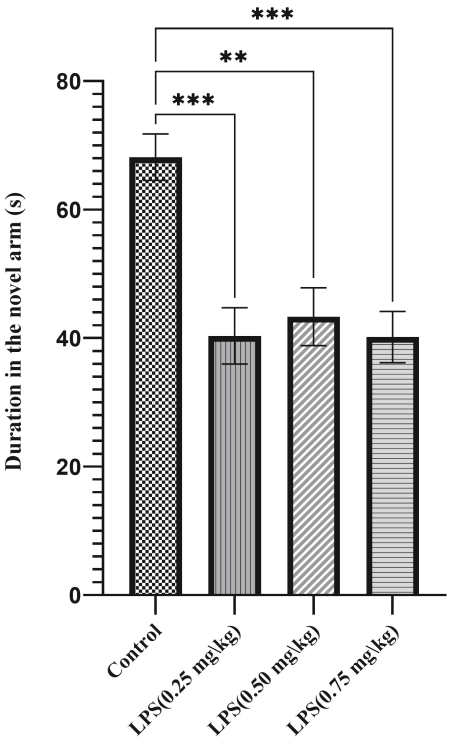

Fig. 5. Novel arm discrimination task. Significant differences were observed in the duration in the novel arm among all the groups compared with the control group. Data are presented as mean ± SEM. One-way ANOVA followed by Tukey's multiple comparisons test was used. $^{**}p < 0.01$, $^{***}p < 0.001$. LPS, lipopolysaccharide; s, seconds.

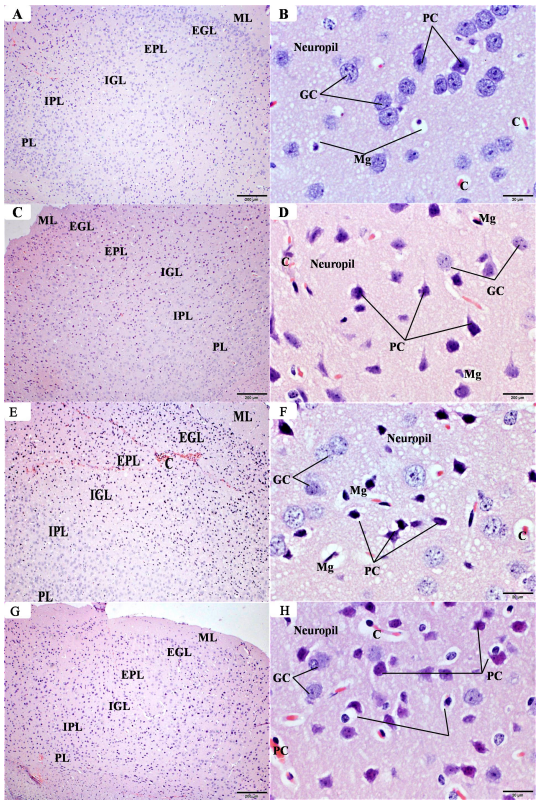

Fig. 6. Representative photomicrographs of H&E-stained sections of the prefrontal cortex (PFC) from mice in the different groups. (A,B) Control group. (A) Normal cortical delineation and cellular distribution of neurons and neuropil in the molecular layer (ML), external granular layer (EGL), external pyramidal layer (EPL), internal granular layer (IGL), internal pyramidal layer (IPL) and polymorphic layer (PL). (B) Pyramidal cells (PC) with a pale vesicular nucleus and a distinct nucleolus. Normal-appearing granular cells (GC), microglial cells (Mg) and blood capillaries (C) were also detected. (C,D) LPS (0.25 mg/kg) group. (C) Poor cortical delineation with increased darker neurons compared with the control tissue. (D) Pyramidal cells (PC) appeared condensed with dark nuclei. The capillaries were more congested with red blood cells compared with the control. The sections also showed more microglial cells and congested capillaries (C). (E,F) LPS (0.5 mg/kg) group. (E) Disorganized delineation of the cortical layers and increased darker neurons compared with control tissue. (F) The pyramidal cells appeared more condensed with dark cytoplasm. Furthermore, the neuropil appeared vacuolated with the presence of congested capillaries (C). (G,H) LPS (0.75 mg/kg) group. (G) Disrupted delineation of cortical layers compared with the control group. (H) Multiple condensed and dark pyramidal cells. These sections also showed more microglial cells (Mg) and congested capillaries (C). (A, C, E, G: ×40; B, D, F, H: ×400).

