Effects of Early Neonatal Infection on Adult Cerebrovascular Health

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for graduation *with distinction* in Psychology in the undergraduate colleges of The Ohio State University

by

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Introduction

Stroke is the third leading cause of death in North America and Europe (Reiter *et al*., 2005). Every year about 600,000 people in America suffer from a new or recurrent stroke. Currently, there are over three million Americans permanently disabled because of stroke (White *et al*., 2000). There are two different types of stroke: ischemic and hemorrhagic. The present study focuses on ischemic stroke, the better understood of the two. An ischemic stroke occurs when the oxygen to the brain is cut off due to the blocking of a blood vessel located within the brain, which in turn leads to neuronal death (Stahl, 1997). Stroke affects 12 common domains including energy, family roles, language, mobility, mood, personality, self-care, social roles, thinking, upper extremity function, vision, and work/productivity. Among these domains hand/arm function, family roles, and language have been reported to be the most affected compared to the others (Williams *et al*., 1999).

During ischemia, the neurons comprising the core infarct, which receive no oxygen at all, suffer the most damage. Surrounding the core, the neurons in the ischemic penumbra, receive some oxygenated blood and thus suffer less damage (Reiter *et al*., 2005). There are two modes of cell death during ischemia: necrosis and apoptosis. Membrane dysfunction and cell swelling characterize necrosis (Stoll *et al*., 1998). The neurons of the ischemic core undergo rapid necrotic cell death due to a reduction in adenosine triphosphate (ATP) that leads to an increase in neuronal cytosolic Ca^{2+} concentration. The influx of Ca^{2+}, via pump failure and depolarization, sets off a chain of events that primes the brain to more damage during reperfusion (White *et al*., 2000). Apoptosis, programmed cell death, occurs after the onset of reperfusion peaking at 24-48 h post-reperfusion (Stoll *et al*., 1998). Also contributing to reperfusion injury is inflammation attributed to the activation of cytokines, inducible nitric oxide synthase (iNOS), and intracellular

adhesion molecule 1 (ICAM-1) (Turley *et al*., 2005). The current study focuses on the effects of neonatal LPS exposure on the development of post-ischemic infarct development.

When an animal is exposed to an inflammagen, such as lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, its immune system responds by initiating an immune response. Specifically, when exposed to LPS, immune cells synthesize cytokines that induce COX-2 to synthesize prostaglandin E_2 (PGE_2) within the central nervous system (CNS) (Boissé *et al*., 2004). PGE_2, by way of inflammation, contributes significantly to the outcome after ischemic insult (Yrjänheikki *et al*., 1999); elevated PGE_2 increases infarct size, while attenuated PGE_2 decreases infarct size (Nogawa *et al*., 1997). The induction of COX-2 has long-term effects: administration of *Escherichia coli* LPS to neonate rats raised their basal levels of COX-2 expression as adults compared to adult rats neonatally administered a pyrogen-free saline (Boissé *et al*., 2004). The basal level of COX-2 is strongly correlated with the level of PGE_2 generation, the rate-limiting enzyme of PGE_2 synthesis (Nogawa *et al*., 1997). Therefore, when exposed to an LPS, an increase in the basal level of COX-2 will result in an increase in the level of PGE_2 response (Boissé *et al*., 2004).

COX-2 plays a role in both the early and late stages of cerebral ischemia. Immediately after the induction of ischemia, the activation of glutamate receptors by COX-2 initiates the ischemic cascade, contributing to damage occurring in the early stages of stroke (Iadecola *et al*., 2001). Indeed, suppressing COX-2 expression decreases ischemic damage. For example, COX-2 null mice have significantly less neuronal damage following experimental stroke relative to their wild-type littermates in both 24 h post-ischemic and 96 h post-ischemic assessments (Iadecola *et al*., 2001).

The goal of the current study was to examine the effects of early life infection on adult ischemic outcome. It was hypothesized that administering LPS as a neonate would increase the size of the stroke-induced infarct as an adult compared to that of an adult administered a pyrogen-free saline solution as a neonate via the up-regulation of the COX-2 enzyme, and it's effects, in turn on PGE_2 expression.

Methods

Animals

Male and female C57BL/6 mice (Charles River, Wilmington, MD, USA) were paired and maintained in a temperature controlled (21˚C) vivarium on a 14:10 light/dark cycle, with *ad libitum* access to food and water. On post-pairing day 15, the males were separated from the pregnant females. All subsequent litters were weaned at 21 d, and males and females housed separately <6 per cage until they reached 22-25 g. The study was conducted in accordance with NIH guidelines for the care and use of animals and under protocols approved by the Institutional Animal Care and Use Committee.

