THE ROLE FOR AMYLOID BETA IN COGNITIVE DYSFUNTION

by

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THE ROLE FOR AMYLOID

BETA IN COGNITIVE

DYSFUNTION

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commitment looks like, and I strive to emulate that. You are the best Lab Mom anyone could hope for, and I thank you for everything.

INTRODUCTION

Over one-hundred years ago, Alois Alzheimer published a remarkable two page paper known as "A Characteristic Disease of the Cerebral Cortex." In his ground breaking paper, Alzheimer described a 51 year old woman, Auguste D, who displayed progressive cognitive impairments, hallucinations, delusions, as well as loss of memory. The patient suddenly started having trouble remembering simple tasks, and as time progressed, she began having behavioral changes as well. Alzheimer began studying her near the end of 1901, and over the next four years he kept a detailed account of her neurological demise. At postmortem, her body was examined. Alzheimer noted the presence of an atrophied brain riddled with senile plaques (which he initially referred to as irregular protein clusters) and neurofibrillary tangles (Alzheimer 1907). Both plaques and tangles are hallmarks of the disease known today as *Alzheimer's*. Though the eponym *Alzheimer* was initially used to represent "presenile dementia," it was later also "applied to dementing processes of old age" (Whitehouse).

Globally, Alzheimer's disease (AD) is the most common form of dementia and currently afflicts upwards of 35 million individuals. As many as one out of eight older Americans has AD. As average life expectancy increases, AD, which is found primarily in those ages 65 and older, is becoming an increasingly prevalent issue. AD is the sixth leading cause of death within the US for those over the age of 65, and is becoming increasingly more prevalent globally as population size continues to escalate. The incidence of AD shows no signs of slowing within the projected future either. It is projected that within the next forty years, AD will affect over 16 million individuals (Alzheimer's Facts & Figures).

Clinically, AD involves progressive memory loss and cognitive deficits. Characterized by an initial loss of short term memory, the symptoms worsen over time and eventually include long term memory loss as well as disorientation, confusion, and communication problems (Perry). The sum of these symptoms costs billions of dollars annually in lost wages and medical bills. It is estimated that last year alone, over two-hundred billion dollars was directly spent by those afflicted with AD. This is expected to reach a staggering $1.1 trillion by 2050 in "aggregate payments for health care, long-term care, and hospice" for those afflicted with AD. Over 15 million Americans "provide unpaid care for a person" with AD which amounts to an estimated "17.4 billion hours of unpaid care" (Alzheimer's Facts and Figures). Currently, treatment is limited in scope and efficacy, though much research is dedicated to better understanding the signs and pathologies of AD in order to help slow, prevent, or potentially reverse the impacts of AD.

AD is a continuous advancing neurodegenerative disease. The development of symptoms is different from patient to patient, but has three distinct stages including mild, moderate, and severe. The progression of the symptoms is usually sequential and continuing demise of "neuronal functions and synaptic connections, and neuronal cell death" in various parts of the brain (Lau). In the mild stages of AD, the disease first manifests with the loss of memory due to the fact that neurons in the brain region responsible for memory, the hippocampus, is the first to be affected. Patients are known to forget names and words with an increasing frequency. The moderate stages, the cortical areas responsible for reasoning start to demise as patients typically have a loss of logical thinking and suffer from confusion. Behavioral and personality changes may also

occur. In the more advanced severe stages of AD, more brain regions atrophy and are damage which result in the inability to control many normal physiological functions. Patients are incapable of daily chores and become unable to speak coherently. Some even require assistance in feeding, walking, and bathing. Death is usually linked with the deterioration of the brain's capability to control vital physiological functions leading to deadly complications like urinary tract infections, pneumonia, or even physically falling (Perry).

What sets AD apart from other neurodegenerative diseases is the presence of trademarks within the brains: neurofibrillary tangles (NFTs) and amyloid plaques. Amyloid plaques are comprised largely of β amyloid (Aβ**)** peptide, while NFTs consist of "intracellular hyperphosphorylated tau in the forms of paired helical filaments." (Lau). According to the amyloid cascade hypothesis, Aβ is the cause of activating the cascade of events, together with forming NFTs, ultimately leading to neurodegeneration and the demise of brain functions in AD patients (Cho). The evidence backing the cascade hypothesis is the fact that the mutations causing AD with 100% penetrance are situated on the gene which encodes proteins involved in regulating Aβ levels. One mutation in particular, for the amyloid precursor protein (APP), is an important substrate for the production of Aβ. The buildup of Aβ peptides into plaques has been shown to damage hippocampal long term potentiating, which is long believed to be the foundation of learning and memory (Lau).

