A Pro-Inflammatory Agent, Lipopolysaccharide, Can Mimic the Effects of Aging on Spatial Reference Memory

By
Emily Escobedo

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APPROVED:

Kathy Magnusson, Biomedical Sciences, CVM — 6/30/14
Date

Katharine G. Field, BRR Director — Date

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Emily Escobedo — 06/13/2014
Date

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Abstract

Humans and rodents experience declines in reference (long-term) and working (short-term) memory and cognitive flexibility during the aging process. Aging changes in GluN1 and GluN2B subunits of N-methyl-D-aspartate (NMDA) receptors show a relationship to both reference and working memory deficits. Sulindac, an anti-inflammatory drug, enhances working memory and NMDA receptor subunit expression in rats. The hypothesis addressed in the present study was that inflammation plays a role in NMDA receptor aging and memory declines. The question addressed was whether a pro-inflammatory treatment in young mice would produce the same changes in memory and NMDA receptor expression as aging.

Lipopolysaccharide (LPS) was the pro-inflammatory treatment that was used to induce inflammation in 3 month old mice by surgical procedure. Male C57BL/6 mice (3 month old) were randomly assigned to 3 treatment groups, lipopolysaccharide (LPS), saline, or non-surgical. Non-surgical (24 month old) mice were also included. These different types of treatment were then randomly assigned to behaviorally characterized or naïve. The behaviorally characterized animals were behaviorally tested with the Morris water maze. The naïve animals were housed for the same amount of time being. The surgical procedure consisted of cannulas that were attached to osmotic pumps, then implanted into the lateral ventricles of the brain for 3 weeks in order to inject all of the LPS or saline solution. One week after pumps were removed, behavioral testing was performed with the Morris water maze. LPS-treated young (cumulative proximity (cm): 6287 ± 625; RANOVA & Fisher's LSD) performed significantly worse than saline young (Mean: 4565 ± 352) and similarly to old mice (Mean: 7519 ± 389) in reference memory place trials. LPS treated young (average proximity (cm): 40 ± 1.4) performed similarly to saline young (35 ± 1.3) and old (45± 1.5) performed the poorest in probe trials for reference memory. LPS

didn't appear to have any effect on reversal, working memory, or cued trials. The average swim speed for reference memory and cued trials showed that the LPS treated mice were the fastest swimmers, suggesting that any deficits were not due to poor motor ability. In situ hybridization for the NMDA subunits GluN2B, GluN2A, GluN1 and receptor autoradiography, using the agonist glutamate and the competitive antagonist [(±)-2-carboxypiperazin-4-yl] propyl-1-phosphonic acid (CPP), were performed on sections from C57Bl/6 mice There were no significant effects of LPS on mRNA densities for the GluN2B, GluN2A, or GluN1 subunits. In conclusion, stimulating inflammation in a young brain produced only some of the memory deficits seen in aging.

Introduction

We live in a world where our population is aging. In 2010, an estimated 524 million people were aged 65 or older-8 % of the world's population. By 2050, this number is expected to nearly triple to about 1.5 billion, representing 16 % of the world's population (1). The sheer number of people achieving older ages will bring increased challenges to health systems and will have an influence on individuals' lives at large. According to Singh-Manoux et al., 2012, "Poor cognitive status is perhaps the single most disabling condition in old age" (2). Studies demonstrate that approximately 40 % of people aged 65 years or older can be diagnosed with some form of age associated memory impairment (3). Declines in memory performance range from normal age-related declines to Alzheimer's Disease (AD), which is the most common form of dementia. Other studies suggest that working memory and the speed of processing information gradually decline throughout the adult life span (4). Spatial memory, a type of memory that represents the ability of the organism to acquire and retain information in order to navigate properly through

space, also declines with age. Rodents experience similar deficits in spatial memory abilities as humans and have been used as a model for age-related memory declines (5).

There is evidence that several brain regions are associated with spatial memory functions. The hippocampus is a very important brain region for spatial memory tasks. The hippocampus is an allocortical structure with three layers, molecular, pyramidal, and polymorphic, located on the medial surface of the temporal lobe. Decades of research have now given us a much broader idea of the structure, cellular function, and circuitry of the hippocampus to help explain the processes of memory (6). There are positive correlations between performance in spatial reference memory tasks and NMDA receptor binding and GluN1 and Glun2B subunits in the hippocampus across aging (7). Because the structure is well conserved throughout all species, studies in animals have been critically informative about human hippocampal functions.

Another brain region that is important for spatial memory functions is the prefrontal cortex. The prefrontal cortex is the part of the cerebral cortex that receives projections from the mediodorsal nucleus of the thalamus (8). The prefrontal cortex is involved in working memory tasks. The prefrontal cortex shows a greater rate of decrease in volume per decade with increasing age in humans (4.3-4.9%) than the hippocampus (2%) (9). Studies show that there are regional differences in function in the prefrontal cortex that may be significant to the cause of age-related memory declines. The lateral prefrontal cortex of mice shows greater significant declines in density of agonist binding to NMDA receptors during aging than another region, the medial cortex (10).

One type of excitatory glutamate receptor, the N-methyl-D-aspartate (NMDA) receptor, is expressed in high density in the cortex and hippocampus and has been shown to be important for learning and memory, including spatial memory (11). The NMDA receptor is important for long-term potentiation, a cellular mechanism for the formation of memory (11). Antagonists of the NMDA receptor block the initiation of long-term potentiation and produce spatial memory deficits. The NMDA receptors appear to be more vulnerable to the aging process than other glutamate receptors and show declines in their binding densities, electrophysiological functions, and influence on other transmitter systems (11).