Study 1

Early life manipulations: Neonatal treatment for longitudinal study

On postnatal day 14 (P14) litters were randomly selected and *Escherichia coli* LPS (Product Number L 2654) was administered intraperitoneally in pyrogen-free sterile saline (1µl/g pup weight) at a dose of 50µg. The other litters were administered an equivalent amount of sterile, pyrogen-free saline.

Surgery

Transient focal cerebral ischemia was induced in the mice by middle cerebral artery occlusion (MCAO). The mice were anesthetized with 1-1.5% halothane in oxygen-enriched air delivered through a facemask. Occlusion of the right middle cerebral artery was achieved by using the intraluminal filament insertion technique. A 6-0 nylon monofilament was inserted into the internal carotid artery, via the external carotid artery. Then the filament tip (approximately 1.0 mm length and 0.25 mm width) was positioned for occlusion at a distance of 6 mm beyond the internal carotid artery-pterygopalatine artery bifurcation. Once the filament was secured, the incision was sutured, and the animal was allowed to emerge from the anesthesia in its home cage. After 60 min of occlusion, the animal was briefly re-anesthetized with halothane in oxygen-enriched air, and reperfusion was initiated via withdrawal of the filament. This surgery protocol typically results in a core infarct limited to the parietal cerebral cortex and caudate putamen of the right hemisphere. For the sham MCAO surgery, the internal carotid artery was exposed but not disturbed. All other aspects of the surgery were similar for the MCAO and sham MCAO groups. Throughout the surgery, rectal temperature was maintained at 37±0.5˚C through the use of a homoeothermic blanket system. All animals were given a 0.5 ml s.c. injection of lactated Ringer's solution at the conclusion of the surgical procedure, and returned to their home cage for recovery.

Determination of stroke volume

Immediately following cervical dislocation, brains were removed, placed in a -70ºC freezer for 2 min, and then sectioned into five 2-mm-thick coronal sections. Sections were incubated for 15 min in 2,3,5-triphenyltetrazolium (TTC), with rotation every 2 min to allow uniform tissue

staining. The TTC solution was maintained at 37ºC throughout the staining process. Following staining, the sections were fixed in 10% buffered formalin solution. The brain slices were photographed and measured using *Inquiry* software (Loats Associates Inc., Westminster, MD, USA). The images were used to determine infarct size as a percentage of the contralateral hemisphere after correcting for edema. Comparisons between infarct sizes via TTC will account for the secondary damage attributed to stroke.

Determination of Post-Stroke Edema

To determine post-stroke edema following 60 min MCAO mice, brain tissue was collected at 24 h reperfusion immediately following transcardial perfusion with 20 ml saline. The brain tissue was immediately divided into right (ischemic) and left (non-ischemic) hemispheres and weighed. The tissue was then dried at 70ºC for 72 h. Once fully dehydrated, an index of edema was calculated using the following equation: $1 = (W/D_R - W/D_L)/(W/D_L) \times 100$.

Behavioral testing

Behavior will be assessed at 24 h pre-induction of experimental stroke and at either 24 h or 72 h post-stroke, depending on whether the initial infarct damage or the secondary damage will be assessed.

Cylinder Test: This test evaluates exploratory behaviors and paw preference. A clear Plexiglas cylinder (8 cm internal diameter, 12 cm height) was placed horizontally on a clean surface, and centered among four cameras to allow simultaneous viewing of the animal from all angles. The mouse was placed inside the cylinder and videotaped for five minutes. Initial placement of the left versus right paw on the cylinder was recorded during rearing to assess preferential paw use

(adapted from Karhunen *et al*., 2003). Only the first paw placement is recorded for each rear. Paw preference data are presented as frequency of left paw placement/total rearing frequency. A decrease in left (contralateral) paw use suggests a functionally significant impairment of the right sensorimotor cortex. Paw preference was determined using the following formula: [left/(left+right+simultaneous)]x100.

Locomotor activity: General locomotor activity was assessed during a 60 min session. The locomotor apparatuses were enclosed in individual sound attenuating chambers equipped with a 15-W fluorescent white light and a ventilation fan. The monitors consisted of a clear 60 cm wide x 60 cm long acrylic box that was cleaned between trials and placed in a metal frame that was lined with 16 equally spaced photo beams along two sides of the box. Four intervals of 900 s each recorded the movement of each mouse testing inclination to stay close to the walls of the chamber or venture into the center. The locomotor chamber serves as an anxiety paradigm; staying close to the walls representing anxious behavior and venturing into the center representing less anxious and more exploratory behavior. This test also counts for total rearing. Total distance traveled (cm) was determined using PAS software (San Diego Instruments, San Diego, CA).

Statistical Analysis

Infarct data was compared among experimental conditions using one way analysis of variance (ANOVA). Behavioral data was compared using ANOVA with repeated measurement factor for behavior (pre vs. post). Relative growth data was compared using both ANOVA with repeated measurement factor for postnatal day and also using one way ANOVA for final weights.