NFTs contain hyperphosphorylated tau and are vital in stabilizing the microtubules within neural cells. (Markesbery). Microtubules help with cellular development and moving molecules to the correct location within the cell. When tau is

hyperphosphorylated, it becomes separated from microtubules leading to disassembly. Without correctly regulating tau, the resultant destabilization of microtubules is believed to lead to the formation of NFTs (Cras et al). Tau plays a significant role in the ultimate demise of neurons because certain genetic mutations on tau are enough to cause neurodegeneration in frontotemporal dementia. The exact cause of how tau causes neurodegeneration is not completely understood, but it is thought to be tied to the loss of necessary functions and gaining lethal functions (Lau). Reduction of tau toxicity is a significant area in the struggle against AD.

In the formation of Aβ plaques, the amyloid precursor protein is degraded and cleaved by a variety of proteins, including γ-secretase. In this cleaving reaction, a varying degree of Aβ peptide lengths are created, some of which have a hydrophobic tendency. Early forms of AD have been thought to involve genetic mutations in γ-secretase genes which may in turn result in an amplified amount of long form Aβ (Seiffert 2000). Opposed to short Aβ peptides, the long strands are much less soluble, specifically the hydrophobic $A\beta_{1\text{-}42}$, which is shown to have the most noteworthy impact on the formation of plaques. In fact, the long stranded Aβ tend to form plaques at a much higher rate and are difficult of for the brain to remove (Heneka and O'Banion, 2007). Plaques are particularly harmful due to the fact that they interfere with neuronal signal transductions which lead to not only cognitive deficits seen in AD, but also eventual cell death. Communication between neurons is blocked by the presence of plaques within the brain, and this leads to the dysfunctional neurons and ultimate cognitive decline (Murphy and Levine, 2010). The collective damage incurred by the neurons as a result of γ-

secretase, NFTs, and plaques are generally the root cause of the degeneration of the brain in AD.

Two major classifications of AD are early onset AD and sporadic AD. Early onset AD is believed to have links to certain gene features that may be passed on to offspring and represents about ten percent of all cases of AD in humans. The sporadic form, however, is the more common form and begins after about the age of 65. Though much is not understood about the onset of sporadic AD, many agree that neither the presence of APP nor γ-secretase gene initiate the disease (Jacobsen 2006).

Two basic discoveries have prompted research into believing that inflammation is the driving cause in the pathology of AD. The first discovery was the identification of "activated microglia in association" in relationship with the plaques. The second was the discovery that those with rheumatoid arthritis were relatively spared from AD (McGeer 271-276). Because of these initial findings, resultant studies have positively identified a correlation between onset of sporadic AD and inflammation. A variety of experiments and surmounting evidence have almost confirmed the positive link between chronic inflammation in the brain being a part of AD pathology. Using a non-steroidal anti-inflammatory drug (NSAID), Rogers and colleagues were the first to propose the idea that anti-inflammatory drugs could delay the progression of AD (Rogers, and Gilman). NSAIDs may help decrease the occurrence of inflammation of AD in some patient decreasing by almost fifty percent. Researchers hypothesize that the anti-inflammatories interfere with the function of key proteins in the AD pathology, thus decreasing their detrimental effects (Turkington). A study published in the May 2007 issue of *Neurology* reported that patients whose blood had detectable signs of inflammation were at a much

higher risk of developing AD opposed to those without signs of inflammation in their blood. They had tested the blood for cytokine levels which are proteins that activate the inflammatory response. Those with higher levels of cytokines in their blood were almost twice as likely to develop AD compared to those with minimal cytokine levels. They also reported that cytokine levels may be a sign for risk of AD in the future (Turkington).

