

A PRO-INFLAMMATORY AGENT, LIPOPOLYSACCHARIDE, CAN MIMIC THE EFFECTS OF AGING ON SPATIAL REFERENCE MEMORY



<http://www.inmonova.com/blog/wp-content/uploads/2013/08/real-estate-nova-notice-brain.jpg>

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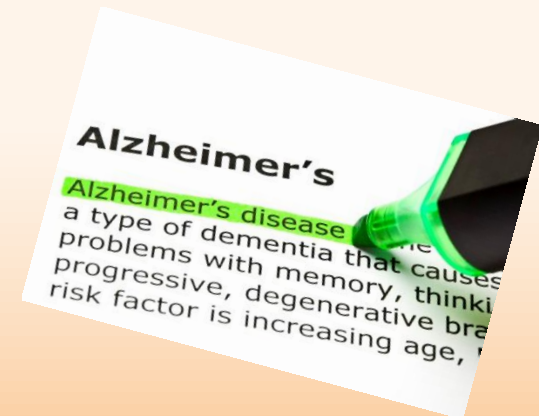
Question...



- The question addressed was whether a pro-inflammatory treatment in mice would produce the same changes in memory (study 1) and NMDA receptor expression as aging (study 2).
- The hypothesis addressed in the present study was that inflammation plays a role in NMDA receptor aging and memory declines.

Aging and Memory

- In 2010, an estimated 524 million people were aged 65 or older.
- By 2050, it is expected to be about 1.5 billion (NIA, WHO, 2012).
- One of the earliest cognitive dysfunctions is a decline in learning and memory performance.
- 40% of people aged 65 or older can be diagnosed with some form of age associated memory impairment (Small, G.W., 2002).



What is Memory?

- Memory: ability to encode, store, retain, and subsequently recall information and past experiences in the human brain.
- 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 (Das, S.R., Magnusson, K.R. 2011).



Anatomical basis of memory

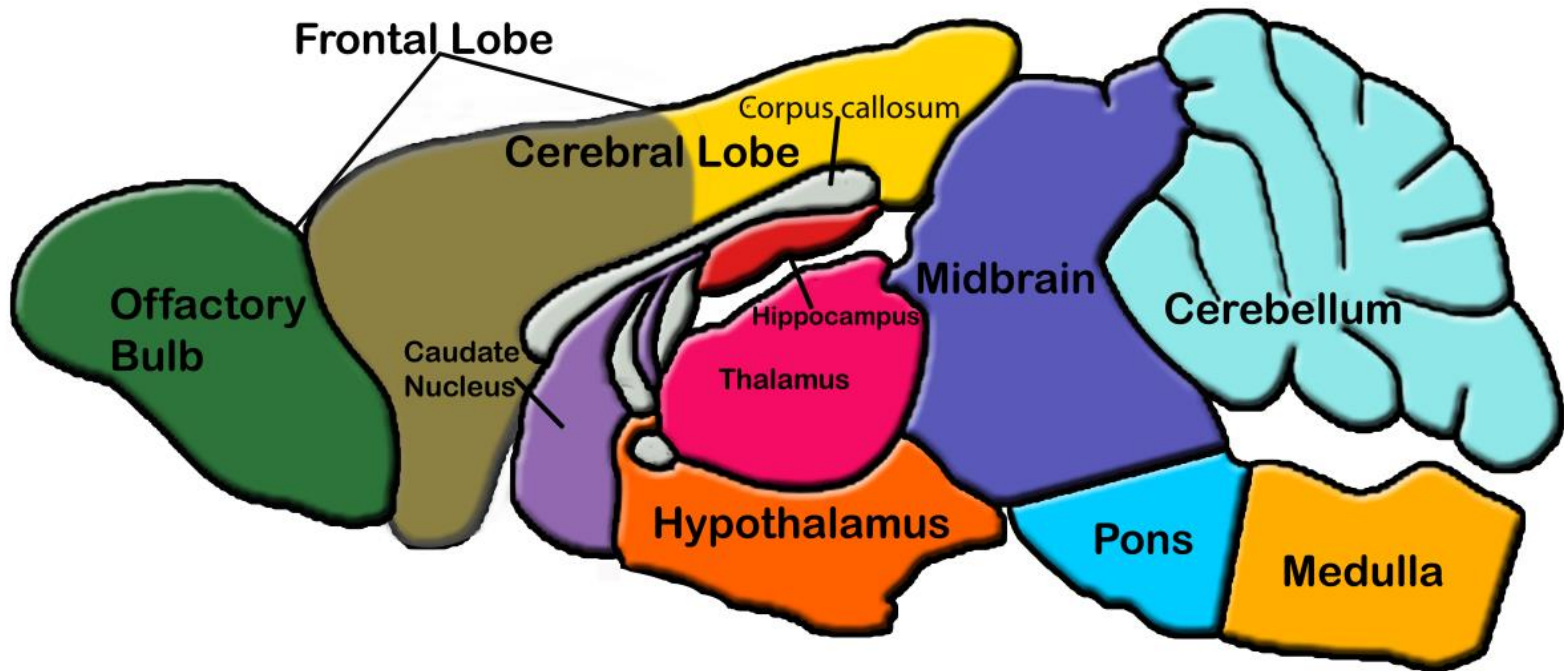


Image adapted from Gene Expression Nervous System Atlas (GENSAT) (Heintz 2004).

Hippocampus

- It plays important roles in long-term memory and spatial navigation.
- Lesions of the hippocampus cause problems with both short-term (working) memory and long-term (reference) memory (Morris RGM et al. 1982).
- 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 (Topic, B et al. 2007).

<http://upload.wikimedia.org/wikipedia/commons/9/99/Hippocampus.gif>

Prefrontal Cortex

- The part of the cerebral cortex that receives projections from the mediodorsal nucleus of the thalamus (Fuster, J. 2008).
- Involved in working memory tasks.
- Shows a greater rate of decrease in volume per decade with increasing age in humans (4.3-4.9 %) than the hippocampus (2%) (Raz, N et al. 1997).

https://upload.wikimedia.org/wikipedia/commons/5/5c/Brodmann_area_10_animation_small.gif

N-Methyl-D-Aspartate (NMDA)

- Type of excitatory glutamate receptor, expressed in high density in the cortex and hippocampus has been shown to be important for learning and memory, including spatial memory (Magnusson, K.R. 2012).
- Important for long-term potentiation, a cellular mechanism for the formation of memory (Magnusson, K.R. 2012).

Subunits

- NMDA composed of protein subunits from different families of proteins, the GluN1, GluN2 and GluN3.
- Studies show 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 (Topic, B et al. 2007), while the GluN2A subunit receptors increase over time.