3.4 LPS chronic administration trigger neuronal death in PFC

In the control group, light microscopic examination revealed normal prefrontal tissue without any sign of pathological change. The control sections contained pyramidal cells with a pale vesicular nucleus and a distinct nucleolus, in addition to normal glial cells (Fig. 6A,B). In contrast, PFC sections from mice in the LPS 0.25 mg/kg group contained an increased number of darker degenerating neurons, compared with sections from control animals. In addition, the sections contained pyramidal cells with a dark nucleus and a prominent nucleolus. Furthermore, basophilic irregular neuronal cells devoid of distinct nuclei (necrotic cells) were detected. Glial cells were also observed (Fig. 6C,D). Sections from the LPS 0.5 mg/kg group contained a higher number of pyramidal cells with a pale vesicular nucleus and a prominent nucleolus compared with the control group. Pyramidal cells with dark vesicular nuclei and multiple processes were also detected. Additionally, some basophilic cells were surrounded by empty spaces (apoptotic cells) (Fig. 6E,F). In comparison, sections from the LPS 0.75 mg/kg group contained an increased number of darker neurons and fewer pyramidal cells with a pale vesicular nucleus an obvious nucleolus. Moreover, these sections contained a larger number of degenerating dark basophilic cells, compared with the control group (Fig. 6G,H).

4. Discussion

In this study, Swiss mice were used to investigate the dose-dependent PFC-related recognition memory deficits produced by chronic peripheral LPS administration. Neuroinflammation caused by chronic microglial hyperactivation has been identified as a leading cause of cognitive impairment and neurodegenerative disease [29]. By releasing numerous proinflammatory cytokines, hyperactivated microglia substantially increase brain neuroinflammatory and neurotoxic processes [5]. Elevation of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β), in addition to inflammatory mediators such as cyclooxygenase-2 (COX-2) and nitric oxide synthase (iNOS), is significantly associated with synaptic degeneration, neuronal death and cognitive impairment [4]. This highlights the importance of having an appropriate animal model to understand the pathogenesis of neuroinflammation-related cognitive impairment [30]. The LPS model is one of the most widely used models of systemic inflammation [31]. LPS administration can be performed either centrally via CNS infusion, or peripherally by single or multiple injections [28]. Accumulating evidence suggests that peripheral LPS disrupts brain signaling, resulting in behavioral, learning and memory deficits [19,31]. Peripheral LPS injection produces systemic inflammation and triggers proinflammatory cytokine synthesis and release within the brain. Furthermore, LPS disrupts the permeability and function of the blood–brain barrier, enabling peripheral proinflammatory cytokines from the circulation to enter the brain parenchyma [18]. Peripheral LPS also increases Iba-1$^+$ microglia and Aβ_{1-16}-containing neurons in the mouse hippocampus [32]. It has been reported that TNF-α levels in the hippocampus and frontal cortex are increased after 7 days of LPS administration [33]. This is associated with increased neuronal death, dopaminergic neuron loss and decreased autophagy biomarkers, all of which contribute to impaired learning and memory performance [18]. In addition, LPS given by acute or chronic peripheral administration can diminish recognition memory performance [28,34].

LPS may lead to multiple non-specific behavioral effects, and can reduce activity, social interaction and exploratory behavior in mice [17]. Thus, the OFT was performed, and body weight and temperature were recorded to evaluate general health and locomotion. Mice receiving LPS showed no significant differences in weight gain, temperature or locomotor activity during the course of LPS administration. Therefore, there was no interaction of these parameters with memory parameters [27].