AD is a disease exclusive to humans, but the use of animal models has contributed greatly to our understanding of certain pathways and pathologies of AD. The most widely used model for AD is the triple transgenic mouse (3xTg-AD) which carries three mutant human genes amyloid precursor protein, presenilin, and tau. This gives the transgenic mouse the unique capability to create Aβ plaques and neurofibrillary tangles similar to AD. (Blurton-Jones 2009). 3xTg-AD mice quickly develop both plaques and tangles in the hippocampus and neocortex in both an age and region dependent method that is similar to that of humans (Chen 2012).

In the last few years, new research has shown that exposure to bacterial endotoxins such as lipopolysaccharide (LPS) had an effect on the amount of Aβ build up within the brain. LPS contains components of gram-negative bacteria cell walls and mimics bacterial infections. When mice are injected with LPS, they have a faux bacterial infection which induces subsequent inflammation. The researchers determined that continued injections of LPS led to an increased build up of Aβ in the hippocampus. In an effort to link peripheral inflammation with AD and cognitive decline, they injected mice with LPS for 3 days and tested their behavior. Their studies showed that the “co-elevated inflammation and amyloidogenesis” led to neuronal cell death, which in turn resulted in impaired hippocampus-dependent learning (Lee et al., 2008).

Previous studies conducted by our lab have presented additional evidence that continuous and repeated exposure to the bacterial endotoxin LPS consequently leads to cognitive decline and an elevation of Aβ in the hippocampus in the mouse. The previously discussed study by Lee did not take into account the effect of sickness behavior when testing for learning deficits. To examine this, our lab conducted studies involving open field behavior tests to determine appropriate timings for the subsequent behavior tests. Using these results, both Morris water maze and contextual fear conditioning (CFC) tests were used to gauge cognitive decline. Both tests revealed significant decline in learning capability in the mice receiving LPS injections. In order to measure Aβ levels in the brain and the levels of central pro-inflammatory cytokines, hippocampus samples were acquired. By the seventh day, the mice did not display any signs of sickness behavior. Mice which received LPS injections had substantially higher amounts of Aβ in their hippocampus. Due to the LPS injections, there were initially elevated levels of both central and peripheral pro-inflammatory cytokines, but by the seventh day when Aβ levels were the highest, the mice developed an endotoxin resistance and no longer exhibited elevated levels of either pro-inflammatory cytokines (Kahn et al., 2012).

The link between bacteria and an accelerated disease progression of AD has been well documented, but the newer area of interest is the effects of viral pathogens and inflammation. Polyinosinic: polycytidylic acid (Poly I:C) is a Toll Like Receptor 3 (TLR3) “agonist and induces type I interferons,” which mimics the inflammatory response to a viral infection . In one study, animals were injected with Poly I:C to stimulate a viral-like infection, which led to a considerable amplification in the

production of central nervous system interferons when compared to animals given just saline. Repeated exposure to Poly I:C caused neurological deficits as well as an accelerated progression of late stage AD signs compared to the control animal group (Field, Campion, Warren, and et al 996-1007).

Poly I:C is a synthetic double stranded RNA analog believed to be identified by TLR3 by making pro-inflammatory cytokines and chemokines (Alexopoulou et al., 2001). Various behavioral studies have been conducted utilizing CFC in order to gauge the cognitive decline of animals when injected with Poly I:C. In order to evaluate hippocampus-dependent learning, behavior testing relying on spatial recognition learning was used. The hippocampus is known to play a key role in converting short-term into long term memory as well as spatial recognition. Experiments have shown that the activation of the hippocampus is "primarily driven by the context exploration component" of a CFC test (Huff, Frank, Hardesty, and et al 1616-1623). CFC most often involves first acclimating the subject to a neutral context, followed by a negative stimulus. The most commonly used negative stimulus involves the use of either one or multiple microshocks. 24 hours following fear conditioning, the subjects are tested for contextual fear memory (Kittelberger, Piazza, and et al). Animals displaying AD-like symptoms have been shown to have noteworthy impaired hippocampal learning in CFC testing as shown in CFC tasks (Jacobsen et al., 2006).