Inflammation

- 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.
- This treatment also enhanced working memory in aged rats. It is not known, however, at what age this is effective or whether it protects at the level of mRNA and/or protein (Mesches MH et al. 2004).



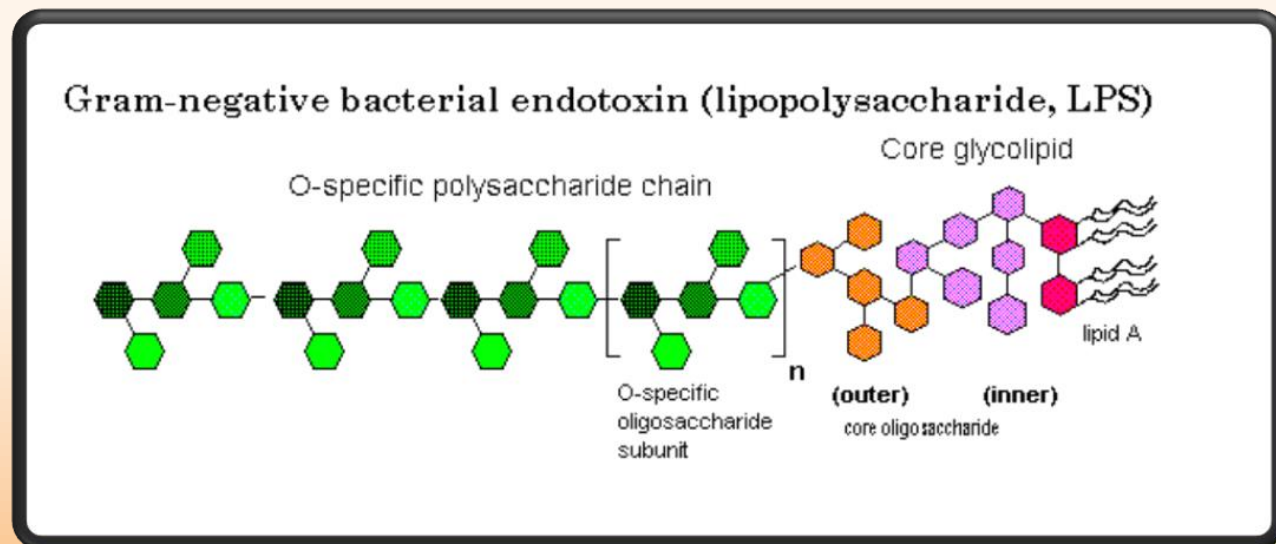
Question...



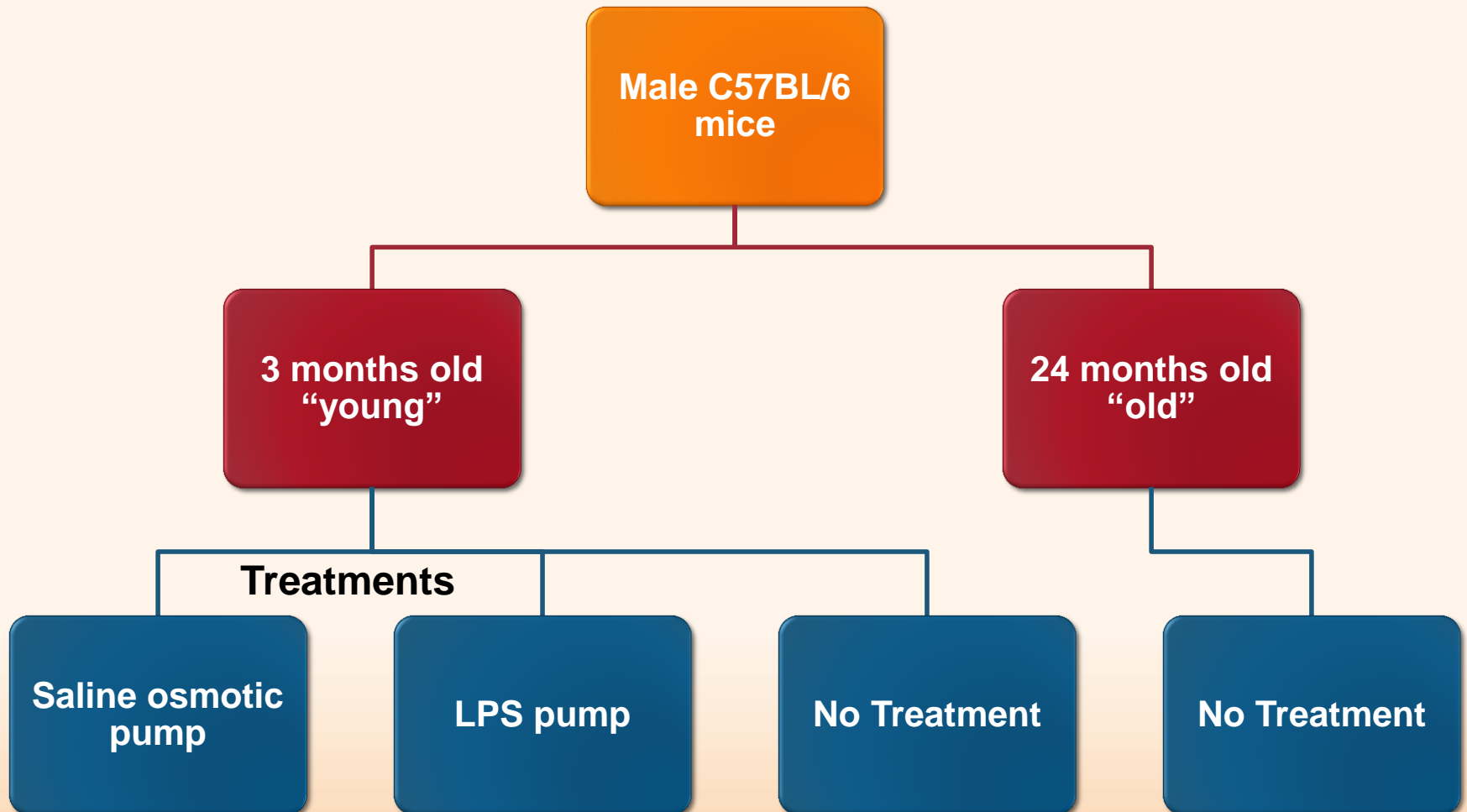
- The question addressed was whether a pro-inflammatory treatment in mice would produce the same changes in memory (study 1) and NMDA receptor expression as aging (study 2).
- The hypothesis addressed in the present study was that inflammation plays a role in NMDA receptor aging and memory declines.