NMDA receptors are excitatory receptors that form ion channels on neurons. They are heteromeric receptors composed of protein subunits, consisting of two GluN1 (earlier names: NMDAR1, NR1) subunits and two other subunits, either from the GluN2 (GluN2A-D, formerly 1-4 in mice) or GluN3 families (GluN3A-B). Eight different splice variants of the GluN1 subunit have been identified in the brain. The GluN1 subunit shows a 99 % amino acid homology between the mouse, rat, and human (12). Studies show that there is decreased expression in C57BL/6 mice of the mRNA for 2 subunits (GluN2B and GluN1) of the NMDA receptor in sub-regions of the hippocampus with increased age (7), while the GluN2A subunit receptors increase over time. The GluN2B subunit of the NMDA receptor shows the greatest age-related declines in expression across multiple brain regions, including the prefrontal cortex, the caudate nucleus, and the hippocampus (13).

There is increasing evidence for a role of inflammation in the aging process. Increased inflammation is a feature of brain aging that is conserved from mouse to man. Inflammatory

responses increase in aged rats, including increases in activated microglia and activated astrocytes (9). Epidemiologic studies suggest that individuals treated with non-steroidal anti-inflammatory agents for arthritis may have a lower incidence of Alzheimer's disease (14). These and similar findings led to the inflammation hypothesis of aging. There is evidence that the protein expression of the GluN1 and GluN2B subunits in aged rats can be increased following treatment with an anti-inflammatory drug, sulindac. It is not known, however, at what age this is effective or whether it protects at the level of mRNA and/or protein (9). Several possible mechanisms may account for an influence of inflammation on NMDA receptor subunit expression including changes in activity of the receptor that could lead to alterations in mRNA expression.

Lipopolysaccharide (LPS) is one of the most powerful bacterial virulence factors with pro-inflammatory properties. LPS is an endotoxin and the major component of the outer cell membrane of gram-negative bacteria. It contains three different regions, lipid A, core, and O-polysaccharide chain. The lipid A region is the highly hydrophobic and endotoxically-active part of the molecule (15). The core is separated into the inner and outer core, with the inner core highly conserved and the outer core more likely to contain sugars such as hexoses. The final region, known as the O-chain, is highly variable and is the primary target for antibody responses against LPS. LPS has been shown with chronic administration in rat brain to produce both inflammation and decreases in the GluN1 protein expression (16). LPS was used in the present study as the pro-inflammatory agent.

The purpose of this research project was to determine whether a pro-inflammatory treatment in young mice would produce the same changes in memory and NMDA receptor mRNA expression as seen in aging. The hypothesis addressed in the present study was that inflammation plays a role in NMDA receptor aging and memory declines. We addressed this hypothesis by treating 3 month old mice with lipopolysaccharide, then challenging the treated and control mice with behavioral tests, and finally measuring the mRNA expression in the brains of treated and control mice. A better understanding of the underlying causes of these memory declines during aging is necessary for the development of appropriate treatments or preventions for memory dysfunction as we grow older. These treatments may also be beneficial in delaying some of the symptoms of Alzheimer's disease (12).

Experimental Procedures

Animals and Treatment Groups:

Male C57BL/6 mice of different ages (3 & 24 months old) were used for the study.

Study 1: The young (3 month old; 60 total) mice were randomly assigned to 3 treatment groups: lipopolysaccharide (LPS), saline, or non-surgical. The 24 month olds (20 total) had no treatment and were designated non-surgical. The animals were divided into two experience groups, behaviorally-characterized (N=12 for treatment) and naïve (N= 8 for each treatment). The animals in the behaviorally-characterized group were subjected to a learning experience with the use of the Morris water maze. The naïve group animals were housed for the same amount of time as the behaviorally-characterized animals. I was involved in the behavioral testing for this study.

Study 2: Three month old mice (20 total) were randomly assigned to 2 treatment groups: LPS or saline and 2 pump groups: 1 pump on right side or 2 pumps, with an N=5 at the start of the study.

One mouse in the saline, 2 pump group lost his cannula and couldn't continue in the testing. These mice were behaviorally tested as described below, but I was only involved in the *in situ* hybridization experiments for this study.

The surgical animals had a unilateral or bilateral cannula, attached to one or two osmotic pumps, implanted with the use of stereotaxic surgery into the lateral ventricles. Each pump contained 600 µg/kg of either the LPS or sterile saline. The pumps delivered solution for 3 weeks, and then the pumps were removed and behavioral testing began a week after.