Results

Adult mice neonatally exposed to LPS show no significant difference in 24 h edema

There was no significant difference in edema between female adults neonatally treated with LPS (n=4) and female adults neonatally treated with saline (n=4; $p>0.05$) (Fig. 1). Similarly there was no significant difference in edema between male adults neonatally treated with LPS (n=5) and male adults neonatally treated with saline (n=5;$p>0.05$) (Fig. 2). These results suggest that early exposure to LPS has no effect on brain water content in response to inflammation following a stroke.

No effect of LPS on locomotor activity 24 h post-ischemia

There was no significant difference in any components of the locomotor activity for adult females neonatally exposed to LPS (n=4) compared to the vehicle females (n=4;$p>0.05$ for all). Similarly there was no significant difference in any components of the locomotor activity for adult males neonatally exposed to LPS (n=5) compared to the vehicle males (n=5;$p>0.05$ for all). The power in both groups was very low indicating that there is a large amount of variability in the data. An increase in n may provide more sufficient results.

Cylinder test shows no significant difference in adults neonatally exposed to LPS 24 h post-ischemia

There was no significant difference in paw use post-ischemia compared to paw use pre-ischemia in adult mice neonatally exposed to LPS compared to the vehicle in males ($p>0.05$) or females ($p>0.05$). Both statistical comparisons suffered from low power due to high variability, thus the null hypothesis is cautiously accepted.

72 h infarct analysis shows that adult females neonatally exposed to LPS are neuroprotected compared to vehicle and to male counterpart

There was a significant decrease in secondary infarct size in female adults neonatally treated with LPS (n=9) compared to female adults neonatally treated with saline (n=7; ($F(1,14)=4.947;p<0.05$) (Fig. 3). In contrast, there was no significant difference in infarct size between male adults neonatally treated with LPS (n=13) and male adults neonatally treated with saline (n=11;$p>0.05$) (Fig. 4). A correlation between LPS exposure and a decrease in infarct size is shown in the male study, but experimental power was very low, which decreases the certainty with which the null hypothesis is accepted. Taken together, these data suggest that early exposure to LPS is acting as a neuroprotective agent against ischemia in adulthood in female, but possibly not male, mice.

Effect of LPS on locomotor activity 72 h post-ischemia

Females

There was no effect on any components of the locomotor test pre-surgery ($p>0.05$ for all). Females treated with LPS MCAO (n=9) show a significant decrease in rearing compared to females treated with LPS sham (n=3;$F(2,16)=4.822;p<0.05$). Female vehicle MCAO (n=7) also show a significant decrease in rearing compared to females treated with LPS sham (n=3;$F(2,16)=4.822;p<0.05$). There was no effect of LPS on any other component of locomotor activity for females.

Males

There was a significant decrease in peripheral movement pre-surgery for LPS (n=13) compared to vehicle (n=14;$F(1,25)=4.997;p<0.05$). There was not, however, any significant effect post-

surgery ($p>0.05$). There was no effect pre- or post-surgery for central locomotor activity, central + peripheral locomotor activity, or percent of total activity spent in the center ($p>0.05$ for all). There was an overall effect of experimental groups for rearing post-surgery ($F(3,23)=9.853;p<0.05$). Post hoc analysis reveals a significant decrease in LPS MCAO (n=9) compared to LPS sham (n=4; $F(1,11)=17.630;p<0.05$). There is also a significant decrease in rearing in vehicle MCAO (n=11) compared to LPS sham (n=4;$F(1,13)=19.161;p<0.05$) and a significant decrease in vehicle MCAO (n=11) compared to vehicle sham (n=3;$F(1,12)=4.212;p<0.05$). These results suggest that rearing decreases following MCAO, but is not affected by LPS treatment.

Cylinder test, 72 h post-surgery, shows severe damage provided by LPS treatment despite neuroprotective capabilities

Females treated with LPS MCAO (n=9) show a significant decrease in contralateral paw use compared to the vehicle MCAO (n=7;$F(2,16)=6.319;p<0.05$). Females treated with LPS MCAO also show a significant decrease in contralateral paw use compared to females treated with LPS sham (n=3;$F(2,16)=6.319;p<0.05$) (Fig. 5). These results suggest a significant effect of MCAO and LPS on paw preference. There was no significant difference in the paw preference in male adults neonatally treated with LPS (n=8) compared to male adults treated neonatally with saline (n=10;$p>0.05$) (Fig. 6). There was also no significant difference in paw preference in males treated with LPS MCAO compared to males treated with LPS sham (n=5;$p>0.05$), nor was there a significant difference in paw preference in male vehicle MCAO compared to male vehicle sham (n=3;$p>0.05$).