Inflammation

- Lipopolysaccharide (LPS): one of most powerful bacterial virulence factors with pro-inflammatory properties.
- LPS: Endotoxin and major component of the bacterial cell wall of gram-negative bacteria.
- LPS been shown with chronic administration in rat brain to produce inflammation and decreases in the GluN1 protein expression (Rosi, S. et al 2004).

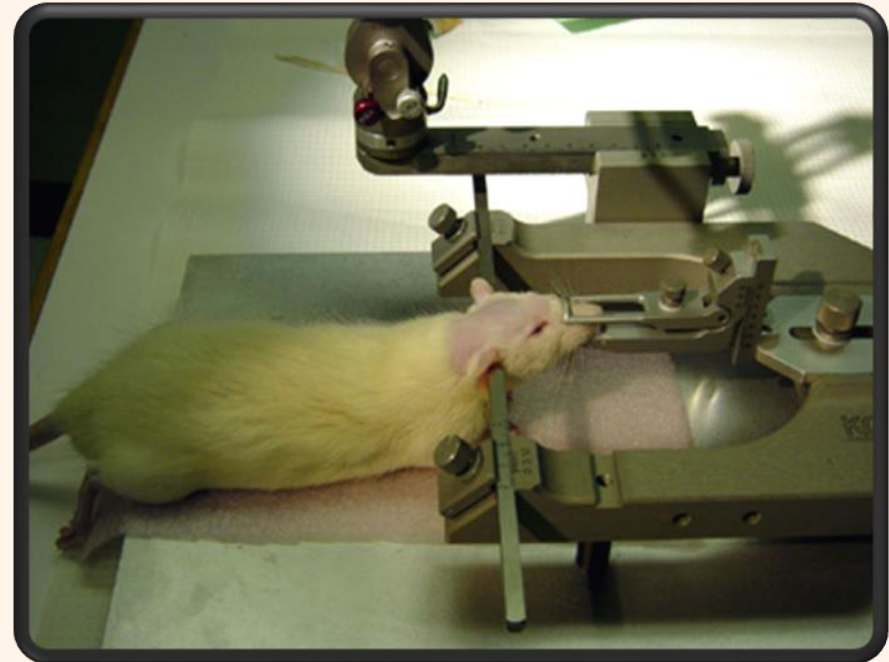


Experiment Set up



Surgery

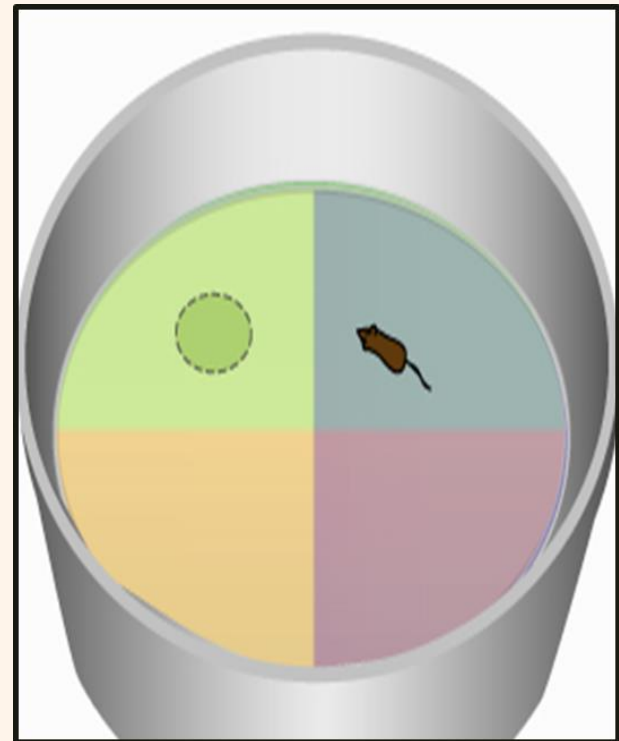
- Surgical animals had a unilateral or bilateral cannula attached to 1 or 2 osmotic pumps, implanted with the use of stereotaxic surgery into the lateral ventricles.
- Each pump contained 600 $\mu\text{g/kg}$ of either the LPS or sterile saline.
- Pumps delivered solution for 3 weeks, then pumps were removed and behavioral testing began a week after.



