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# The Effects of Acute Lipopolysaccharide Induced Inflammation on Spinal Cord Excitability

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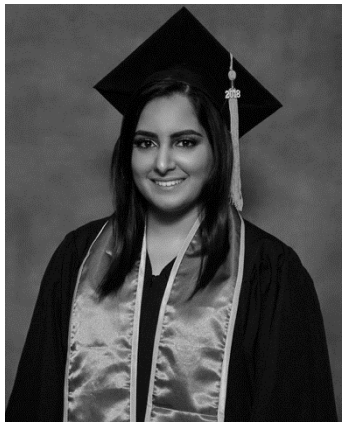

## *Puneet Sanghera*

Major:
Molecular Biology
Mentor: **Dr. Katherine Wilkinson**

The Effects of Acute Lipopolysaccharide Induced Inflammation on Spinal Cord Excitability

***Biography***

*Puneet Sanghera is an alumna of the NSF-REU RUMBA (Research for Undergraduate students using Molecular Biology Applications) at San José State University where she studied the effects of acute inflammation on spinal cord excitability. She also attended the NSF-REU Integrative Biology Program at the UC Berkeley where she studied the effects of repetitive mild traumatic brain injury. She has presented her research at SACNAS, ABRCMS, the CSU-Biotechnology Symposium and West Coast Biological Sciences Undergraduate Research Conference. She is a recipient of the CSUPERB-Howell fellowship and the University of the Provost Undergraduate Research Grant. She served on the board of Biology Student Association, Global Medical Brigades and is a founder of the Beta Upsilon chapter of Alpha Omega Epsilon. She hopes to complete an M.D./Ph.D. program and become a practicing surgeon while also doing clinical research on neurodegenerative diseases.*

# *The Effects of Lipopolysaccharide Induced Inflammation on Spinal Cord Excitability*


## Abstract

Peripheral inflammation alters the excitability of dorsal horn interneurons and increases flexor reflex strength (Dubner & Ruda, 1992); however, its effect on the spinal stretch reflex is not well understood. The stretch reflex is a muscle contraction in response to muscle stretch. We hypothesize that the acute inflammation caused by an injection of lipopolysaccharide (LPS) will cause an increase in spinal cord excitability. To test this hypothesis, we measured Hoffman's (H) reflex, the electric analog of the stretch reflex in adult mice receiving an injection of LPS (.5mg/kg) or saline (200µl). Adult male and female mice (C57Bl/6) were anesthetized; then, the sciatic nerve was exposed and stimulated at current strengths from H-wave threshold (T) to 8T (20 x 0.1 ms pulses at 0.1 Hz). Recording electrodes were placed in the foot. We measured the maximum M wave amplitude (Mmax), maximum H wave amplitude (Hmax) and latencies of both waves. We compared the ratio of the maximal H wave over the maximal M wave (Hmax/Mmax), which reports the percentage of motor neurons activated by electrical stimulation of Group Ia muscle sensory neurons. Increased spinal cord excitability would be reflected in a larger Hmax/Mmax. We found that LPS-induced inflammation does not alter the Hmax/Mmax. While we found no evidence of changes in spinal cord excitability, inflammation could be altering Group Ia muscle spindle afferent responses to stretch. Future studies will test whether stretch reflex strength is altered by inflammation.


**Introduction**

Hoffman's reflex (H-reflex) is an electrically induced reflex. The H reflex estimates of alpha motor excitability, which can be used to evaluate the response of the nervous system to different neurological conditions. The M wave is a contraction caused by direct stimulation of motor neuron axons and the H wave is derived from the reflex activation of the motor neurons by electrical stimulation of Group Ia afferents (Palmeri, Ingersoll, & Hoffman, 2004).