Recognition memory is generally defined as the ability to judge whether an item or event is previously experienced [35]. Damage to the PFC has been found to affect recognition memory tasks [36]. The NORT is a behavioral task commonly used to evaluate visual object recognition memory in rodents, including PFC-dependent recognition memory [37,38]. Mice with normal PFC functions prefer to investigate the novel object rather than the familiar object, whereas mice with a damaged PFC are predicted to have no preference [39]. Our NORT results demonstrate recognition memory impairment in mice administered 0.75 mg/kg LPS. In particular, analysis of the frequency of sniffing (%) showed that the LPS 0.75 mg/kg group had no significant preference for either object, indicating a recognition memory deficit. To the best of our knowledge, this is the first study to demonstrate that LPS at a dose of 0.75 mg/kg causes PFC-related memory impairment and histological alterations. The LPS groups given the 0.25 and 0.5 mg/kg doses exhibited performance similar to the control group (i.e., a preference for the novel object over the familiar object). In line with our results, a previous study showed that adult male Swiss albino mice explored the novel object markedly more than the familiar object after 3 days of LPS 0.25 mg/kg injections [28]. In comparison, 7 days of LPS 0.75 mg/kg administration significantly impairs the ability to discriminate the novel and familiar objects [9]. In line with the previous study, we found here that LPS 0.75 mg/kg caused a significant decrease in the DI. While mice in the LPS 0.25 and 0.50 mg/kg groups showed reduced novel object discrimination, the differences were not significant. There is a lack of concordance in the literature on the effects of acute and chronic administration of 0.25 and 0.5 mg/kg LPS on recognition memory performance. For example, Czerniawski *et al.* [22] showed that acute LPS administration, by either i.p. or intracerebroventricular (i.c.v.) administration, impairs novel object recognition. In male Wistar rats injected with a single dose of LPS 0.50 mg/kg, there is a significant decrease in the ability to discriminate between the novel and familiar objects [40]. Moreover, several reports found that chronic administration of LPS 0.25 mg/kg for 7 days reduced discrimination in the novel object recognition task [8,24,41]. Feng *et al.* [42] reported that a single peripheral injection of $<$1 mg/kg failed to have a neurodegenerative effect. Whereas the majority of studies demonstrate a neuroinflammatory effect, Chen *et al.* [43] reported a neuroprotective action of LPS. Wang *et al.* [44] showed that pretreatment with 0.25 and 0.5 mg/kg LPS markedly mitigated inflammation in mice, and the authors also suggested that the 0.5 mg/kg dose had the optimal protective effect. This could explain our finding here that administration of 0.25 and 0.5 mg/kg did not affect performance in the NORT. Therefore, the experimental conditions (e.g., mouse strain, LPS dose, age, sex and genetic background) may affect whether peripheral LPS injections in mice have a neuroinflammatory or neuroprotective effect [19].

Here, we also assessed whether chronic peripheral LPS administration affected another type of recognition memory—spatial recognition. NADT is a simple two-trial hippocampus-dependent task commonly used to assess spatial recognition memory based on rodents' innate tendency

to investigate a new environment [45,46]. Our findings revealed that compared with the control group, all LPS groups significantly spent a shorter duration in the NA, reflecting a spatial recognition memory deficit. LPS administration resulted in no preference for the new arm throughout the exploration time.

To link the PFC neurodegeneration with the cognitive deficits, histological examination was conducted using H&E staining. In general, PFC neurodegeneration was observed in all sections from LPS-treated mice. However, each dose produced different degrees of degeneration. In particular, mice given the 0.75 mg/kg dose showed more dark neurons compared with the control group. In agreement with the behavioral results, the 0.75 mg/kg dose had the greatest impairment effect.

Taken together, our results demonstrate that the peripheral administration of 0.75 mg/kg LPS impaired both object and spatial recognition memory, while the 0.25 and 0.50 mg/kg doses significantly impaired spatial recognition memory only. Thus, we hypothesize that recognition memory deficits following chronic administration of 0.25 and 0.50 mg/kg LPS are likely to be associated mainly with hippocampal insult, whereas the 0.75 mg/kg dose impacts both the hippocampus and prefrontal cortex. Indeed, the 0.25 and 0.50 mg/kg groups had a frequency of sniffing (%) similar to the control group, indicating that a greater dose of LPS is needed to induce PFC-related recognition memory deficits. To the best of our knowledge, this is the first study linking the chronic administration of LPS at 0.50 and 0.75 mg/kg doses with PFC-related recognition memory impairment in mice.