Surgical Procedure:

Mice were given 0.1 mg/kg buprenorphine, subcutaneous, 10-15 minutes prior to anesthesia induction. Buprenorphine was used to provide pain relief of moderate to severe pain in the mice. The mice were anesthetized with isoflurane at 4% and oxygen at 1 L/minute and then placed in the stereotaxic apparatus. An incision was made in the skin down the center of the skull from between the eyes to the occipital ridge. Following the incision, a pocket was created between the skin and subcutaneous fascia on the dorsum between the shoulder blades in order to place the osmotic pumps. Once the pumps were in place, the cannula was secured in the cannula holder on the stereotaxic arm, and the medial/lateral, and anterior/posterior measurements were measured using the sutures on the skull (-.22 mm AP, ±1 mm ML, -2.5 to 3.0 mm DV). Afterwards, the location of the lateral ventricles relative to the suture measurements were calculated, with the use of a mouse stereotaxic atlas (17) and the cannulae were moved to the new coordinates. The dorsal/ventral coordinates on the skull were measured at both sites. Two holes were drilled through the skull. The cannulae were lowered into the ventricles through the drilled holes. After

the cannulae were in place, superglue was used to attach the cannulae to the skull and on the incised skin around it. When everything was securely in place, and the superglue was dry, the isoflurane was turned off and the oxygen delivery rate was moved up to 2 L/minute. The animal was removed from the apparatus and placed in a recovery cage, set to 37^{o} C, until fully awake. After the animal started ambulating, 0.1 ml of buprenorphine was given and then the animal was placed in a clean cage. Buprenorphine injections were continued twice daily for 3 days for the saline mice and 7 days for the LPS mice. Saline mice were only given the buprenorphine for 3 days because we were concerned about the side effects of giving opiates in the absence of considerable pain. The animal's rectal temperature was monitored continuously with a digital thermometer (Physitemp Instruments, Clifton, N.J.).

Behavioral Testing:

The Morris water maze was used to perform behavioral testing. A four-foot diameter tank was filled with water (between 16-18^{o} C) and made opaque white with non-toxic paint. The water level was approximately 1 cm above the level of the hidden platform. There were spatial cues located high on the walls, the ceiling, and high on the tank. These cues included circular objects, a star, towel, etc. The trials were videotaped with a video camera placed on the ceiling directly above the center of the tank and analyzed with the use of the "SMART" Video Tracking System (San Diego, Instruments, San Diego, CA). Each mouse individually began the trial to find the platform by being placed into the tank facing the wall at different entry points. If the mouse did not find the platform within the time limit, they were led to it by the handler. Following each mouse's trial, the handler put the mouse back in its temporary cage and placed a heating lamp above the cage. The behavioral testing included acclimation, reference memory, reversal, and working memory testing, and a cued control task.

Acclimation: The first two days consisted of acclimation in which the mouse swam for 60 seconds with no platform present. Then the platform was placed in the center of the tank and the mice were trained to remain on the platform for 30 continuous seconds. This testing allowed the mice to become familiar with the maze environment without spatial cues and the platform rules.

Reference Memory Testing: Mice underwent 8 place trials per day for 4 days from day 3 through 6. The platform was kept in the SE quadrant for each place trial. The entry points for the mice were randomly assigned, as SW, NE, or NW. Each mouse had a maximum of 60 seconds to find the platform, and then 30 seconds remaining on the platform. The mice performed 4 trials, rested for an hour, performed 4 more trials, and rested for another hour before performing a probe trial. An initial probe trial was conducted before reference training on the first day. The rest of the probe trials occurred after the last place trial each day. During the probe trials, the platform was removed and the mice were allowed to search in the water for 30 seconds from the NE start position. The probe trials were conducted to assess the animal's ability to show a bias for the platform location.

Cognitive Flexibility Testing: Mice were tested for 1 day in reversal trials on day 7. The protocol was the same as described above for place trials. The platform was located in the quadrant diagonally opposite (NW) to the reference memory trials and entry points were NE, SE and SW.

Working Memory Testing: From day 8 to 11, mice were tested for working memory. The working memory task had 2 sessions per day, an a.m. and a p.m. session, for 4 days. The platform position was changed between each session. For each session, there were 4 trials for each mouse. All trials had a 60 second time limit to search for the platform, and then spent 30 seconds on the platform after each trial (except for the 4th trial). The trials included a naïve trial, followed by cage rest for 10 minutes. In the second trial designated as the delay trial, the mouse

was placed in a different entry point and was allowed to search for the platform. The mouse remained on the platform for 30 seconds, but was not allowed cage rest after the second trial, and was put in the water a third and fourth time from different entry points. Following the four trials, the mice were placed in their cages until the next session later that day.

Cued Control Trials: Cued trials were used to test motivation and physical ability for the task. On day 12, the cued control task was done by removing all the spatial cues from around the room and tank and performing 6 trials in one day. For each trial the platform was placed in one of six locations with a flag located in the center of the platform. For each trial, the mouse was placed into the tank facing the wall at one of the entry points and was allowed to search for the platform for 60 seconds, without having to remain on the platform for 30 seconds as in previous testing.

Analysis:

With the use of the SMART tracking program, cumulative proximity to the platform was obtained for place, reversal, working, and cued trials. Average proximity was calculated for probe trials. Age-related differences in performance in working and reference memory, cognitive flexibility, and cued tasks were analyzed separately by repeated measures ANOVA and two-way ANOVA followed by post-hoc analysis with Fisher's protected least significant difference test with the use of Statview software.