Previous studies conducted by our lab sought to determine if a lengthened viral inflammation would have similar results to that of bacterial inflammation in regards to the AD progression. Induced inflammation in mice through Poly I:C has been known to induce sickness behavior which can distort results obtained from CFC. In order to

account for this and make sure sickness behavior is not misinterpreted as improved learning in CFC, burrowing studies were employed to determine the proper test time following Poly I:C injections. When animals are sick, they tend to burrow less, and it is a very sensitive hippocampus dependent task used to help detect cytokine-induced disruptions (McLinden et al., 2012). Our lab did not use burrowing for LPS tested animals due to the fact that by the seventh day of injections, they had developed an endotoxin tolerance and did not display signs of sickness behavior. We have determined that repeated injections of Poly I:C results in an increased Aβ production, however there were no cognitive deficits displayed during CFC training (unpublished data).

The overall amount of Aβ produced by LPS injected mice is elevated in comparison to those injected with Poly I:C. Due to this irregularity, it is difficult to compare the effects of bacterial endotoxins to that of viral infection. Therefore, we hypothesize that the amount of Aβ produced has a role in an animal's ability to learn, and in order to test that, we attempted to normalize the potency of LPS in order to obtain similar Aβ production levels between LPS and Poly I:C. LPS dosage will be sequentially decreased until the same level of Aβ is produced between the LPS and Poly I:C groups. CFC will then be used to measure hippocampus-dependent learning between mice receiving LPS, Poly I:C, and those receiving saline injections. An ELISA assay will be used to determine the amount of Aβ. We believe that there is a base level of Aβ that does not hinder cognitive abilities, but anything above this level would lead to deficits.

MATERIAL AND METHODS

Subjects and housing

All experiments employed the use of male C57BL/6J mice from the Texas Christian University vivarium. Animals were treated and housed following the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and in agreement with standards approved by the Institutional Animal Care and Use Committee (IACUC) of Texas Christian University.

Animals were housed in groups of three or four in standard cages sized 12.5cm x 15cm x 25 cm. All the experimental groups were housed under the same conditions. Lights were on only from 6:00 AM to 6:00 PM daily and food and water were constantly available for mice to consume as they desired.

Biological Assays

Treatment conditions

Prior to behavioral testing, doses of either 250μg/kg LPS, 67.5 μg/kg LPS, Poly I:C, or saline injections were given for seven days consecutively. In order to properly assess the effects of both Poly I:C and LPS, a control group of mice was used which was given saline injection for seven consecutive days prior to behavioral testing to make certain that behavioral differences were not due to the stress of consecutive injections. The mice received intraperitoneal injections of either the previously utilized dose (250μg/kg) or a lower dose (67.5μg/kg) of LPS, Poly I:C (12 mg/kg), or saline. Following seven consecutive days of a once-daily injection, mice were tested for hippocampus-dependent cognitive impairments utilizing the contextual conditioning behavioral paradigm.

Tissue preparations

Following the injections at the selected times, the mice were euthanized using IACUC protocol. Samples of hippocampus tissues were harvested for the purpose of protein assays and ELISA procedures. The tissue was then homogenized with protein extraction solution (PRO-PREP, Boca Scientific, Boca Raton, FL.) which contained protease inhibitors. The samples were on ice for thirty minutes in order to further lyse. The lysates were centrifuged at 16,000 rpm for 30 minutes. The clear lysate was then removed for protein assay.

Aβ ELISA procedure

The BetaMark Aβ ELISA (Covance Research Products, Dedham, Massachusetts) involved the use of a 96-well plate over a period of 48 hours. Coating each well with antibody, the wells were then filled with either the sample or standards of known concentration. The incubation buffer and the standard intermediates were made before beginning the assay. The Standard Diluent was used in order to make the standard curve as well as the Aβ standard. Samples were subsequently diluted in the buffer, which included the HRP-labeled detection antibody at a ratio of 2 to 1. The plates were then incubated overnight at 2-8 degrees Celsius. The proceeding day, each well was washed 5 times using 1X wash buffer. After the washes, 200 μL of TMB was added. TMB is the HRP enzyme substrate. The plate then incubated for forty-five minutes at room temperature within a dark room. Following this, an optical density of 620 nm was used to read the plates.

Statistics

Using an analysis of variance (ANOVA) statistics, we were able to quantify significant differences both between and within experimental groups. If a significant omnibus F was found, then the use of Fisher's PLSD post-hoc test was implemented to conclude which groups were significantly different.