5. Conclusions

In summary, we investigated the effects of administration of different doses of LPS on recognition memory and PFC histological changes in mice. Results of behavioral assessment and histopathological examination show that chronic peripheral administration of LPS at 0.75 mg/kg is the optimal dose for studying neuroinflammation-associated recognition memory deficits. Further study by immunostaining is needed to evaluate the effect of LPS on different neuronal and glial cells. Furthermore, electrophysiological investigations are required to clarify the contributions of the PFC and hippocampus to the recognition memory impairment induced by peripheral LPS administration.

Abbreviations

AD, Alzheimer disease; LPS, lipopolysaccharide; NORT, novel object recognition test; NDAT, novel arm discrimination test; OFT, open field; PFC, prefrontal cortex; NOR, novel object recognition; IP, aspartate transaminase; ICV, total bilirubin; mPFC, medial prefrontal cortex.

Author contributions

NAA, KAB, RAM and BSA designed the research study. NAA performed the research. KAB helped carried out the experiments. NAA analyzed the data and wrote the manuscript. RAM and BSA supervised the project. HMA provided critical feedback and helped shape the research. RAM, BSA and HMA contributed to editorial changes in the final manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All mouse experiments followed the protocols and guidelines of the Animal Unit Department at King Fahad Medical Research Center, Jeddah, SA. The study was approved by the Unit of Biomedical Ethics, Faculty of Medicine, King Abdul-Aziz University, Jeddah, SA (Reference No. 603-20), which complied with the "System of Ethics of Research on Living Creatures" guidelines prepared by King Abdulaziz City for Science and Technology, and were approved by Royal Decree No. M/59 dated 24/08/2010.

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Conflict of interest

The author declares no conflict of interest.

References

[1] DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. Journal of Neurochemistry. 2016; 139: 136–153.

[2] Kempuraj D, Thangavel R, Natteru PA, Selvakumar GP, Saeed D, Zahoor H, *et al*. Neuroinflammation Induces Neurodegeneration. Journal of Neurology, Neurosurgery and Spine. 2016; 1: 1003.

[3] Jin X, Yamashita T. Microglia in central nervous system repair after injury. Journal of Biochemistry. 2016; 159: 491–496.

[4] Huang X, Hussain B, Chang J. Peripheral inflammation and blood–brain barrier disruption: effects and mechanisms. CNS Neuroscience & Therapeutics. 2021; 27: 36–47.

[5] Huang C, Irwin MG, Wong GTC, Chang RCC. Evidence of the impact of systemic inflammation on neuroinflammation from a non-bacterial endotoxin animal model. Journal of Neuroinflammation. 2018; 15: 147.

[6] Muthuraju S, Zakaria R, Karuppan MKM, Al-Rahbi B. The Role of Neuroinflammation in Cellular Damage in Neurodegenerative Diseases. BioMed Research International. 2020; 2020: 9231452.