In situ Hybridization:

Following the cued trials, mice were euthanized by exposure to CO_2. The brains were removed, frozen in dry ice, and stored in -80^{o} C freezer. For Study 2, one half of each brain was sectioned horizontally through the frontal cortex, intermediate hippocampus and cerebellum. ^{14}C standards- and ^{33}P-dATP were used on each film. *In situ* hybridization was performed according to the

method of Watanabe, et al., 1992 (18). Each solution step was performed with gentle rotation on a rotating table except for the fixation and hybridization steps. Slides with sections were thawed, air-dried, fixed in 4% paraformaldehyde–PBS, pH 7.2 (25°C) for 15 min, placed in 2 mg/ml glycine in PBS, pH 7.2 (25°C) for 20 min, and placed in 0.25% acetic anhydride–0.1 M triethanolamine, pH 8.0 (25°C) for 10 min. Slides were placed in coplin jars for 2 hr in a prehybridization solution that consisted of 50% formamide, 0.1 M Tris-HCl, pH 7.5, 4XSSC (1X SSC = 150 mM NaCl and 15 mM sodium citrate), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 2% sarkosyl, and 250 μg/ml salmon testes DNA. Chemicals were purchased from Sigma (St. Louis, MO). Slides were then successively washed for 5 min each in 2X SSC, 70 and 100% ethanol, and air-dried for 15 min. Hybridization was performed by placing 100 μl of prehybridization solution with 10% dextran sulfate and 1 X 10^6 dpm of ^{33}P-labeled oligonucleotide probe added onto the slides, coverslipping the slides with parafilm, and incubating them for 18 hr in a 42°C oven humidified with 5X SSC. Coverslips were removed, and slides were rinsed for 40 min each in 2X SSC and 0.1% sarkosyl (25°C) and twice in 0.1X SSC and 0.1% sarkosyl (55°C) and air-dried. Nonspecific binding was determined by addition of 20-fold excess cold oligo to the hybridization solution on some slides. Sections were exposed to Hyperfilm-β max (Amersham, Piscataway, NJ) for 3–5 d along with brain paste standards. The standards were prepared by homogenizing whole brain from 12 3-month-old mice and mixing in measured amounts of ^{33}P-dATP (Marks et al., 1992). The actual concentrations were determined by scintillation counting of weighed aliquots and ranged from 95,000 to 14 cpm/mg wet weight of brain tissue. Brain and standard images were captured using a Macintosh IIci computer with a Quickcapture board, a Panasonic CCD camera, and NIH Image software. Quantitative densitometry was performed on the images from four sections for total binding and two sections

for nonspecific binding from each animal with the use of NIH Image software. The standards were used to convert optical density to counts per minute per milligram of tissue. Specific signal was determined by subtracting nonspecific binding from total binding. Slides were then dipped in photographic emulsion (NTB2; Eastman Kodak, Rochester, NY), exposed for 8–12 weeks, developed in D19 developer (Eastman Kodak), and counterstained with a Giemsa stain. Images of cells and grains within specific brain regions were captured on the computer as described above. Grain area was determined by filtering images to highlight grains (Smolen and Beaston-Wimmer, 1990), thresholding, and analyzing particles within 12 cells per slide for a total of 48 cells for total binding and 24 cells for nonspecific binding for each animal (Watanabe et al., 1993). An overall average for cells and for animals was calculated, and specific binding was determined as described for film analysis (18).

Results

Reference Memory:

The ANOVA showed a main effect of age/treatment type ($F_{(2,31)}$= 8.9, P = 0.0009) and day ($F_{(2,31)}$= 8.139, P = 0.0014) for reference memory place trials. Mean ±SEM values for reference memory place trials by days are shown in Table 1. All groups showed declines in cumulative proximity over days 1 to day 3 (Table 1). A large decrease was observed across all treatment groups and place trial days, and this is visualized in Figure 1. There were also significant differences between LPS treated young, saline young, and old ($F_{(2,31)}$= 9.7, P = 0.0005) mice in reference memory probe trials when averaged over all the respective trials (Figure 2). LPS-treated young (17.5 ± 0.41 cm/s) and saline young (15.5 ± 0.75 cm/s) mice were observed to have significantly faster swim speeds than old mice (14.1 ± 0.43 cm/s) when averaged over the respective trials ($F_{(2,31)}$= 10.7, P = 0.0003) (Figure 3).

Table 1: Mean cumulative proximity (cm) values for Reference memory place trials by days.

Treatment type	Day 1	Day 2	Day 3	Day 4
LPS young	10,249±765	6,550±997	4,321±765	4,027±922
Saline young	7,357±851	4,431±737	3,409±601	3,800±644
Old no treatment	9,904±833	8,537±872	6,128±559	5,507±617