No difference in growth between mice neonatally exposed to LPS compared to vehicle

There was no significant difference in relative growth rate between female mice neonatally exposed to LPS (n=24) compared to their vehicle counterpart (n=16;$p>0.05$). There was also no significant difference in relative growth rates between male mice neonatally exposed to LPS (n=18) compared to the vehicle in males (n=17; $p>0.05$). These results suggest that neonatal infection does not influence mouse growth rate.

Study 2

This study is a repeat of the first differing only in the concentration of LPS administered.
On postnatal day 14 (P14) litters were randomly selected and *Escherichia coli* LPS (Product Number L 2654) was administered intraperitoneally in sterile saline (1µl/g pup weight) at a dose of 100µg. The other litters were administered an equal amount of sterile, pyrogen-free saline. All other methods are exactly as written above.

Results

Adult mice neonatally exposed to LPS show no significant difference in 24 h edema

Female mice neonatally exposed to LPS (n=2) show no significant difference in edema brain weight compared to the vehicle female (n=4; $p>0.05$). The power is very low (<0.1) implying much variability suggesting that an increase in animal number may provide more significant results. There are no results as of yet for male mice neonatally exposed to LPS.

*Note: Due to an uneven ratio of male to female pups there were not enough males to assess any effects of stroke 24 h post-surgery.

No effect of LPS on locomotor activity 24 h post ischemia

There was no significant difference in locomotor activity 24 h post-ischemia for adult females neonatally exposed to LPS (n=2) compared to the vehicle females (n=5; $p>0.05$). A low power indicates much variability.

No effect of LPS on contralateral paw use 24 h

There was no significant difference in the right versus left paw use 24 h post-ischemia in adult females neonatally exposed to LPS (n=2) compared to vehicle female (n=5; $p>0.05$).

72 h infarct analysis shows that adult mice neonatally exposed to LPS show no significant difference in infarct size compared to vehicle

Female adult mice neonatally exposed to LPS (n=6) showed no significant difference in infarct size compared to the vehicle female (n=6; $p>0.05$). Similarly, male adult mice neonatally exposed to LPS (n=5) showed no significant difference in infarct size compared to the vehicle male (n=11; $p>0.05$).

No effect of LPS on locomotor activity 72 h post ischemia

There was no significant difference in locomotor activity 72 h post-ischemia for adult females neonatally exposed to LPS (n=6) compared to the vehicle females (n=6; $p>0.05$). There was no significant difference in locomotor activity 72 h post-ischemia for adult males neonatally exposed to LPS (n=5) compared to the vehicle males MCAO (n=11; $p>0.05$). Also there was no significant difference in locomotor activity 72 h post-ischemia for adult males neonatally exposed to LPS (n=5) compared to vehicle males sham (n=3;$p>0.05$). .

No effect of LPS on contralateral paw use 72 h post-ischemia

There was no significant difference in right versus left paw use 72 h post-ischemia in adult females neonatally exposed to LPS (n=5) compared to vehicle females (n=7: $p>0.05$). There was no significant decrease in contralateral paw use in males treated with LPS (n=5) compared to male vehicles sham (n=3;$p>0.05$). There was also no significant decrease in contralateral paw use in males treated with LPS (n=5) compared to male vehicles MCAO (n=7;$p>0.05$).

Exposure to LPS as a neonate does effect animal growth

Female mice neonatally exposed to LPS (n=16) show a significant difference in relative growth rate compared to the vehicle (n=16;$F(1,30)=12.175$;$p<0.05$). Post hoc analysis reveals a significantly heavier weight in LPS females compared to vehicle at each 7 day interval from P14 to P42 ($p<0.05$ for all) and a significantly lighter weight in LPS females compared to vehicle at P49 ($p<0.05$) (Fig. 7). Male mice neonatally exposed to LPS (n=7) show no difference in relative growth rate compared to the vehicle (n=7) (Fig. 8).

Discussion

In this study, we examined the effects that neonatal infection has on adult stroke outcome in mice. Stroke damage was compared between mice that had been infected as neonates with LPS versus the vehicle control. In females treated neonatally with low LPS there was a significant decrease in infarct size 72 h post-surgery. These data suggest that a low dose of LPS is providing neuroprotection in females against ischemia in adulthood. Contrary to these data, however, there was a significant decrease in contralateral paw use 72 h post-surgery in females treated with the low dose of LPS compared to vehicle. This implies that although LPS is

preventing neuronal death, the damage to the neurons is such that behavioral deficits common following stroke still develop. There was no effect on of LPS treatment in females on edema 24 h post-behavior, nor was there any effect on locomotor or paw preference behavior 24 h post surgery. One female was not included in the 72 h cylinder testing because the total post was greater than two times the standard deviation making the animal an outlier.