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Lipopolysaccharide (LPS) is a bacterial endotoxin found on the capsule of gram-negative bacteria (Gao et al., 2002). LPS is also known to cause an immune response in animals. Inflammation is synonymous with many neurodegenerative diseases (Qin et al,. 2004). When LPS is injected in an animal, cytokines, specifically TNFα, is released into the body (Qin et al., 2004). These cytokines cause a low level of chronic inflammation, much like a person gets when they catch the flu. Peripheral inflammation alters the excitability of dorsal horn interneurons and increases flexor reflex strength (Dubner & Ruda, 1992); however, its effect on the spinal stretch reflex is not well understood. My hypothesis is that LPS induced inflammation will increase spinal cord excitability; I expect to see an increase in HMax/MMax and earlier latencies in drug groups as compared to control groups. Additionally, I hypothesize that female mice will show an increase in spinal cord excitability as compared to males because females have a more robust immune system in response to bacterial infections (Klein, 2000).

**Methods**

C57/B16 adult (2-3 months) male mice were injected with lipopolysaccharide (LPS; $7.5 \times 10^{-5}$ EU/kg in 200 µl saline) or control (200 µl saline) 18 hours before the experiment LPS. Mice were anesthetized with an intraperitoneal injection ketamine (100mg/kg) and xylazine (10mg/kg). The sciatic nerve was exposed and stimulating electrodes were placed around the sciatic nerve and recording electrodes are placed in the 4th dorsal interossei muscle of the foot (Figure 1). Electrical stimulations were induced to find threshold, the lowest voltage at which a stable H wave was elicited. Electrical stimulations were given at threshold and multiplied by 1.3, 1.5, 2, 3, 5, 6, 7 and 8T to find the maximum H-wave. Trains of 20 stimulations were given at 0.1 Hz. All data was recorded using LabChart. Hmax/Mmax is the ratio of peak amplitude of the H wave divided by the myotatic wave (M-wave). Amplitude of H and M waves were measured from peak to trough (Figure 2) (Turski, Bressler, Klockgether, & Stephens, 1990). Hmax/Mmax of LPS and controls were compared. Latency was measured from stimulation to start of each waveform (Figure 2) (Lee et al., 2009).

The percentage of the motor neurons activated electrically was measured by normalizing the amplitude in millivolts of the H wave to the

amplitude of the millivolts of the muscle contraction (M wave), with the amplitude of the M wave theoretically representing the maximum number of motor neurons that could be activated and the H wave the percentage of motor neurons that are actually activated.

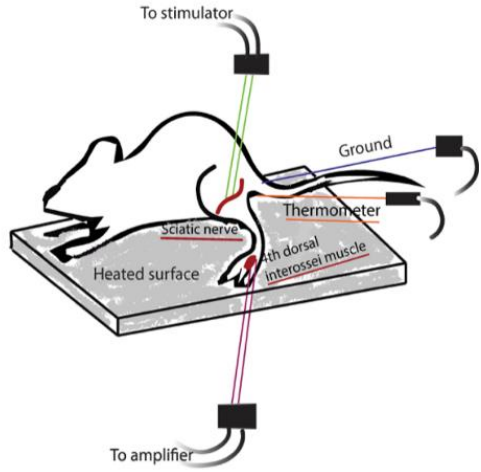


**Figure 1:** Description of the mouse anesthetized on a temperature controlled, heated surface with stimulating, recording and ground electrodes attached.

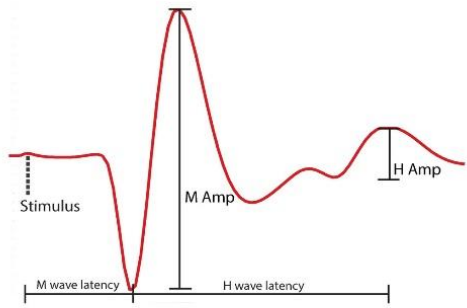


**Figure 2:** Measurement of amplitudes and latencies in Hoffman's wave.



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## Results

### *Hmax/Mmax ratio not significantly different between saline and lipopolysaccharide injected mice in either sex*

Using an in vivo method, the percent of motor neurons activated by electrical stimulation of the sciatic nerve is not significantly different between saline and lipopolysaccharide injected groups, p = .219 (Figure 3). There were also no differences between the sexes p = .905.