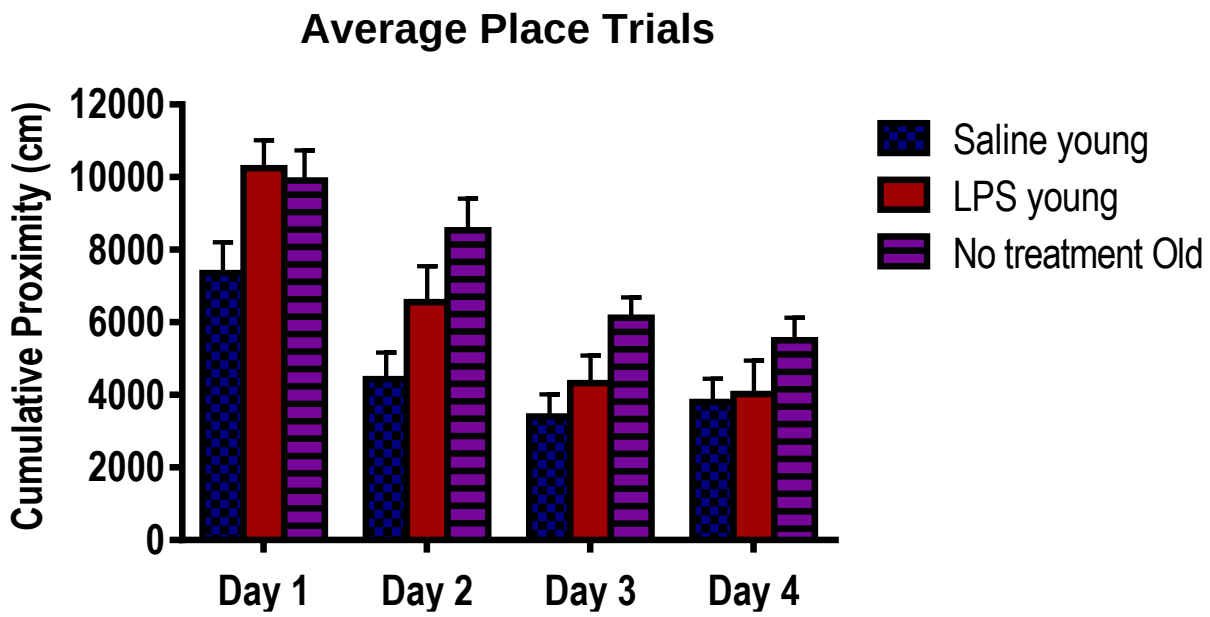


Figure 1: Place trials for reference memory. Graph showing treatment effects on performance. The place trials were averaged for each reference memory testing day. Mean ±SEM, ANOVA & Fisher's PLSD, N=12.

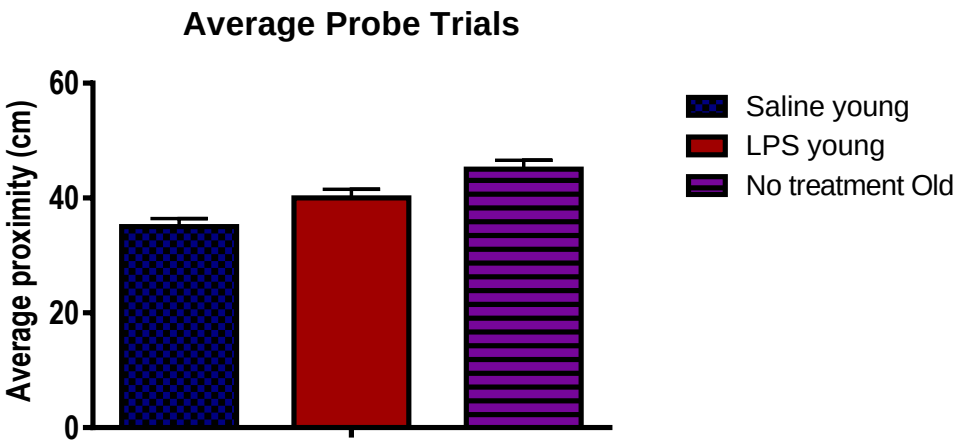


Figure 2: Probe trials for reference memory. The probe trials were assessed by average proximity in cm. Mean ± SEM, ANOVA & Fisher's PLSD, N=12.

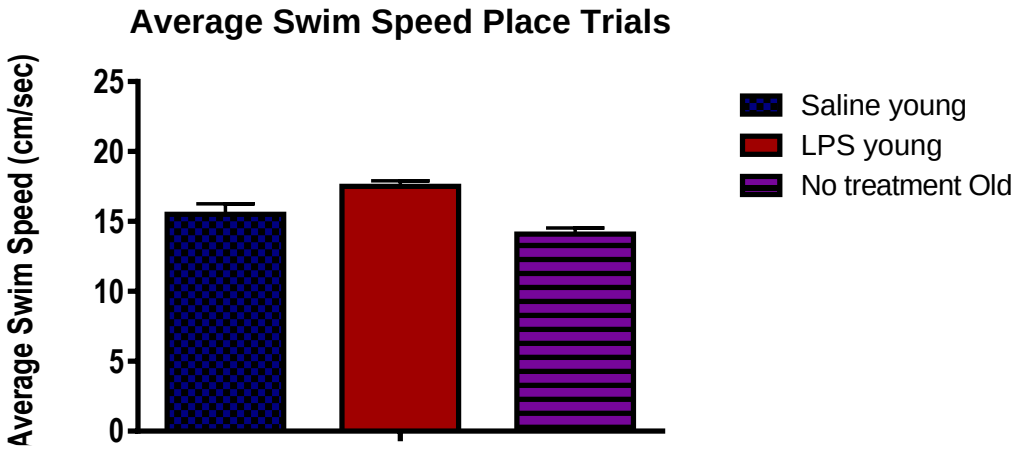


Figure 3: Swim speed in reference memory trials. The graph shows the average swim speed for place trials measured in cm/sec. Mean ±SEM, ANOVA & Fisher's PLSD, N=12.

Cognitive Flexibility, Working Memory, and Control Task:

The ANOVA for Reversal trials showed no significant difference in cumulative proximity between LPS young and Saline young mice ($F_{(1,21)}$= .001, P = 0.98) (Figure 4). Working memory trials showed performance from the delayed trial to be significantly lower than the naive trial ($F_{(1,18)}$= 11.14, P = 0.0037). However, neither the naïve nor the delayed showed significant

difference between the treatment types ($F_{(1,18)}$= .673, P = 0.42). This can be observed in Figure 5.A.