<http://play.psych.mun.ca/~smilway/rat-pre-cut.jpg>

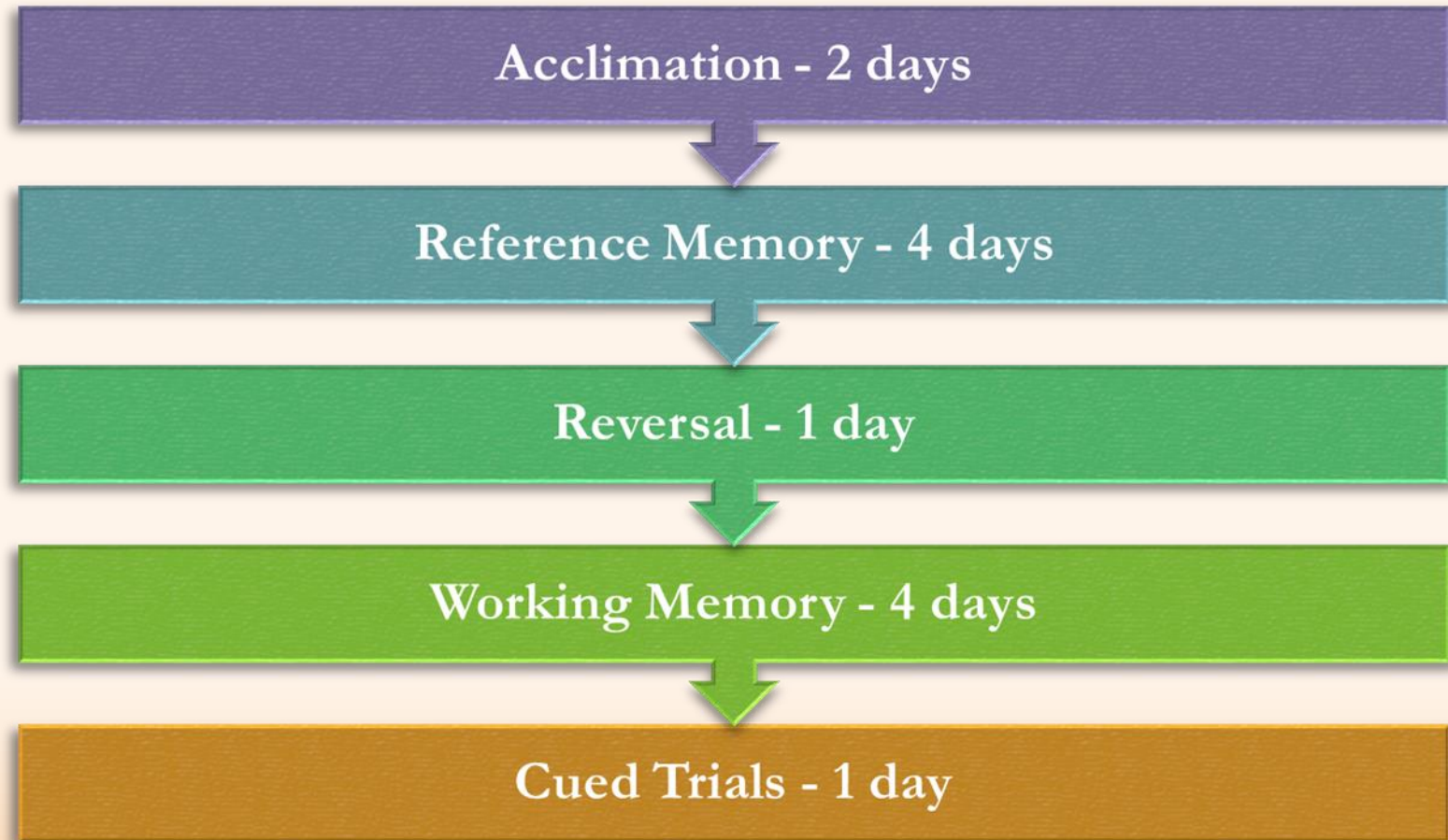
Morris Water Maze

- Used to perform behavioral testing.
- 4 foot diameter tank filled with water, made opaque white with non-toxic paint.
- The trials were videotaped with a video camera placed on the ceiling directly above the center of the tank.



<http://btc.psych.ucla.edu/mwm-c.jpg>

Behavioral Testing Overview



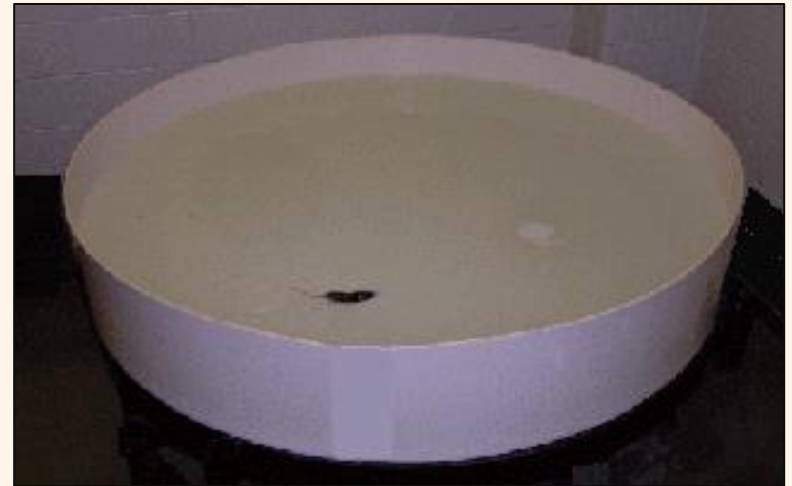
Acclimation

- Swim for 60 seconds with no platform present.
- Platform then placed in center of tank, mice trained to remain on platform for 30 continuous seconds.



Reference Memory Testing

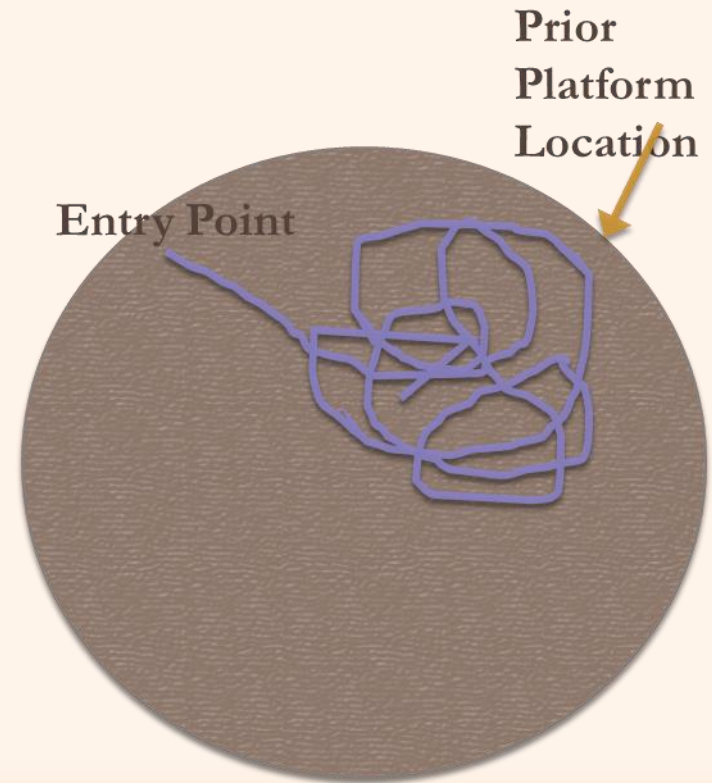
- 8 Place trials per day for 4 days.
- Platform kept in SE quadrant.
- Entry points randomly assigned as SW, NE, NW.
- Maximum of 60 sec to find platform, then 30 seconds on platform.
- Measures long-term spatial memory.



<http://www.watermaze.org/smallwtr.gif>

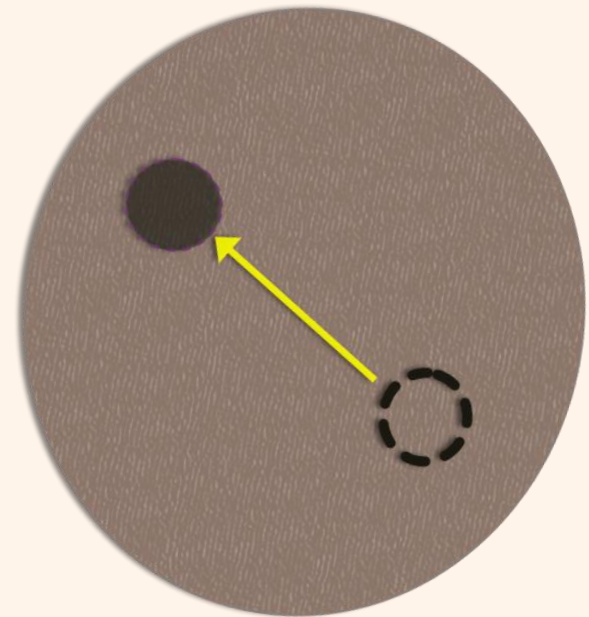
Probe Trials

- Done after the last place trial each day (also an initial probe done before place trials on 1st day).
- Platform removed.
- Mice allowed to search for 30 seconds.



