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

E. R. Escobedo[1], K. Magnusson[2,3] —[1]BioResource Research, [2]Department of Biomedical Sciences, College of Veterinary Medicine, [3]Linus Pauling Institute, Oregon State University, Corvallis, Oregon 97331

Introduction

Humans and rodents experience declines in memory and other cognitive functions during the aging process (1). This includes problems with reference (long-term) and working (short-term) memory and cognitive flexibility. N-methyl-D-aspartate (NMDA) receptors, a type of excitatory glutamate receptor, have been shown to be important for learning and memory. Aging changes in specific subunits of the NMDA receptors, GluN1 and GluN2B show a relationship to both reference and working memory deficits (2). An anti-inflammatory drug, Sulindac, enhances expression of these two subunits in aged rodents (3). 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.

Materials and Methods

Animals: Male mice of 2 different ages (3 & 24 months) were purchased. There were two experience groups, behaviorally characterized and naïve. The mice were randomly assigned to three treatment groups: lipopolysaccharide (LPS), saline, or non-surgical. The surgical animals had a cannula attached to an osmotic pump, implanted into the lateral ventricles of the brain with the use of stereotaxic surgery. The pumps delivered solution for 3 weeks, and behavioral testing began a week after pumps were removed.

Behavioral Testing: The Morris water maze (Fig.1.0 A) was used to perform testing. A four-foot diameter tank was filled with water (between 16-18º C) and non-toxic paint. The water was 1 cm above the level of the platform. There were spatial cues located high on the walls and on the tank. Trials were videotaped and analyzed with the use of a "SMART" Video Tracking System.

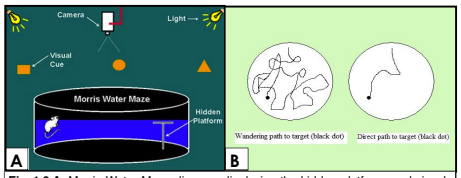

Fig. 1.0 A. Morris Water Maze diagram displaying the hidden platform and visual cues surrounding the tank **B.** Representative search paths from tracking system

The mice began the trial by being placed into different entry points facing the wall of the tank. If the mice did not find the platform within the time limit, they were led to it by the handler. Mice were tested for reference memory with 8 place trials and 1-2 probe trials per day for 4 days. The platform remained in the same position throughout testing. Cognitive flexibility was addressed in reversal trials by moving the platform to the opposite quadrant and testing for 1 day. Working memory testing involved moving the platform each session and performing naïve trial, 10 minute rest, then a delay trial. The cued control task involved a visible platform and a change in position each trial for 6 trials within one day.

Analysis: Cumulative proximity to the platform was obtained for place, reversal, working, and cued trials. Average proximity was calculated for probe trials. Statistical analysis was performed with RANOVA and Fisher's LSD post-hoc tests with Statview software.

***In Situ* Hybridization:** Following cued testing, mice were euthanized and their brains removed. In Situ was done as described in Das, Magnusson, 2011.

Behavior Results

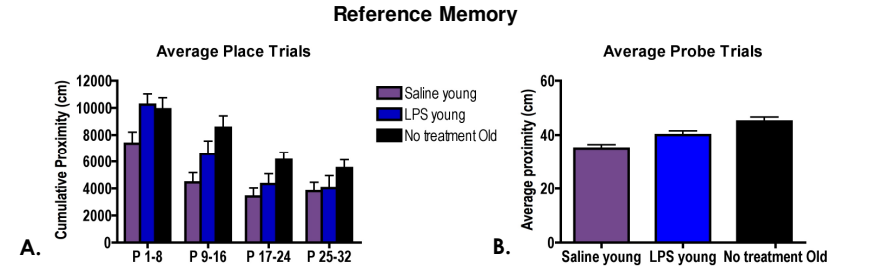

Figure 2.0 A. Graph showing treatment effects on performance. Overall, the LPS-treated young mice performed worse than saline-treated young and the same as old. The place trials were averaged for each reference memory testing day. **B.** The probe trials were measured by average proximity, with the old no treatment performing the worst, then LPS young, and Saline young. Mean ±SEM, ANOVA & Fisher's PLSD.