The first study found no significant difference in the stroke outcome or behavior for male adults treated neonatally with LPS compared to vehicle, regardless of whether assessment was made 24 h post-surgery or 72 h post-surgery. Future studies will employ a higher dose of LPS in males.

To test for dose dependent differences, in the second study a higher dose of LPS was administered to the neonates. The higher dose showed no effect of treatment on edema 24 h post-surgery and there was also no effect on infarct 72 h post-surgery. This higher dose also showed no effect on behavior or anxiety in either of the assessments. That there was no effect on 72 h post-surgery mice treated with the higher dose of LPS leads us to believe that the dose was high enough to no longer provide neuroprotection yet not high enough to affect behavior in non-ischemic mice.

The significant decrease in locomotor activity for both male and female 72 h post-MCAO surgery compared to 72 h post-sham surgery indicates an effect of surgical technique on mice treated with the low dose of LPS. There was not, however, any effect of surgical technique on mice treated with the higher dose of LPS.

There appears to be inconclusive, yet promising, data for the impact on up-regulated COX-2 in both initial and secondary ischemic pathways. The next step is to increase each data set to reduce the variability, increase statistical power, and then to increase the dose of LPS

administered. There is an apparent difference in results between the two doses suggesting the amount of LPS injected has a significant effect on stroke outcome. Assessing the role of COX-2 post-stroke will afford much information about the exact role that COX-2 plays and how it is comparatively up-regulated with each LPS dose. Also assessing the level of PGE_2 in the brain post-stroke will prove useful in identifying the level of COX-2 and its role in ischemia, as PGE_2 is a by-product of COX-2 and the inflammatory agent in secondary stroke outcome. Specifically, PGE_2 EP1 receptors have been denoted the underlying factor in neurotoxicity, that in fact COX-2-derived PGE_2 is not neurotoxic in the absence of EP1 (Kawano *et al*, 2006. Studies show that administration of an EP1 receptor inhibitor SC51089 reduces the infarct size in ischemic stroke (Kawano *et al*, 2006). It would therefore be interesting to determine the effect LPS has on EP1 receptors.

If administering a small amount of LPS as a neonate is neuroprotective to ischemia, it could prove to be a very important therapeutic agent in human stroke. As an influenza vaccination protects against the influenza virus, perhaps the cell wall of a gram-negative bacteria will protect against stroke. In recent years, COX-2 inhibitors have been associated with improved stroke outcome. However, COX-2 inhibitors suppress vasodilators such as prostacyclin (PGI_2) that counter the cardiovascular effects of the platelet agonist vasoconstrictor thromboxane (TxA_2). Thus inhibition of COX-2 may indirectly enhance the risk of thrombosis (Fitzgerald, 2003), and subsequently stroke or cardiac arrest. It will be interesting to determine the relationship between LPS as a protective agent and the subsequent increase in COX-2 as a protective agent against stroke.

It is necessary to determine the role that COX-2 has in the mechanisms underlying stroke damage, as COX-2 may not be the only player. Studies have shown that when exposed to LPS

iNOS can be induced to express in macrophage resulting in the production of large amounts of nitric oxide (NO) (Luo et al. 2005). NO derived from activated macrophages strongly influences immune function through inhibiting cell proliferation, antibody production, and regulating cytokine production. Therefore, one may speculate that exposure to LPS increases the level of NO produced in the system resulting in a larger infarct size post-stroke due to an attenuated immune response. Perhaps a small amount of LPS inhibits nitric oxide attenuating the level of PGE_2 concentration.

The present study has shown that a low dose LPS acts as a neuroprotective agent against ischemia in females. Despite reduced neuronal death in low dose LPS females, behavioral deficits still developed, indicating that neurons were still damaged. Also, because there was no effect on females with a high dose LPS it can be implied that the effects of LPS are dose-dependent, and having no effect on males whatsoever, implies a gender-bias. Future research will be aimed at determining the mechanism underlying the protective qualities of neonatal exposure to LPS in female mice.