[7] Murray C, Sanderson DJ, Barkus C, Deacon RMJ, Rawlins JNP,

Bannerman DM, *et al*. Systemic inflammation induces acute working memory deficits in the primed brain: relevance for delirium. Neurobiology of Aging. 2012; 33: 603–616.e3.
[8] Frühauf PKS, Porto Ineu R, Tomazi L, Duarte T, Mello CF, Rubin MA. Spermine reverses lipopolysaccharide-induced memory deficit in mice. Journal of Neuroinflammation. 2015; 12: 3.
[9] Tyrtyshnaia A, Bondar A, Konovalova S, Sultanov R, Manzhulo I. N-Docosahexanoylethanolamine Reduces Microglial Activation and Improves Hippocampal Plasticity in a Murine Model of Neuroinflammation. International Journal of Molecular Sciences. 2020; 21: 9703.
[10] Barker GRI, Bird F, Alexander V, Warburton EC. Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. The Journal of Neuroscience. 2007; 27: 2948–2957.
[11] Xiang JZ, Brown MW. Differential neuronal encoding of novelty, familiarity and recency in regions of the anterior temporal lobe. Neuropharmacology. 1998; 37: 657–676.
[12] Wagner AD, Desmond JE, Glover GH, Gabrieli JD. Prefrontal cortex and recognition memory. Functional-MRI evidence for context-dependent retrieval processes. Brain. 1998; 121: 1985–2002.
[13] Duarte A, Ranganath C, Knight RT. Effects of unilateral prefrontal lesions on familiarity, recollection, and source memory. The Journal of Neuroscience. 2005; 25: 8333–8337.
[14] Euston DR, Gruber AJ, McNaughton BL. The role of medial prefrontal cortex in memory and decision making. Neuron. 2012; 76: 1057–1070.
[15] Zuberi A, Lutz C. Mouse Models for Drug Discovery. Can New Tools and Technology Improve Translational Power? ILAR Journal. 2016; 57: 178–185.
[16] Zhao J, Bi W, Xiao S, Lan X, Cheng X, Zhang J, *et al*. Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. Scientific Reports. 2019; 9: 5790.
[17] Zakaria R, Wan Yaacob WM, Othman Z, Long I, Ahmad AH, Al-Rahbi B. Lipopolysaccharide-induced memory impairment in rats: a model of Alzheimer's disease. Physiological Research. 2017; 66: 553–565.
[18] Batista C, Gomes GF, Candelario-Jalil E, Fiebich BL, de Oliveira A. Lipopolysaccharide-Induced Neuroinflammation as a Bridge to Understand Neurodegeneration. International Journal of Molecular Sciences. 2019; 20: 2293.
[19] Catorce MN, Gevorkian G. LPS-induced Murine Neuroinflammation Model: Main Features and Suitability for Pre-clinical Assessment of Nutraceuticals. Current Neuropharmacology. 2016; 14: 155–164.
[20] Lee JW, Lee YK, Yuk DY, Choi DY, Ban SB, Oh KW, *et al*. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. Journal of Neuroinflammation. 2008; 5: 37.
[21] De Luca SN, Soch A, Sominsky L, Nguyen T, Bosakhar A, Spencer SJ. Glial remodeling enhances short-term memory performance in Wistar rats. Journal of Neuroinflammation. 2020; 17: 52.
[22] Czerniawski J, Miyashita T, Lewandowski G, Guzowski JF. Systemic lipopolysaccharide administration impairs retrieval of context-object discrimination, but not spatial, memory: Evidence for selective disruption of specific hippocampus-dependent memory functions during acute neuroinflammation. Brain, Behavior, and Immunity. 2015; 44: 159–166.
[23] Pugh CR, Kumagawa K, Fleshner M, Watkins LR, Maier SF, Rudy JW. Selective effects of peripheral lipopolysaccharide administration on contextual and auditory-cue fear conditioning. Brain, Behavior, and Immunity. 1998; 12: 212–229.
[24] Salmani H, Hosseini M, Baghcheghi Y, Moradi-Marjaneh R, Mokhtari-Zaer A. Losartan modulates brain inflammation and improves mood disorders and memory impairment induced by innate immune activation: The role of PPAR-gamma activation. Cytokine. 2020; 125: 154860.
[25] Heisler JM, O'Connor JC. Indoleamine 2,3-dioxygenase-dependent neurotoxic kynurenine metabolism mediates inflammation-induced deficit in recognition memory. Brain, Behavior, and Immunity. 2015; 50: 115–124.
[26] Ramírez K, Quesada-Yamasaki D, Fornaguera-Trías J. A Protocol to Perform Systemic Lipopolysacharide (LPS) Challenge in Rats. Odovtos - International Journal of Dental Sciences. 2019; 21: 53–66.
[27] Labban S, Alghamdi BS, Alshehri FS, Kurdi M. Effects of melatonin and resveratrol on recognition memory and passive avoidance performance in a mouse model of Alzheimer's disease. Behavioural Brain Research. 2021; 402: 113100.
[28] Borikar SP, Dongare SI, Danao KR. Reversal of lipopolysaccharide-induced learning and memory deficits by agmatine in mice. The International Journal of Neuroscience. 2021; 1–12. (in press)
[29] Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N. Neuroinflammation as a Common Feature of Neurodegenerative Disorders. Frontiers in Pharmacology. 2019; 10: 1008.
[30] Saito T, Saido TC. Neuroinflammation in mouse models of Alzheimer's disease. Clinical & Experimental Neuroimmunology. 2018; 9: 211–218.
[31] Nazem A, Sankowski R, Bacher M, Al-Abed Y. Rodent models of neuroinflammation for Alzheimer's disease. Journal of Neuroinflammation. 2015; 12: 74.
[32] Katafuchi T, Ifuku M, Mawatari S, Noda M, Miake K, Sugiyama M, *et al*. Effects of plasmalogens on systemic lipopolysaccharide-induced glial activation and β-amyloid accumulation in adult mice. Annals of the New York Academy of Sciences. 2012; 1262: 85–92.
[33] Bossù P, Cutuli D, Palladino I, Caporali P, Angelucci F, Laricchiuta D, *et al*. A single intraperitoneal injection of endotoxin in rats induces long-lasting modifications in behavior and brain protein levels of TNF-α and IL-18. Journal of Neuroinflammation. 2012; 9: 101.
[34] Sorrenti V, Contarini G, Sut S, Dall'Acqua S, Confortin F, Pagetta A, *et al*. Curcumin Prevents Acute Neuroinflammation and Long-Term Memory Impairment Induced by Systemic Lipopolysaccharide in Mice. Frontiers in Pharmacology. 2018; 9: 183.
[35] Biederman I. Recognition-by-components: a theory of human image understanding. Psychological Review. 1987; 94: 115–147.
[36] Szczepanski SM, Knight RT. Insights into human behavior from lesions to the prefrontal cortex. Neuron. 2014; 83: 1002–1018.
[37] Alghamdi BS, AboTaleb HA. Melatonin improves memory defects in a mouse model of multiple sclerosis by up-regulating cAMP-response element-binding protein and synapse-associated proteins in the prefrontal cortex. Journal of Integrative Neuroscience. 2020; 19: 229–237.
[38] Pezze MA, Marshall HJ, Fone KC, Cassaday HJ. Role of the anterior cingulate cortex in the retrieval of novel object recognition memory after a long delay. Learning & Memory. 2017; 24: 310–317.
[39] Reid JM, Jacklin DL, Winters BD. Delineating prefrontal cortex region contributions to crossmodal object recognition in rats. Cerebral Cortex. 2014; 24: 2108–2119.
[40] Jamali-Raeufy N, Kardgar S, Baluchnejadmojarad T, Roghani M, Goudarzi M. Troxerutin exerts neuroprotection against lipopolysaccharide (LPS) induced oxidative stress and neuroinflammation through targeting SIRT1/SIRT3 signaling pathway. Metabolic Brain Disease. 2019; 34: 1505–1513.