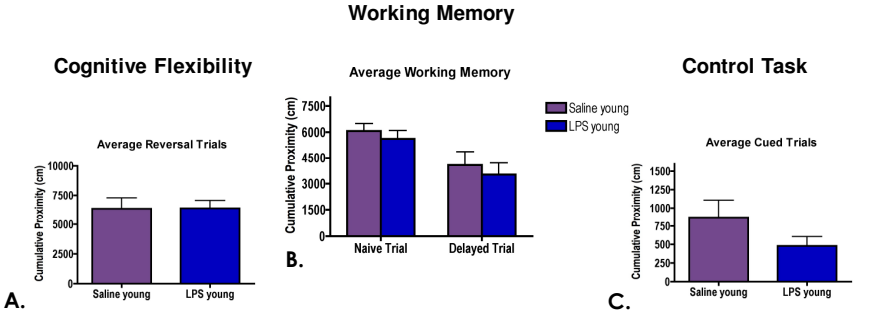

Figure 3.0 A. Changing the platform position to the opposite quadrant, showing saline and LPS treated animals performed similar. **B.** Averages of 8 working memory sessions, one am and one pm session per day. **C.** Control Task showed no effect of treatment in LPS- versus saline-treated young mice. Mean ±SEM, ANOVA & Fisher's PLSD.

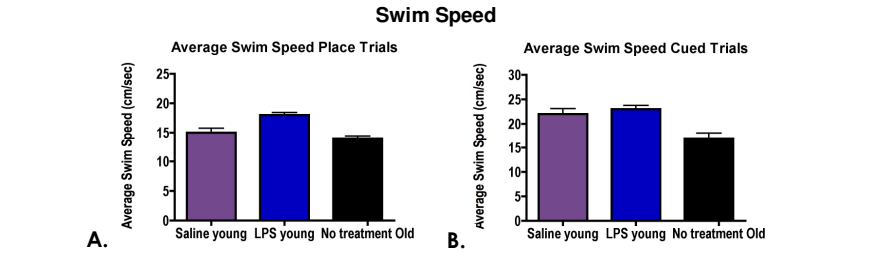

Figure 4.0 A. & B. In the Place trials LPS-treated young swam the fastest, with old no treatment being the slowest for both place and cued trials.

In situ Hybridization Results

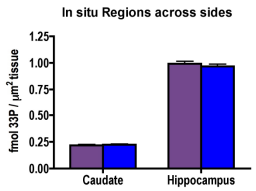

Figure 5.0 A. Graph showing no effect of LPS on mRNA densities for the GluN2B subunit in the caudate nucleus and hippocampus.

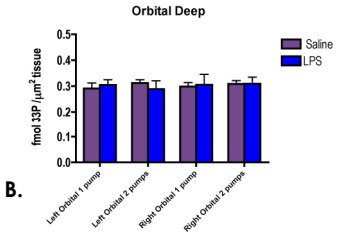

Figure 5.0 B. Graph showing no effects of LPS, brain side, or pump number on mRNA for the GluN2B subunit in the deep layers (IV-VI) of the orbital cortex.

Conclusions

1. The LPS treatment as a pro-inflammatory agent in young mice produced reference memory deficits similar to old mice.
2. Old mice performed worse than LPS treated and control mice in probe trials.
3. No effect of LPS on working memory, cognitive flexibility, or associative memory.
4. Swim speed doesn't account for the deficits in reference memory.
5. There was no effect of LPS on mRNA densities for GluN2B subunit.
6. Stimulating inflammation in a young brain only produced some of the memory deficits seen in aging.

References

1. Das SR, Magnusson KR. Changes in expression of splice cassettes of NMDA receptor GluN1 subunits within the frontal lobe and memory in mice during aging. Behavioural Brain Research 2011 Jan; 222(1):122-133.
2. Magnusson KR, Brim BL, Das SR. Selective Vulnerabilities of N-methyl-D-aspartate (NMDA) Receptors During Brain Aging. Frontiers in aging neuroscience 2010 Jan; 2:11.
3. Meses MH, Gemma C, Veng LM, Allgeier C. Sulindac improves memory and increases NMDA receptor subunits in aged Fischer 344 rats. Neurobiology of Aging 2004 Mar; 25(3):315-324.

Acknowledgments

Funding for this project was provided in part by:

Grant AG016322 from National Institutes of Health to Dr. Kathy R. Magnusson.