Behavioral Paradigms

Contextual fear conditioning

CFC (contextual fear conditioning) relies on the freezing behaviors of mice. The use of Freeze Monitor System and software (7 in x 7 in x 12 in) was implemented to measure freezing behaviors. The CFC unit (Coulbourn Instruments, Whitehall, PA) has a floor containing an electric grid which emits a negative stimulus to the mouse. The ceiling of the unit houses both a small light bulb as well as a camera to track and monitor the movements of mouse. The first day is used as training, and the subsequent second day is used as testing. 48 hours following the final Poly I:C injection and 24 hours following the final LPS and saline injection, the mice were put in the unit for day one (training).

A single shock experiment protocol was used. During the training session, there was a 120 second acclimation period preceding a 2 second 0.7 mA mild shock to their feet, which was then followed by a 60 second phase where they remained within the unit. The total training time was 182 seconds. The following testing day, the mice were once again placed in the unit. On the testing day, no shock was administered. Instead, the animals' movements were recorded for 90 seconds continuously in order to observe freezing behaviors. The freezing behavior was documented as the percentage of the total time in which the animal was freezing. This indicates hippocampus-dependent contextual

learning. The longer an animal freezes implies an increase in learning in an hippocampus-dependent task.

RESULTS

Appearance of animals and weight loss following injections

For all seven injections, before the injection was administered, the animals were weighed and visually inspected in all four experimental groups. Animals receiving seven injection of Poly I:C and LPS showed classic signs of sickness such as decreased weight and lethargy. As expected, there were no weight differences between groups before any injections were administered ($F(3,28) = 2.142$, p=ns ; see figure 1A). However, there was an overall significant weight difference found when comparing weights from day 1 to weights on day 7 ($F(3,28) = 4.9.09$, $p < .01$; see figure 1B). Utilizing a Fisher's PLSD, significant weight differences were detected between 250μg LPS and 67.5ug LPS groups ($p < .05$), 250μg LPS and saline ($p < .01$), 67.5 μg LPS and Poly I:C ($p < .05$), and Poly I:C and saline ($p < .01$). However, there were no significant differences in weight loss between the groups 250 μg LPS and Poly I:C (ns) or between groups 67.5 μg LPS and saline (ns). Weight loss from administration of both LPS and Poly I:C was expected as they both induce inflammatory processes leading to sickness behavior including decreased eating, drinking, and lethargy.

Poly I:C & LPS induced Aβ production

Four experimental groups, 250μg LPS, 67.5 μg LPS, Poly I:C or saline injected mice, were used to determine if daily injections would all lead to changes in Aβ production in the hippocampus. The animals were administered one injection consecutively for seven days. Forty-eight hours after Poly I:C and 24 hours after the final

LPS and saline injections, the mice were euthanized in accordance to IACUC protocols, tissue from the hippocampus was immediately harvested and an ELISA was conducted to deduce the total amount of Aβ. As shown in Figure 2, there was an overall significant difference in the amount of hippocampal Aβ ($F(3,26) = 8.637$, $p<.001$). Further Fisher's PLSD post-hoc analysis revealed significant differences between 250μg LPS and Poly I:C ($p <.01$), 250μg LPS and saline ($p< .0001$), 67.5 μg LPS and saline ($p< .01$), and Poly I:C and saline ($p<.05$). Surprisingly, there were no significant differences in Aβ amounts between the groups 250 μg LPS and 67.5 LPS (ns) and 67.5ug LPS and Poly I:C (ns). Both doses of LPS and the dose of Poly I:C were able to significantly increase the amount of hippocampal Aβ as compared to saline, however, we were not able to significantly reduce the amount of Aβ produced from a lower dose of LPS.