Reversal Trials

- Tested for cognitive flexibility for 1 day on day 7.
- Protocol same as above for place trials.
- Platform moved to NW (diagonally opposite) position.

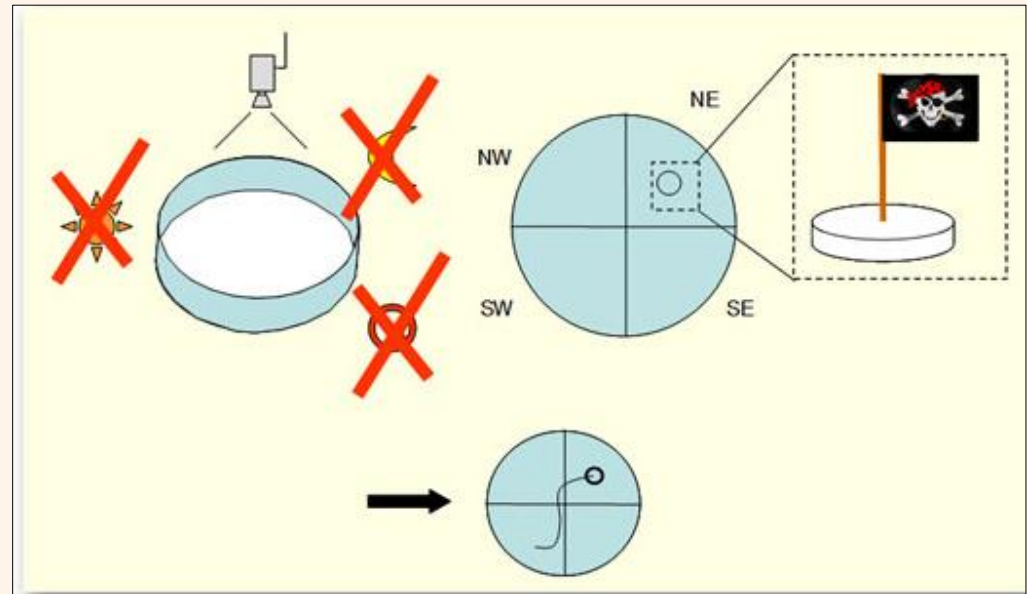


Working Memory Testing

- From day 8-11, 2 sessions per day (a.m. and p.m.).
- Platform position changed between each session.
- Each session has a series of 4 trials.
 - Naïve Trial (T0)
 - Delay Trial (Tdelay)
 - Trial (T2)
 - Trial (T3)
- Mice have new entry point for each trial.

Cued Trials

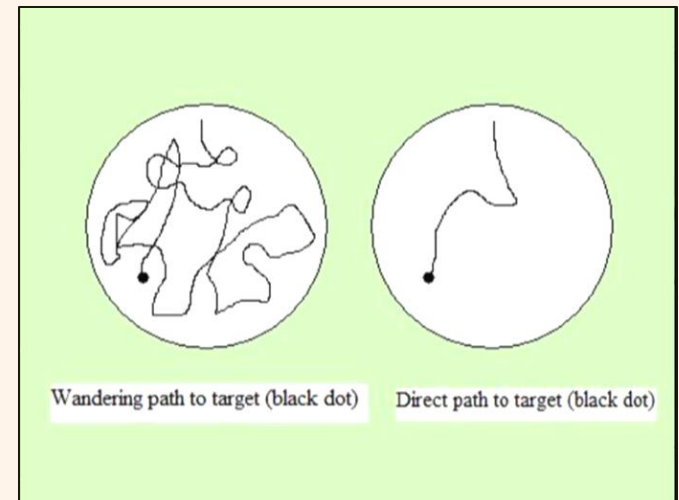
- Day 12, all spatial cues around room and tank removed.
- 6 trials, each trial platform placed in one of 6 locations with flag on platform.
- Allowed to search for platform for 60 seconds.
- Did not need to remain on platform once found.



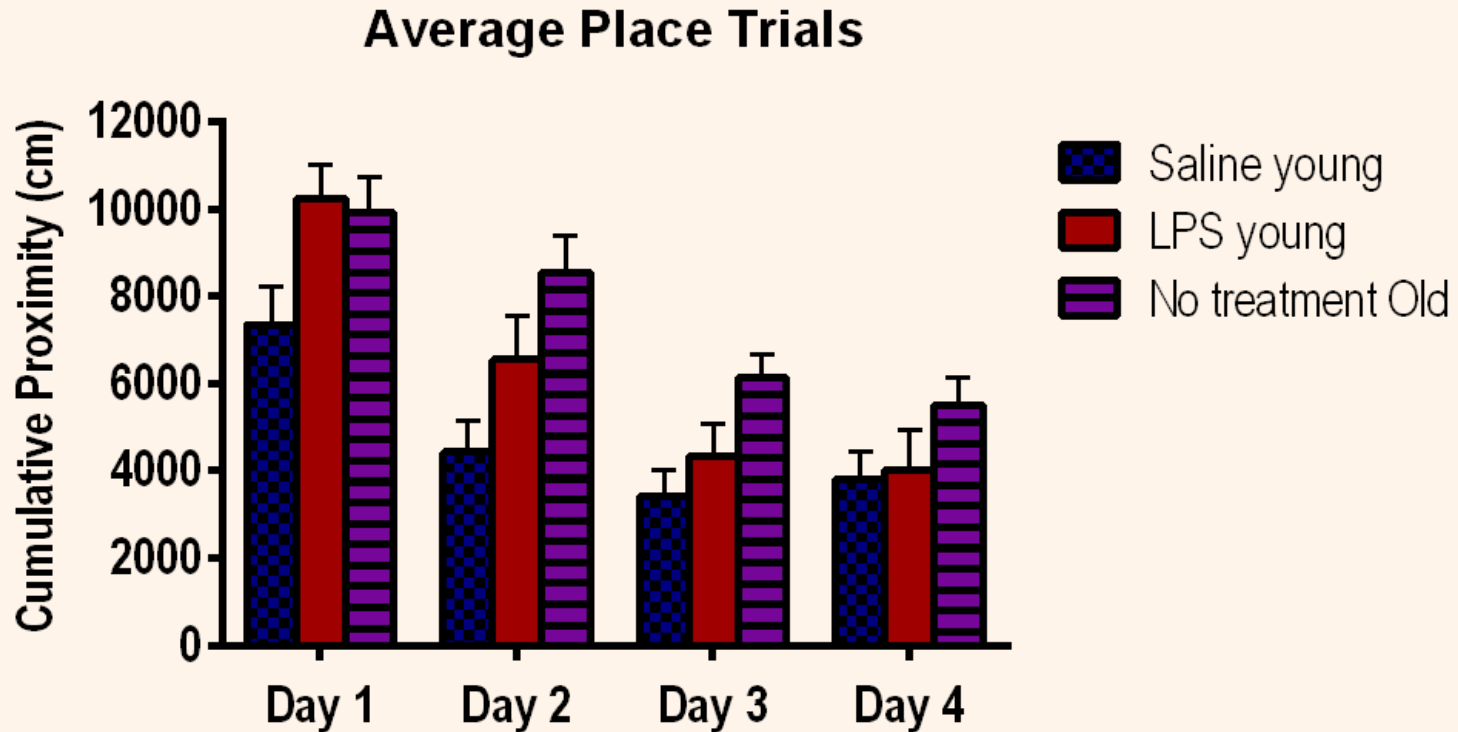
<http://www.panlab.com/en/tests-solutions/morris-water-maze-test>