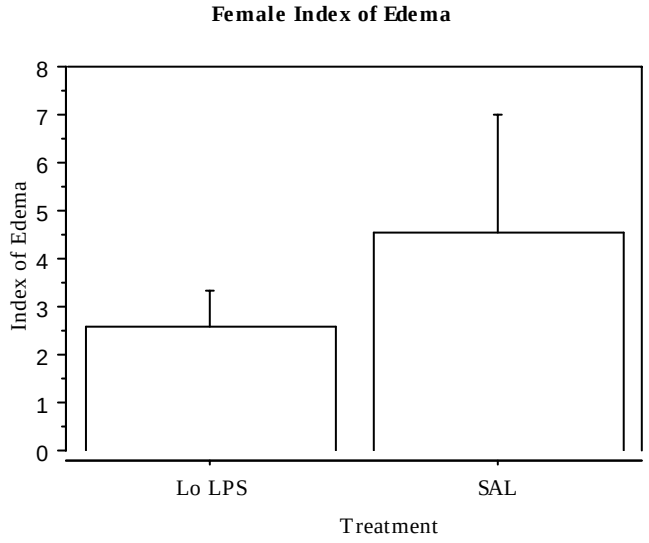

Figure 1: No effect of LPS in female edema

Mean ± SEM

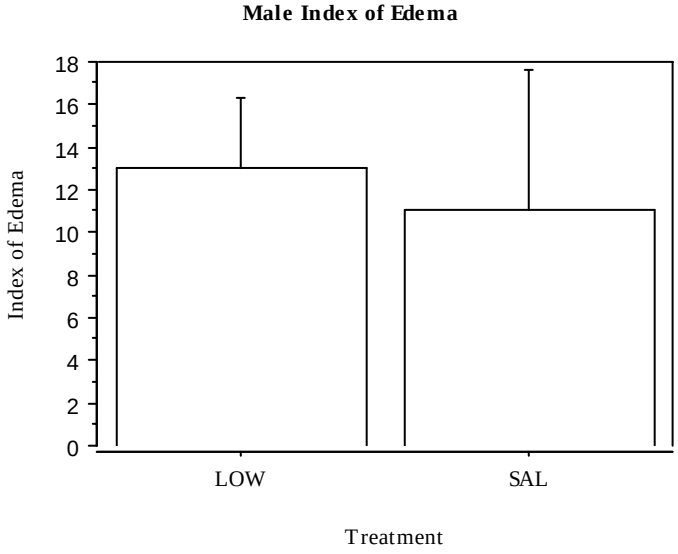

Figure 2: No effect of LPS in male edema

Mean ± SEM

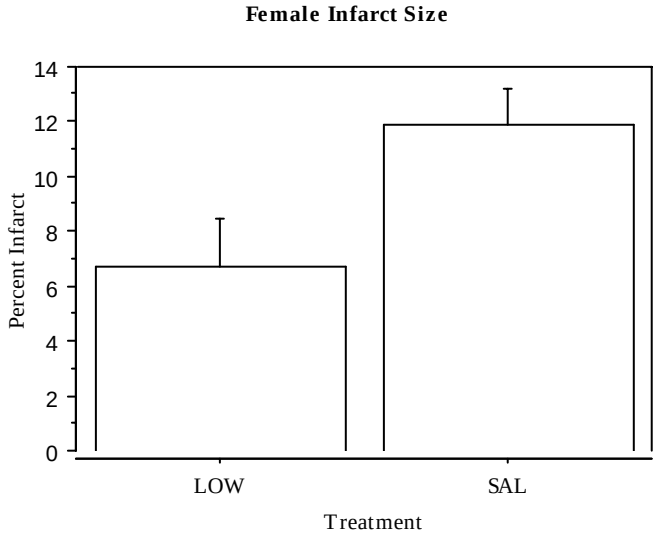

Figure 3: Females treated with LPS show a reduction in infarct

72 h post-surgery compared to vehicle

Mean ± SEM

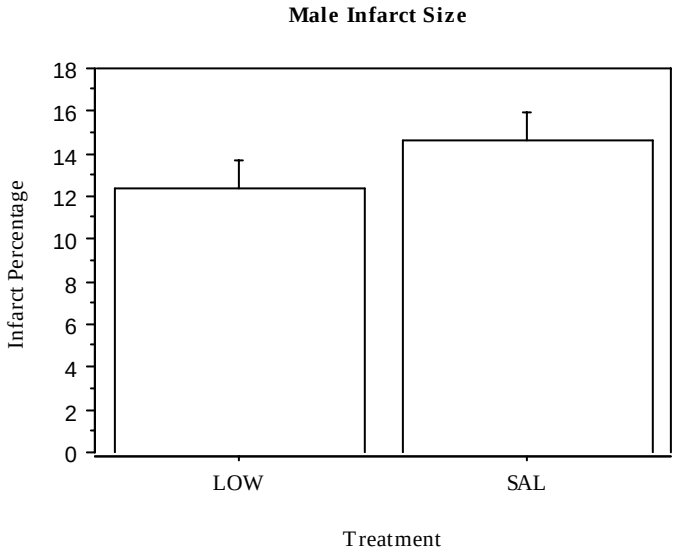

Figure 4: No effect of LPS on male infarct size

72 h post-surgery compared to vehicle