Contextual Fear Conditioning

The hippocampus dependent task contextual fear conditioning was used to determine if there were any variations in learning between the LPS, Poly I:C, and saline injected mice. Daily injections were administered to animals for seven consecutive days which were completed 48 hours prior to testing for Poly I:C injected mice and 24 hours prior to testing for LPS and saline injected mice. Results from the training session of CFC, as expected, depict no significant differences in freezing time ($F(3,26) =.178$, $p=$ ns; see Figure 3A). During the testing day, however, there was an overall significant difference in freezing time detected between the groups ($F(3,26) = 4.061$, $p < .05$, see Figure 3B). Using Fisher's PLSD post-hoc analysis we determined that that there was significantly less freezing in animals injected with 250 μg LPS group as compared to the 67.5 μg LPS and saline groups ($p<.05$). Animals administered Poly I:C also froze

significantly less than both the 67.5 μg LPS and saline injected groups ($p < .05$). There were no differences in learning between animals administered 250 μg LPS or Poly I:C (p= ns), or between animals administered 67.5 μg LPS when compared with animals administered saline (p=ns). Freezing Data from CFC, which is used to measure learning in rodents, revealed that animals administered either 250 μg LPS or Poly I:C froze less, and therefore displayed cognitive deficits, as compared to animals administered either 67.5 μg LPS or saline.

DISCUSSION

In the current study, we wanted to determine if multiple peripheral injections of Poly I:C can lead to hippocampus-dependent learning deficits, as previously seen after LPS injections. We also wanted to determine if there was a minimum amount of Aβ protein required to alter hippocampus-dependent learning. Our lab has previously shown that peripheral injections of a bacterial mimetic (lipopolysaccharide; LPS) and a viral mimetic (polyinosinic: polycytidylic acid; Poly I:C) led to elevated levels of Aβ in the mouse hippocampus. Previous murine studies have also shown that peripheral injections of the bacterial-mimetic lipopolysaccharide (LPS) lead to hippocampus-dependent learning deficits.(Kahn et al., 2012). Though LPS acts as a bacterial infection and Poly I:C acts as a viral infection, we saw a different response in physiological changes from peripheral inflammation between the two. This project attempted to normalize the Aβ production between LPS and Poly I:C in order to compare both cognitive and physiological findings for inflammation. We sought to better comprehend the possible role of peripheral inflammation in the beginning of Alzheimer's disease pathologies, particularly the elevation of Aβ within the hippocampus.

Our findings showed that 7 consecutive days of once daily injections of Poly I:C, high dose LPS, or low dose LPS resulted in a significantly elevated amount of Aβ in the hippocampus. Hippocampal tissue was harvested in order to assess the Hippocampal-Aβ levels. Just as in studies demonstrating the connection between elevated Aβ and cognitive deficits, we had predicted that the high dose LPS animals would display learning deficits while the Poly I:C animals would not. We hypothesized that the low LPS dose, since it produced Aβ similar to that of Poly I:C, should not display cognitive deficits either.

In order to asses that sickness behavior was evident, weight data was collected. The results indicated that before the start of the experiment, there was no significant difference in weight between groups. By the end of the experiment, however, the total weight lost between groups varied. There was a significant difference in weight lost between the low dose LPS group and the high dose LPS group indicating that the low dose LPS group was less sick than that of the high dose LPS group. This was expected due to the fact that one group is receiving a smaller dose of the same compound. When comparing the low dose LPS to Poly I:C, there was a significant difference in total weight loss indicating that the low dose LPS group lost significantly less weight than Poly I:C. This data suggested that the low dose LPS group experienced less sickness than that of both Poly I:C and the high dose of LPS. However, when looking at the ELISA analysis for Aβ production, both the low dose LPS and Poly I:C were not significantly different from each other in Aβ production. Both groups produced a similar level of Aβ, yet the low dose LPS experienced less sickness in comparison. One explanation for this might be that a minimum level of Aβ must be present in the hippocampus, and this Aβ production level is near that level. Interestingly, statistically, the low dose LPS and high dose LPS

groups are not significantly different from each other in Aβ production; however, we attributed this to be due to the low sample size that was used.

Contextual fear conditioning (CFC) was used to determine potential alterations in Hippocampal function following injections. Our results indicated that both the high dose LPS and Poly I:C groups suffered cognitive deficits while the low dose LPS group showed no apparent cognitive decline. Even though both the low dose LPS and Poly I:C produced a similar level of Aβ, the low dose LPS group showed no cognitive deficits in comparison. One theory was that a minimum level of Aβ must be present in the hippocampus to impair learning in a hippocampus-dependent task, though it is not likely the sole factor.

Future steps for our lab would be to execute further studies utilizing a larger sample size to replicate the results. Additionally, studies trying to narrow the region of the minimum Aβ necessary to induce cognitive deficits should be conducted.