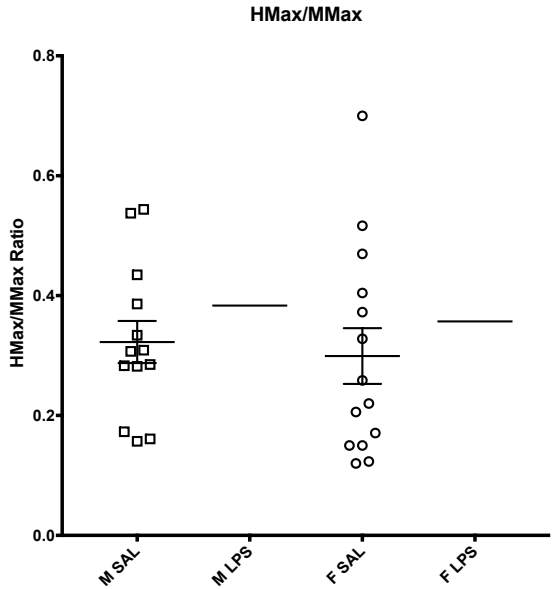


**Figure 3: No changes in Hmax/Mmax ratios with LPS injection**
Hmax/Mmax ratio are not significantly different in SAL and LPS mice. Individual animal values are shown along with means and standard error of mean.

### *M latency is unchanged by injection of LPS*

The muscle contraction provides the normalization factor against which the H wave is compared. The latency of this wave is dependent on the distance it has to travel (nerve length) and the contraction is a direct result of the electrical stimulation, larger mice should have a greater M latency than smaller mice. There are no significant differences between LPS and control groups p = .580. There are no significant differences between male and female groups p = .878.

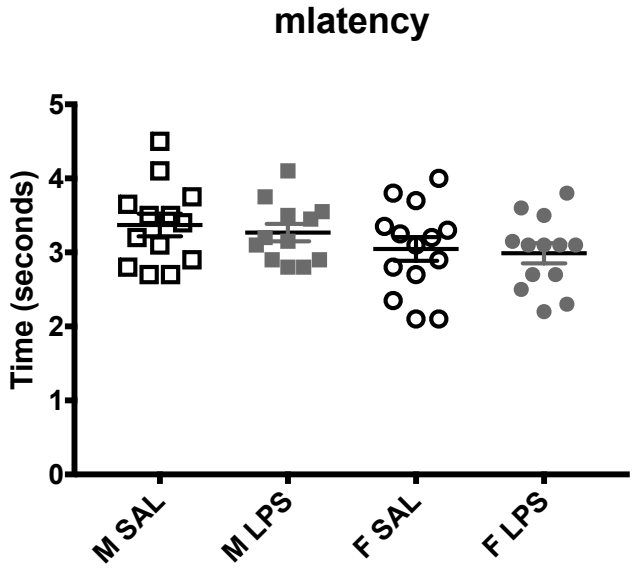


**Figure 4. No changes in M latency with LPS injection** Hmax/Mmax ratio are not significantly different in SAL and LPS mice. Individual animal values are shown along with means and standard error of mean.

***H latency is unchanged by injection of LPS***

There are no significant differences between LPS and control groups p = .105. There are no significant differences between male and female groups p =.726.