Mean ± SEM

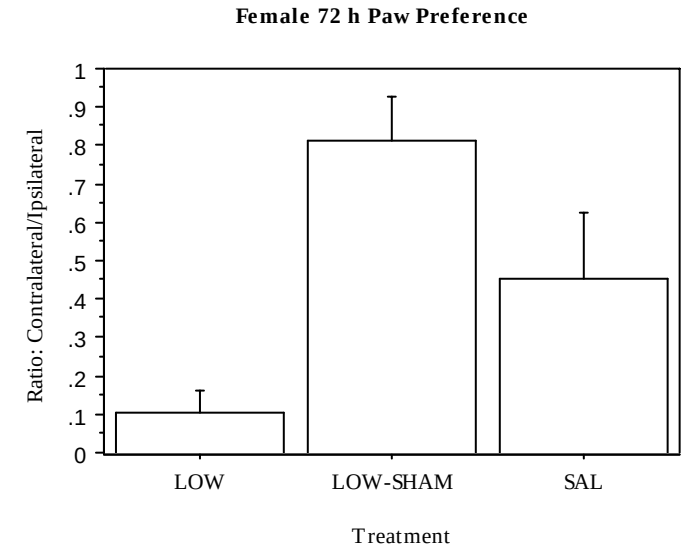

Figure 5: Females treated with LPS show a reduction in contralateral paw use 72 h post-surgery compared to vehicle

Mean ± SEM

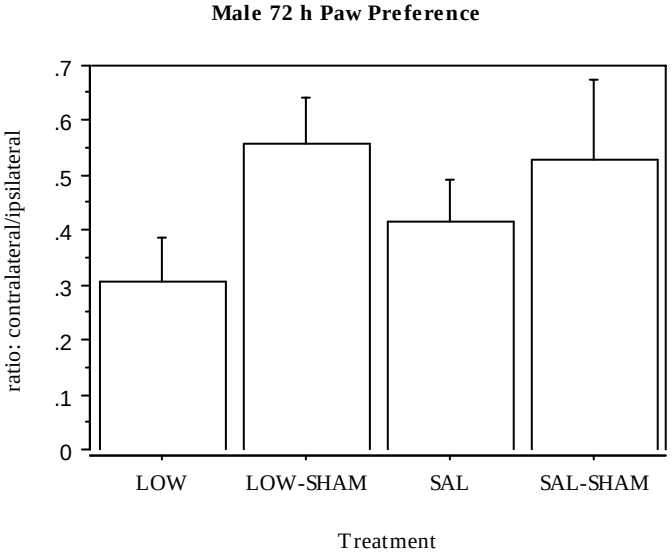

Figure 6: No effect of LPS on contralateral paw use 72 h post-surgery

Mean ± SEM

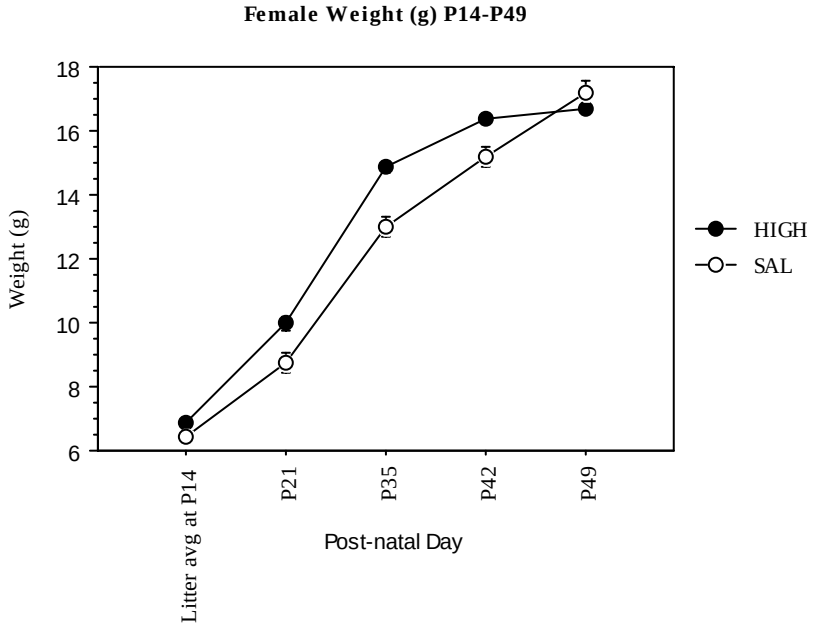

Figure 7: Females treated with high LPS were significantly heavier than vehicle from P21-P42, and significantly lighter on P49