This data builds upon previously collected data which indicated the damaging effects of peripheral inflammation. Considerable evidence gained over the past decade indicates that peripheral inflammation leads to an elevation in Aβ within the brain. This may have a function in the sequence of Alzheimer's disease like pathology, including cognitive decline and the loss of memory. Those afflicted with chronic inflammatory diseases such as Diabetes Type II, hypertension, and Lupus, need to be conscious of the potential risk. These findings will be valuable as we work towards targeting inflammation or Aβ production in order to prevent cognitive decline associated with AD. Understanding the connection between neuroinflammation and neurodegeneration is fundamental for continued research.

Figure 1

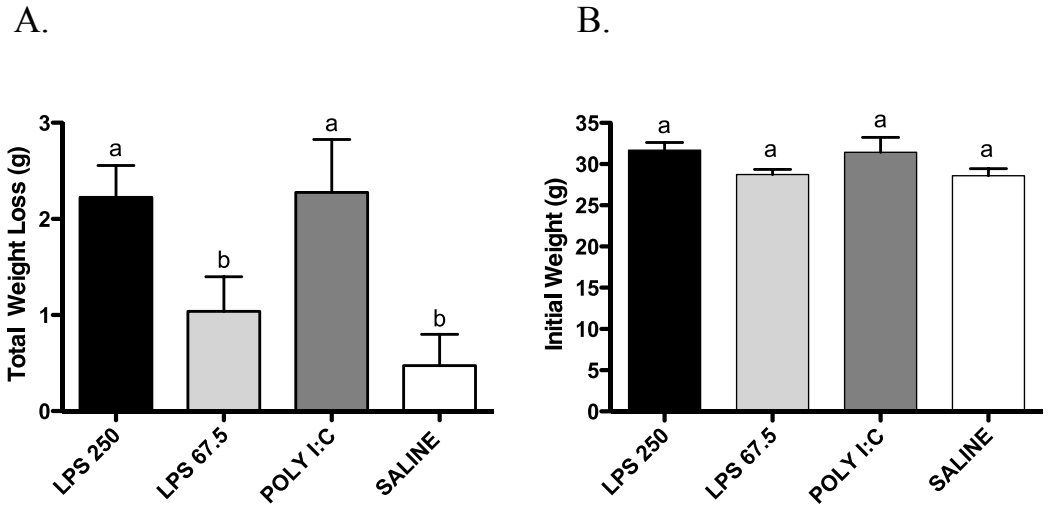

Figure 1. Measured weight of animals receiving either 250 µg/kg LPS, 67.5 µg/kg LPS, 12 mg/kg Poly I:C or saline injections. No difference in initial weight of animals prior to injections was recorded (**A**). Following 7 days of LPS (250 µg/kg) and Poly I:C treatment (**B**), animals lost a significant amount of weight indicating they were sick. Means with different letters (a,b) are significantly different (at least $p<0.05$) from each other. Bars represent mean ±SEM. (*Abbreviations*: LPS: lipopolysaccharide; Poly I:C : polyinosinic: polycytidylic acid.)