The mice analyzed above in the reference and working memory tasks, did not show a significant effect of treatment ($F_{(1,21)}$= 1.94, P = 0.18) on cumulative proximity scores in the cued control trials (Figure 5.B). LPS-treated young (22.5 ± 0.76 cm/s) and saline young (21.7 ± 1.1 cm/s) mice were observed to have significantly faster swim speeds than old mice (17.0 ± 1.1 cm/s) when averaged over the respective cued trials ($F_{(2,32)}$= 9.5, P < 0.05) (Figure 5.C).

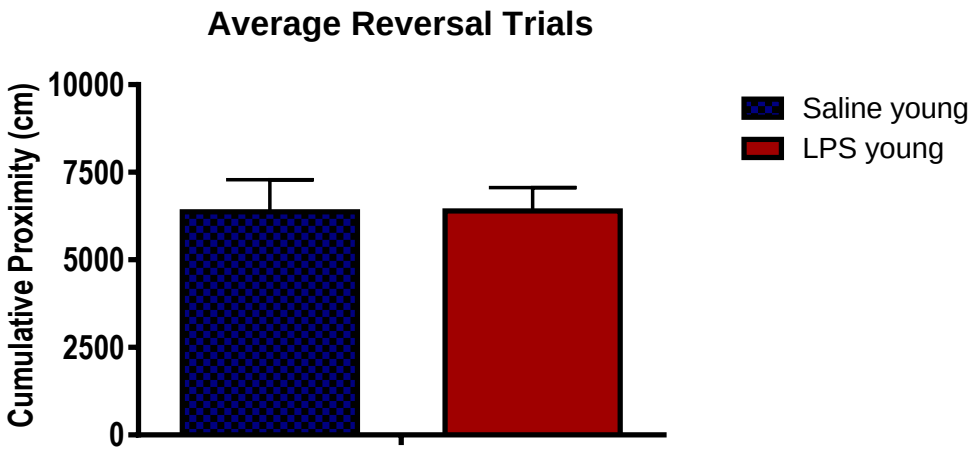
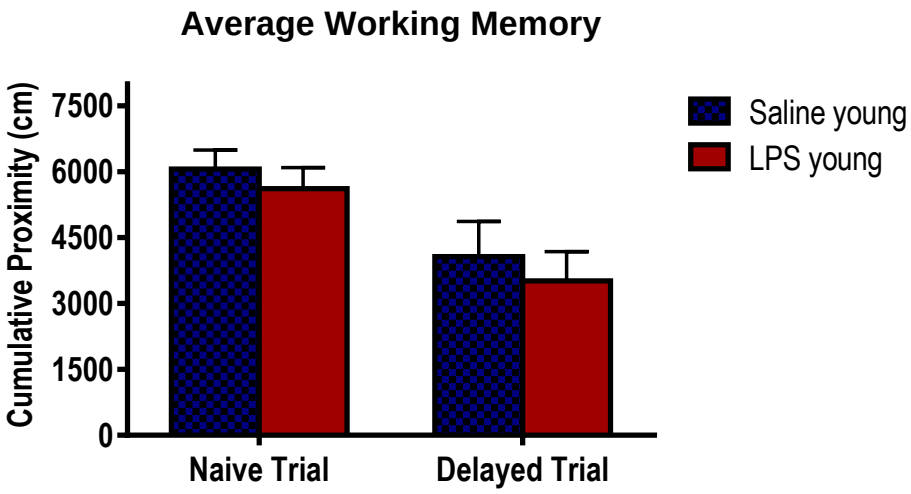


Figure 4: Average Reversal trials required changing the platform position to the opposite quadrant. Mean ±SEM, ANOVA & Fisher's PLSD, N=12.

Figure 5:

A.



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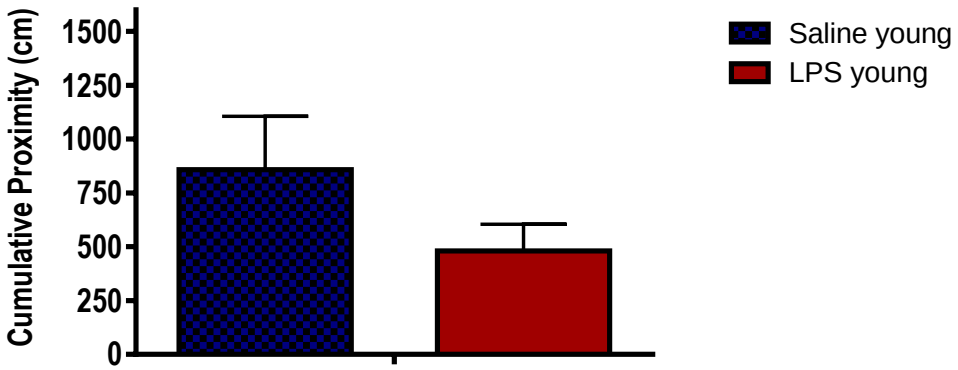

C.