[41] Tan W, Zhang Q, Dong Z, Yan Y, Fu Y, Liu X, *et al*. Phosphatidylcholine Ameliorates LPS-Induced Systemic Inflammation and Cognitive Impairments via Mediating the Gut–Brain Axis Balance. Journal of Agricultural and Food Chemistry. 2020; 68: 14884–14895.

[42] Feng X, Hu H, Chen J, Long C, Yang L, Wang L. Acute neuroinflammation increases excitability of prefrontal parvalbumin interneurons and their functional recruitment during novel object recognition. Brain, Behavior, and Immunity. 2021; 98: 48–58.

[43] Chen Z, Jalabi W, Shpargel KB, Farabaugh KT, Dutta R, Yin X, *et al*. Lipopolysaccharide-induced microglial activation and neuroprotection against experimental brain injury is independent of hematogenous TLR4. The Journal of Neuroscience. 2012; 32: 11706–11715.

[44] Wang D, Liu Y, Zhao Y, Zhou J. Low dose of lipopolysaccharide pretreatment can alleviate the in? Ammatory response in wound infection mouse model. Chinese Journal of Traumatology. 2016; 19: 193–198.

[45] Sarnyai Z, Sibille EL, Pavlides C, Fenster RJ, McEwen BS, Toth M. Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin (1a) receptors. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97: 14731–14736.

[46] Wang JH, Ma YY, van den Buuse M. Improved spatial recognition memory in mice lacking adenosine a2a receptors. Experimental Neurology. 2006; 199: 438–445.