Mean ± SEM

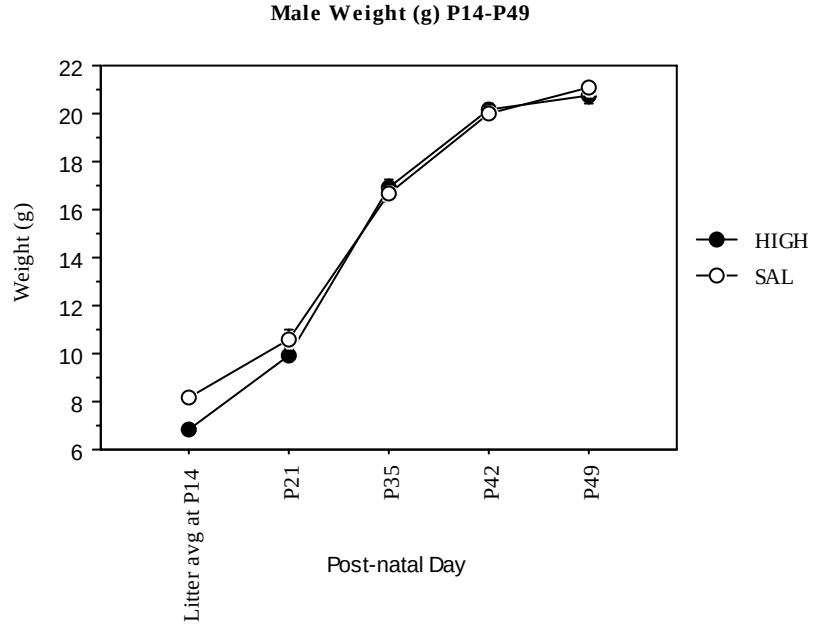

Figure 8: No effect of LPS on growth

Mean ± SEM

References

Boissé, L.; Mouihate, A.; Ellis, S. and Pittman, Q. J. (2004). Long-term alterations in neuroimmune responses after neonatal exposure to lipopolysaccharide. *Journal of Neuroscience*, 24(21). 1928-1934

Fitzgerald, G. A., (2003). COX-2 and Beyond: Approaches to prostaglandin inhibition in human disease. *Nature Reviews Drug Discovery*, 2. 879-890

Iadecola, C.; Niwa, K.; Nogawa, S.; Zhao, X.; Nagayama, M.; Araki, E.; Morham, S.; and Ross, M. E. (2001). Reduced susceptibility to ischemic brain injury and *N*-methyl-$_{D}$ -aspartate-mediated neurotoxicity in cyclooxygenase-2-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 1294-1299.

Kawano, T.; Anrather, J.; Ping, Z.; Park, L.; Gang, W.; Frys, K. A.; Kunz, A.; Cho, S.; Orio, M.; and Iadecola, C., (2006). Prostaglandin E[2] EP1 receptors: downstream effectors of COX-2 neurotoxicity. *Nature Medicine*, 12(2). 225-229

Luo, G.; Peng, D.; Zheng, J.; Chen, X.; Wu, J.; Elster, E.; and Tadaki, D., (2005). The role of NO in macrophage dysfunction at early stage after burn injury. *Burns*, 31(2). 138-144

Nogawa, Shigeru; Zhang, Fangyi; Ross, M. Elizabeth; and Iadecola, Constantino, (1997). Cyclo-Oxygenase-2 gene expression in neurons contributes to ischemia brain damage. *Journal of Neuroscience*, 17(8). (2746-2755)

Reiter, Russel J.; Tan, Dun-xian; Leon, Joseph; Kilic, Ulkan; and Kilic, Ertugrul, (2005). When melatonin gets on your nerves: its beneficial actions on experimental models of stroke. *Experimental Biological Medicine*, 230. (104-117)

Stahl, S. M. (1997). Apoptosis: Neuronal Death by Design. *Journal of Clinical Psychiatry*, 58(5). 183-184

Stoll, Guido; Jander, Sebastian; and Schroeter, Michael, (1998). Inflammation and glial responses in ischemic brain lesions. *Progress in Neurobiology*, 56. (146-171)

Turley, Kenneth R.; Toledo-Pereyra, Luis H.; and Kothari, Rashmi U., (2005). Molecular mechanisms in the pathogenesis and treatment of acute ischemic stroke. *Journal of Investigative Surgery*, 18. (207-218)

White, Blaine C.; Sullivan, Jonathon M.; DeGracia, Donald J.; O'Neil, Brian J.; Neumar, Robert W.; Grossman, Lawrence I.; Rafols, José A.; and Krause, Gary S., (2000). Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *Journal of the Neurological Sciences*, 179. (1-33)

Williams, Linda S.; Weinberger, Morris; Harris, Lisa E.; Clark, Daniel O.; and Biller, José; (1999). Development of a stroke-specific quality of life scale. *Stroke*, 30. (1362-1369)

Yrjänheikki, J.; Tikka, T.; Keinänen, R.; Goldsteins, G.; Chan, P. H.; and Koistinaho, J., (1999). A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proceedings of the National Academy of the Sciences*, 96(23). (13496-13500)