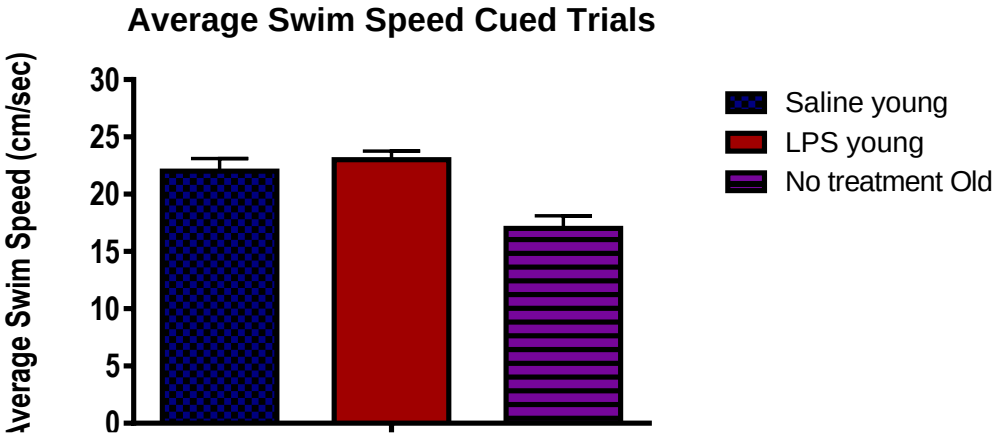

*Figure 5: **A**. shows the averages of eight working memory sessions, one a.m. and one p.m. session per day. **B**. Control task showed no effect of treatment in LPS versus saline treated young mice. **C**. Shows the average swim speed in cm/sec for the cued trials. Mean ±SEM, ANOVA & Fisher's PLSD, N=12.*

In Situ Hybridization:

GluN2B subunit:

The ANOVA for all brain regions showed no significant effect of LPS treatment ($F_{(1,16)} = .36$, $P = 0.56$) or brain side/pump number ($F_{(1,16)} = .002$, $P = 0.96$) on mRNA densities for GluN2B subunit. There were also no significant interactions between treatment and brain side/pump number ($F_{(1,16)} = .63$, $P = .44$). This can be observed for the deep layers of orbital cortex in Figure 6.B. LPS showed no significant effect on mRNA densities in the caudate nucleus ($F_{(1,18)} = .23$, $P= .64$) and hippocampal brain regions ($F_{(1,18)} = .71$, $P= .41$) when data was collapsed across brain side and pump number (Figure 6.A). The frontal cortical regions also showed no significant effect of LPS on mRNA density for GluN2B ($F_{(1,18)} = .2$, $P = .66$; Saline: .42±.02, LPS: .41±.02; not illustrated in graphs).

GluN1 subunit:

The ANOVA for all brain regions showed no significant effect of LPS treatment ($F_{(1,16)} = .36$, $P = .55$) or brain side/pump number ($F_{(1,16)} = .37$, $P = 0.55$) on mRNA densities for all GluN1 subunits. There were no significant interactions between treatment and brain side/pump number on the mRNA for GluN1pan subunit ($F_{(1,16)} = .0004$, $P = .98$), as illustrated by the deep layers for the orbital cortex (Figure 6.D). The averages for caudate nucleus and hippocampus are shown, collapsed across brain regions and side/pump numbers (Figure 6.C). The frontal cortical regions also showed no significant effect of LPS on mRNA density for GluN1 ($F_{(1,18)} = .8$, $P = .38$); Saline: .45±.02, LPS: .42±.03; not illustrated in graphs).

GluN2A subunit:

The ANOVA for all brain regions showed no significant effect of LPS treatment ($F_{(1,16)} = .00001$, $P = .997$) or brain side/pump number ($F_{(1,16)} = 2.9$, $P = .11$) on mRNA densities for all GluN2A subunits. Analysis of the GluN2A subunit showed no significant differences between LPS and saline treatment on mRNA densities in the caudate nucleus (Saline: .0059±.001, LPS: .0064±.002; not illustrated in graphs) and hippocampus brain regions (Saline: .58±.015, LPS: .58±.014; not illustrated in graphs) on GluN2A mRNA density. The frontal cortical regions also showed no significant effect of LPS on mRNA density for GluN2A ($F_{(1,18)} = .8$, $P = .38$; Saline: .08±.005, LPS: .08±.006; not illustrated in graphs).

Figure 6.