Analysis of Behavioral Data

- Paths of the trials were analyzed by using the “SMART” video tracking system.
- Cumulative proximity to platform was obtained for place, reversal, working, and cued trials.
 - Higher values represent poorer learning ability.
- Average proximity was used for probe trials.

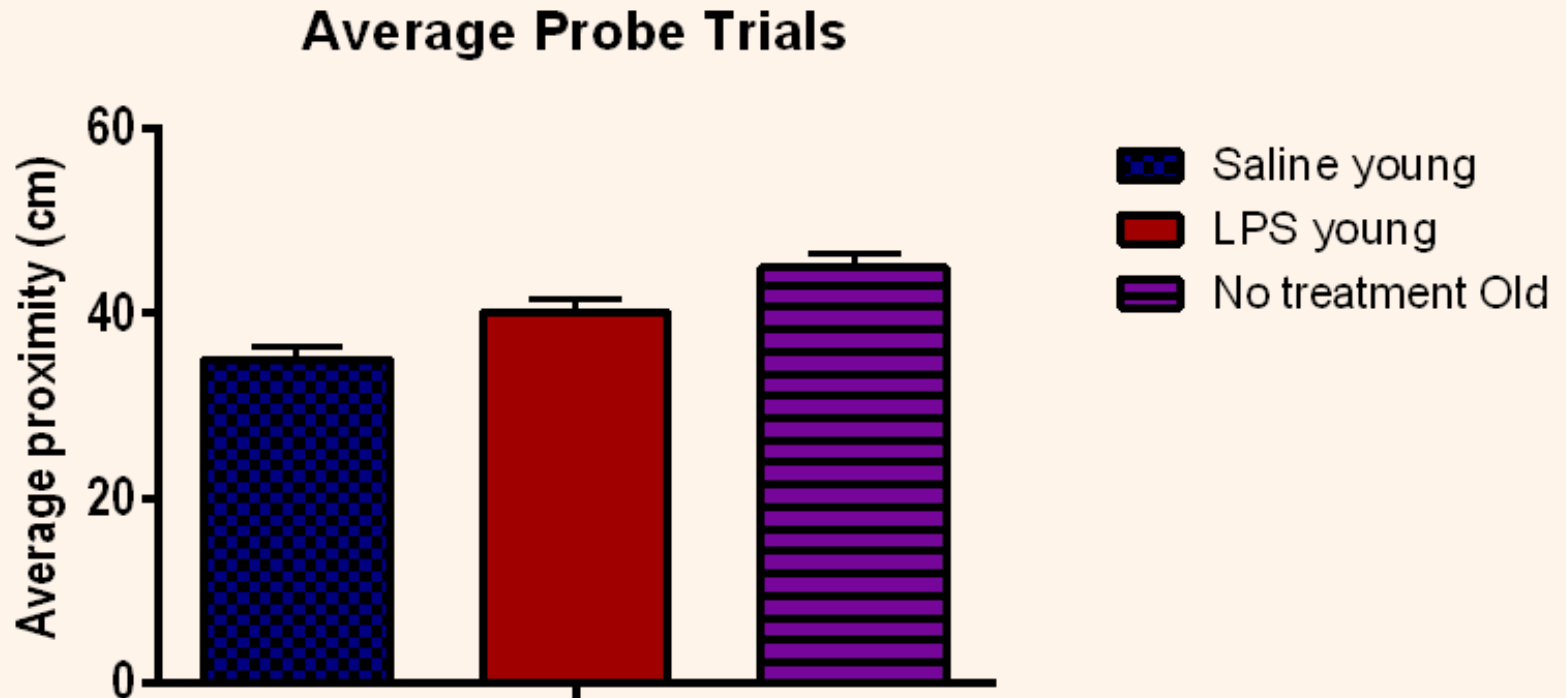


Results: Behavioral Data



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

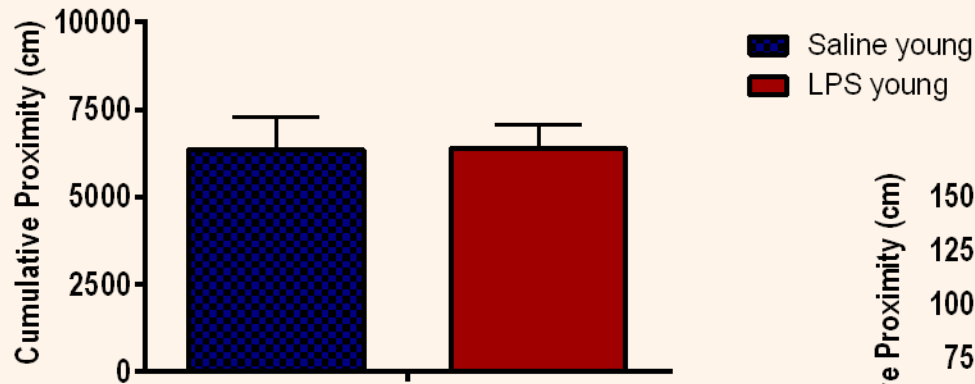
Results: Behavioral Data



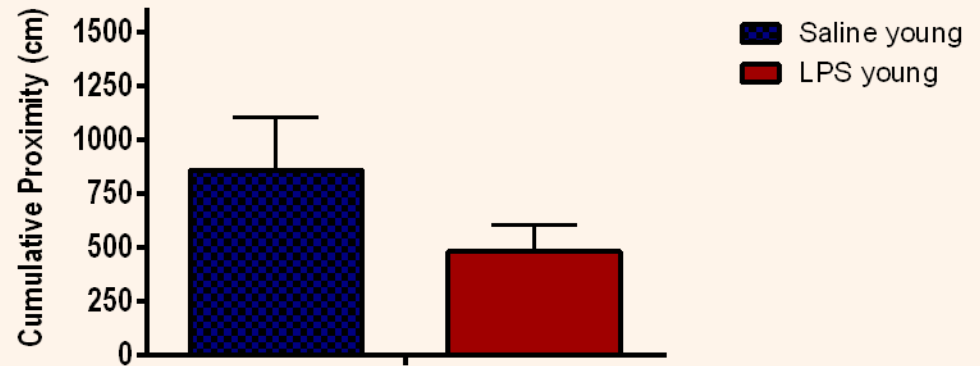
Probe trials for reference memory. The probe trials were assessed by average proximity in cm. Mean \pm SEM, ANOVA & Fisher's PLSD, N=12.