Figure 2

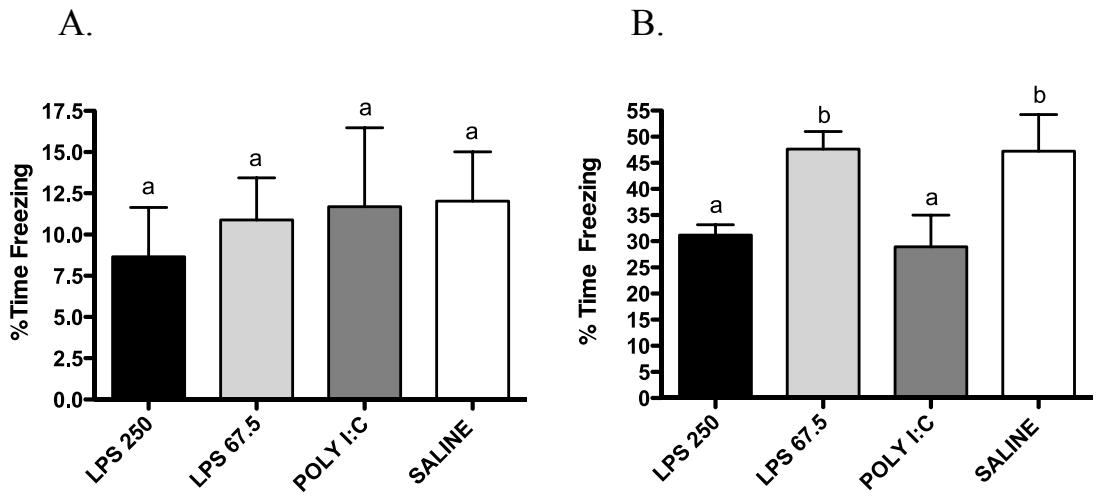

Figure 2. Percent time freezing in contextual fear conditioning. (**A**) No significant differences in activity was found between groups during the training session of CFC. However, during CFC testing (**B**) there was a significant difference detected in freezing behaviors. Both 250 µg/kg LPS and 12 mg/kg Poly I:C produced significantly less freezing time compared to 67.5µg/kg LPS and saline treatments, indicating a learning deficit. Means with different letters (a,b) are significantly different (at least $p<0.05$) from each other. Bars represent mean±SEM. (*Abbreviations*: LPS: lipopolysaccharide; Poly I:C : polyinosinic: polycytidylic acid.)

Figure 3

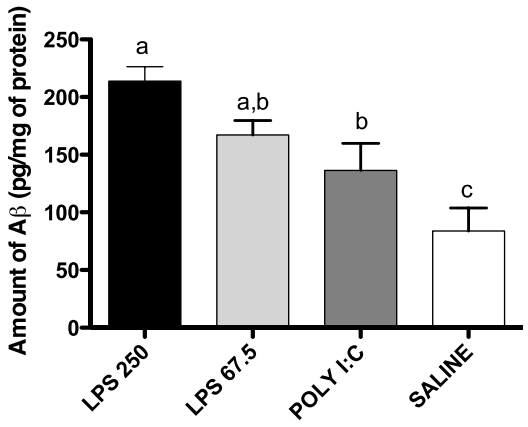

Figure 3. LPS and Poly I:C induced Aβ production. Immediately following CFC testing, Aβ was significantly elevated in the hippocampus of all three groups as compared to saline treated controls. No difference in Aβ is noted between 250 μg/kg LPS and 67.5 μg/kg LPS or between 67.5 μg/kg LPS and 12 mg/kg Poly I:C treated. 250 μg/kg LPS treatment lead to a significantly higher amount of Aβ compared to Poly I:C. Means with different letters (a,b,c) are significantly different (at least $p<0.05$) from each other. Bars represent mean±SEM. (*Abbreviations*: LPS: lipopolysaccharide; Poly I:C : polyinosinic: polycytidylic acid.)

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease which involves the atrophy of parts of the brain such as the hippocampus and cortex. The presence of two trademarks, neurofibrillary tangles (NFTs) and amyloid plaques (Aβ), inhibit cell signaling properties which lead to learning and memory deficits and eventually cellular death. Inflammation has been linked to the progression of AD pathologies. Our lab has shown that peripheral injections of both bacterial and viral mimetics lead to an elevated level of Aβ levels in the hippocampus of mice. Previous studies have shown hippocampus-dependent learning deficits in those injected with the bacterial mimetic, LPS; however, those injected with polyinosinic: polycytidylic acid (Poly I:C) have not shown hippocampus dependent learning deficits. The current study is aimed at investigating if there is a minimum Aβ level needed in order to induce these cognitive learning deficits. By first conducting a pilot study to normalize the potency of LPS to obtain similar Aβ levels to that of Poly I:C, we were able to set up the control group, the Poly I:C group, the original LPS dose and the new normalized LPS dose. Mice were then give seven consecutive days of intraperitoneal injections of one of the four groups followed by contextual fear conditioning in order to test for hippocampus learning deficits. The lower dose of LPS produced a lower more comparable amount of Aβ to Poly I:C, however their learning behaviors were different. Both the original higher dose LPS and Poly I:C groups failed to learn whereas the lower dose LPS group displayed much less cognitive learning deficits. We believe that Aβ levels are not the only factor impacting learning but that external factors such as cytokine levels may also be involved in the learning deficits normally observed with AD.