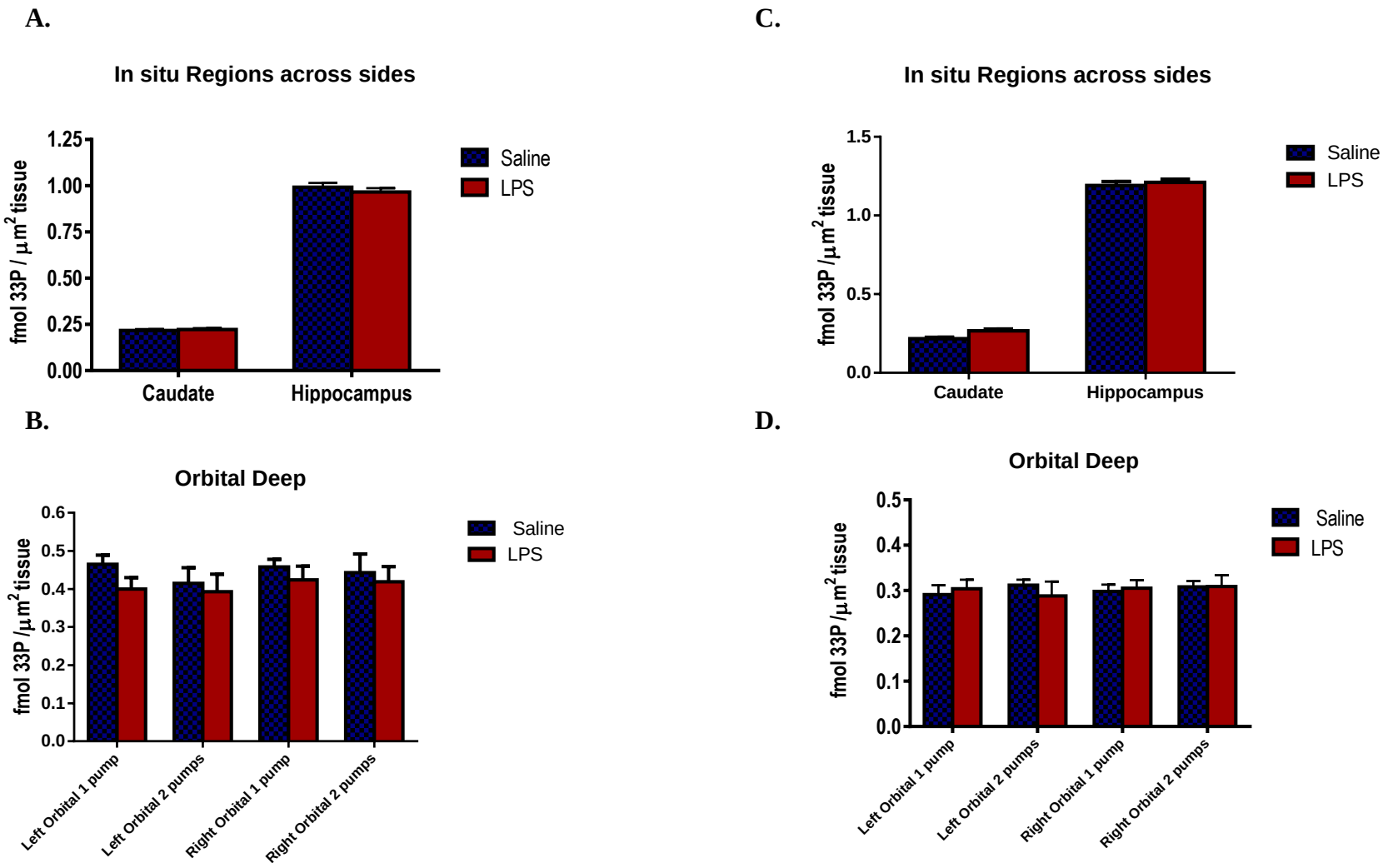

Figure 6: Graphs showing densities of mRNA expression of GluN2B (A,B) and GluN1pan (C,D). ***A****. and* ***C****. in caudate and hippocampus regions of mice brain.* ***B****. and* ***D****. for the deep layers (IV-VI) of the orbital cortex.*

Discussion

The Morris water maze was used to test the spatial reference memory performance in mice. Using this task, we observed that the LPS treatment interfered with reference memory. There was a trend for more interference early in the learning process, but LPS treated mice performed similarly to saline controls by Day 4. Previous studies show that hippocampal NMDA receptors appear to be important for early learning (13). This would suggest that the LPS treated mice need more repetition, but not as much as the old mice. LPS mice had significantly faster swim speeds than saline and old mice in the place trials. This would suggest that swim speed doesn't account for the deficits in reference memory.

LPS did not affect all cognitive functions tested. There was no effect of LPS on working memory and reversal task. These functions are more associated with the frontal cortex region (19).
In the cued control task, the LPS treated showed trends of better performance than the saline controls. This indicated that LPS had no negative effects on visual acuity, physical ability for the task, or motivation, unless it may have increased their motivation.

Swim speed differences were observed in the water maze tasks used. Because of this, path length was not considered a good option. The reason for this is that when initially placed in the tank, older mice tended to float. Cumulative and average proximity measures used in this study were corrected for ideal path cumulative proximity by using individual speed for that trial. This is the best option because this measure is less affected by swim speed than latency and was more reflective of the bias for the platform than path length (20). The probe trials were less affected by the differences in swim speed because the trial time was the same for all probe trials.

Although not shown in the results above, the young non-surgical mice performed similar to old controls and worse than previous work (21). We can speculate why the young non-surgical mice performed so poorly. There is a new study showing that the exposure of rodents to male but not female experimenters induces stress. Male related stimuli induced a robust physiological stress response that results in stress-induced analgesia (22). We had one male that was involved with the working memory tasks, while females were involved with the rest of the behavioral tasks. With males involved in our study this could suggest that experimenter gender can affect baseline responses in behavioral testing. Because the non-surgical controls were not what we expected, we cannot interpret the rest of the results as more than preliminary findings. We would need to try again, and hope to have more promising results with the controls working.

Our results showed no effect of LPS on NMDA receptor subunits. Our lab, in previous studies, has observed significant effects of aging on GluN1 subunit protein expression (23; 24), but has also seen no effect of aging on GluN1 mRNA expression in other studies (10; 25). We expected to see a difference between sides on one pump LPS animals, but did not. Other studies showed a decrease in GluN1 protein so we can suggest that the effect is not at the mRNA level (16). This can be a reason for not seeing an effect of LPS.

Although we saw that stimulating inflammation in a young brain only produced some of the memory deficits seen in aging, these studies will provide vital information about how different aging processes influence the expression patterns of specific subunits of the NMDA receptor, and whether it will influence the age-related declines in learning and memory. This study was beneficial towards the labs main goal of finding interventions into aging that will help to

maintain the quality of life into old age, and also in helping understand the function of the NMDA receptor in different brain regions.

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