Results: Behavioral Data

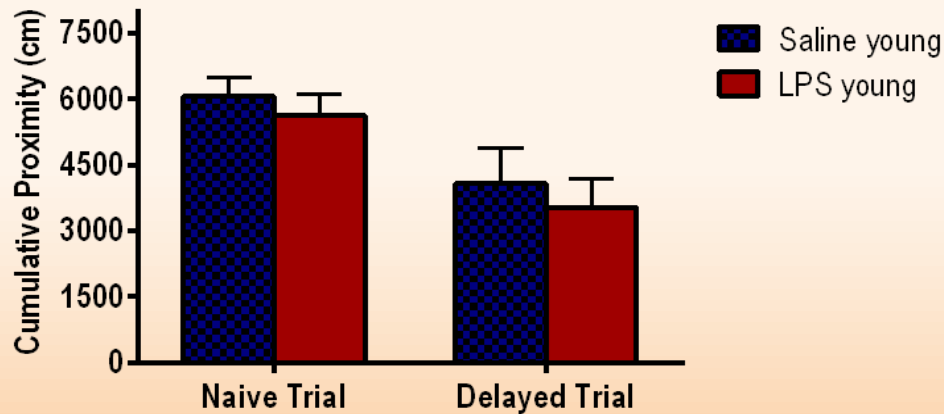
Average Reversal Trials



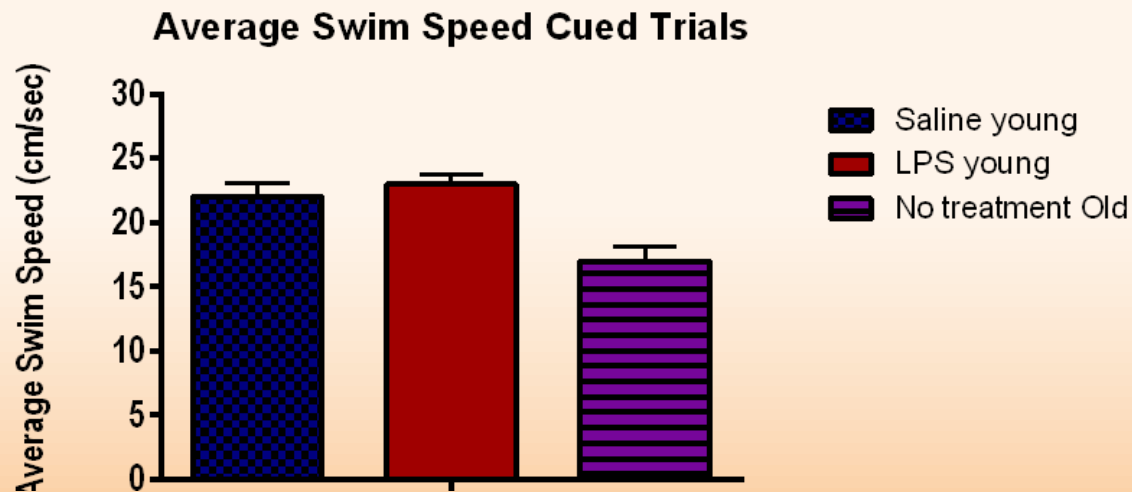
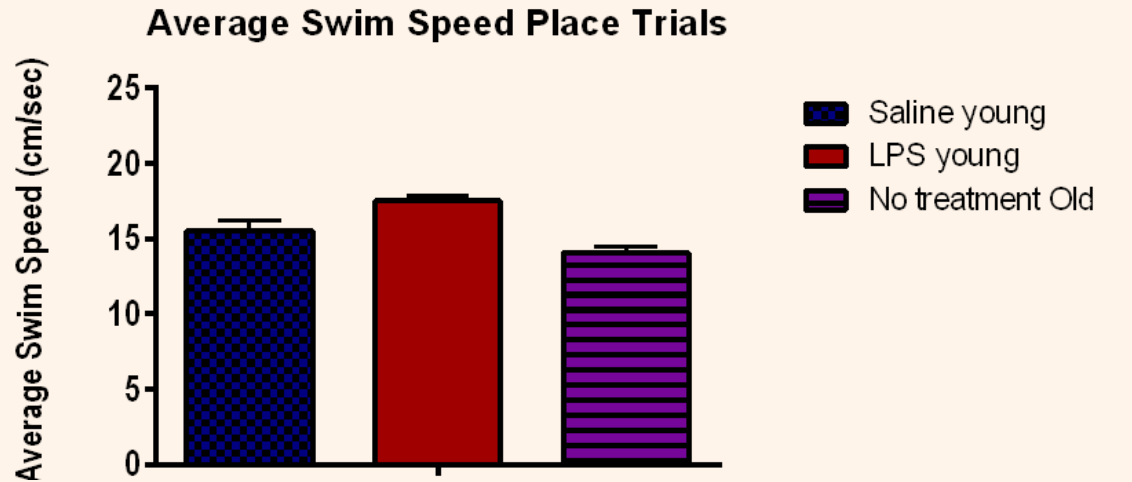
Average Cued Trials



Average Working Memory



Results: Behavioral Data

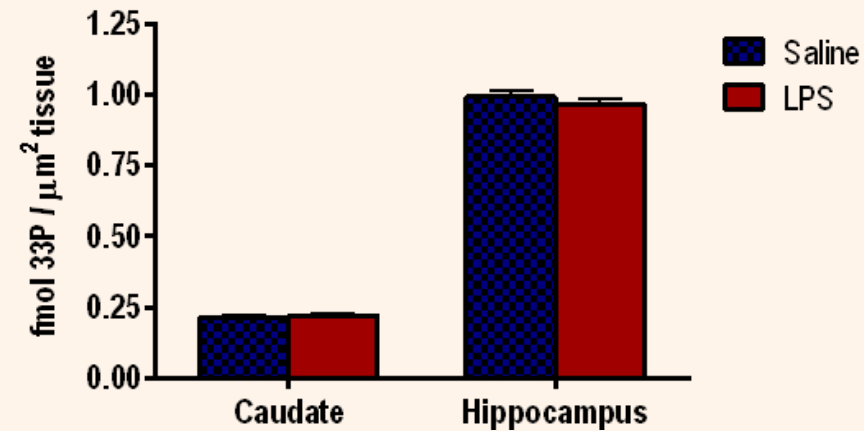


In Situ Hybridization

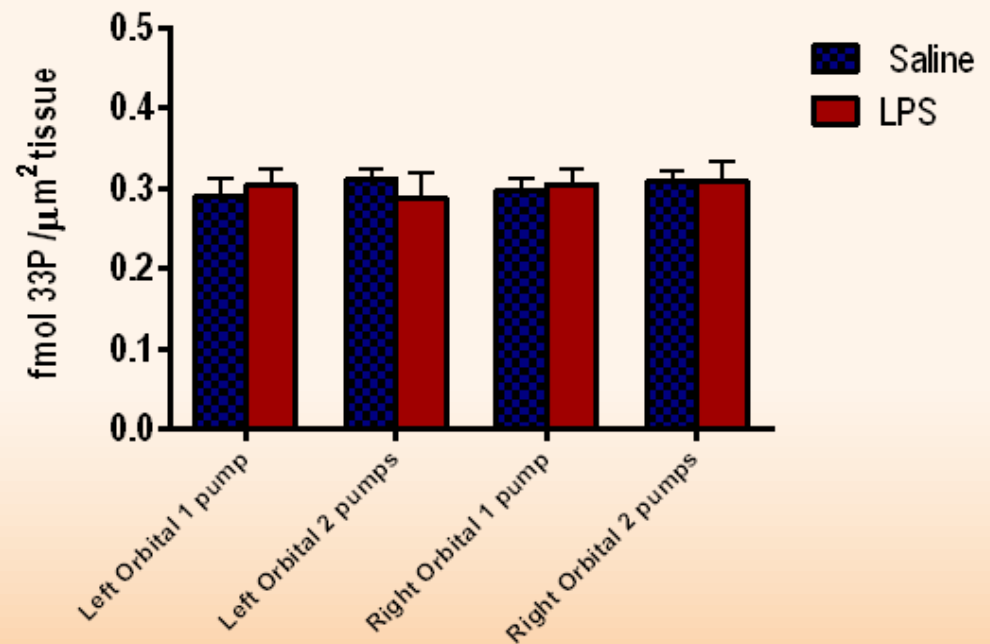
- Following cued trials, mice euthanized by exposure to CO₂.
- Brains removed, frozen in dry ice, and stored in -80° C freezer.
- One half of each brain was sectioned horizontally through the frontal cortex, intermediate hippocampus and cerebellum.
- Probes were used to measure the levels of mRNA for GluN2B, GluN2A, and GluN1 subunits.
- *In situ* hybridization was performed according to the method of Watanabe, et al., 1992.

Results: GluN2B Subunit

In situ Regions across sides

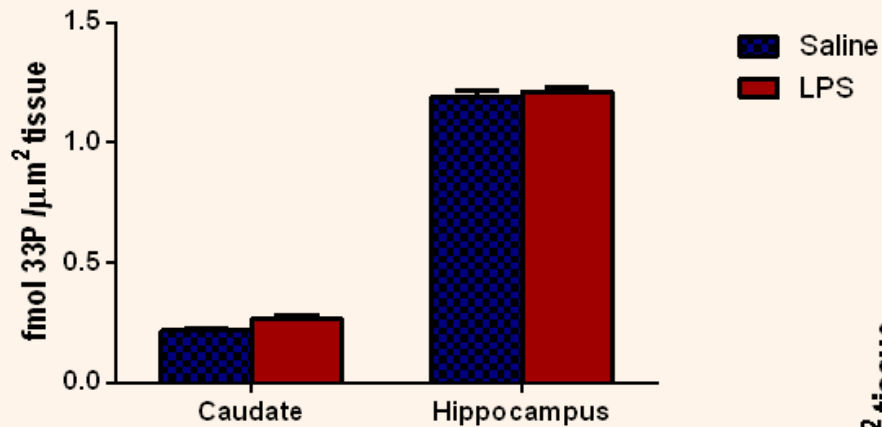


Orbital Deep

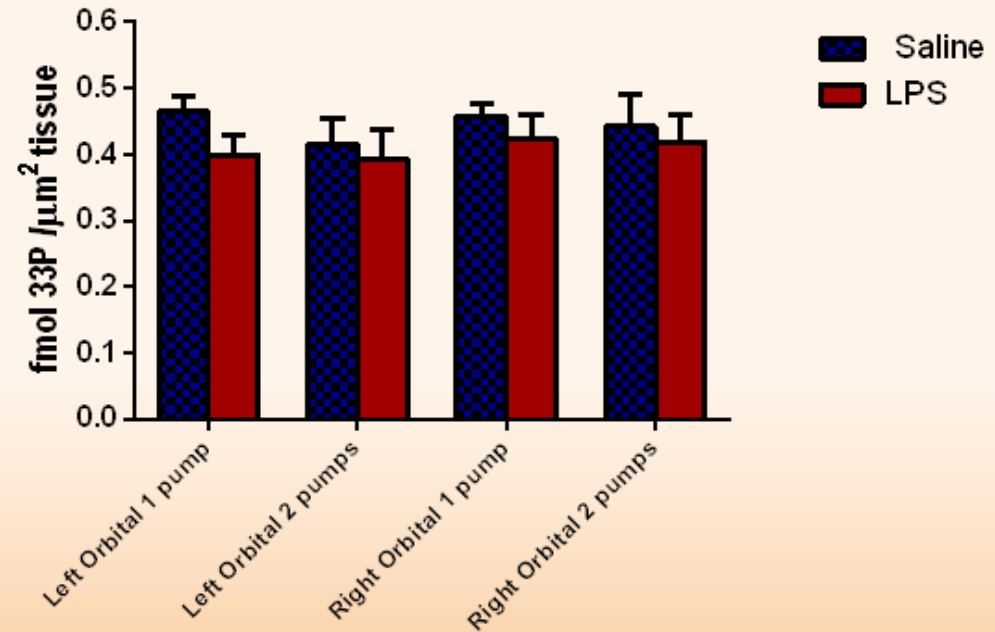


Results: GluN1 Subunit

In situ Regions across sides



Orbital Deep



Results: 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.
- No significant differences between LPS and saline treatment on mRNA densities in caudate nucleus, hippocampus, and frontal cortical regions.

Discussion

- Using Morris water maze, LPS treatment interfered with reference memory.
- LPS mice had faster swim speeds than saline and old mice in place trials. This would suggest that swim speed doesn't account for deficits in reference memory.
- No effect of LPS on working memory and reversal task.

Discussion

- LPS treated showed trends of better performances than the saline controls in cued control task.
- No effect of LPS on NMDA receptor subunits.
- Stimulating inflammation in a young brain only produced some of the memory deficits seen in aging.

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