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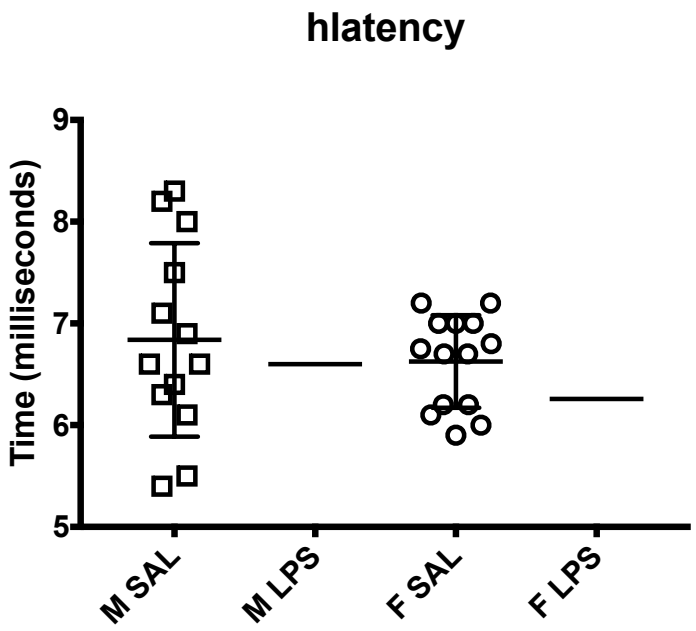


**Figure 5. No changes in H latency with LPS injection**
Hmax/Mmax ratio are not significantly different in SAL and LPS mice. Individual animal values are shown along with means and standard error of mean.

## Discussion

Input from the stretch sensitive muscle spindle afferents is crucial for sufficient proprioception. Changes to spinal cord processing of the muscle spindle afferent sensory information may lead to unwanted motor responses and loss of balance. The stretch reflex is a muscle contraction in response to stretching within the muscle. This reflex is extremely important as a protective mechanism for muscles to prevent tearing. The stretch reflex is also an important feedback mechanism for coordinating and correcting movements, such as keeping posture. In this study we found no evidence for alterations in spinal cord excitability in lipopolysaccharide injected mouse models.

We measured spinal cord excitability by analyzing Hoffman's Reflex and found no differences in any response we measured. The time it takes for the H wave to travel up the sensory afferent neuron to the spinal cord, across the synapse to α-motorneuron, and then back down to the muscle can also be an indicator of spinal cord excitability. The time it takes between the stimulus and the resulting H wave, H wave latency, can be induced by increased spinal cord excitability causing a faster monosynaptic transmission between the sensory and motor neurons (Levin and Hui-Chan, 1993). In this experiment, H latency was not changed by lipopolysaccharide induced inflammation. Likewise, there were no significant differences in the proportion of motor neurons that could be recruited by electrical stimulation of the Group Ia muscle spindle afferents in lipopolysaccharide injected mice. Any method of spinal cord excitability measurement has several components that are difficult to control and can ultimately result in perplexing results, such as muscle tension, level of anesthesia and electrode placement (Hultborn, Meunier, Morin, & Pierrot-Deseilligny, 1987; Ho & Waite, 2002).

This experiment should include another type of analysis such as using neural recruitment curves, also known as rate dependent depression to evaluate the frequency of spinal cord excitability. Short term synaptic plasticity of rate dependent depression would allow us to investigate changes in spinal cord excitability more reliably than the Hmax/Mmax ratios alone. High frequency electrical stimulation would normally result in a decrease of the H wave amplitudes (Lakie & Robson, 1990). This decrease can be due to many different factors, including a decrease in release-ready neurotransmitter vesicles and changes in the receptor sensitization (Campbell & Moss, 2000). Reflex testing can also cause hyperreflexia, which is known as overactive reflexes.

In conclusion, inflammation is a common symptom of many diseases. Lipopolysaccharide injections also cause low levels of system inflammation. Spinal cord excitability was largely unchanged by the injections of LPS; this was shown by no significant variations in HMax/MMax ratios and no significant dissimilarities in latencies for both the myotatic wave and the H-wave.

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Wilkinson Lab
San Jose State Veterinary Staff
CSUPERB-Howell

**Statistical Analysis**
**Hmax/Mmax**

***Between-Subjects Factors***

| | | N |
|---|---|---|
| sex | .00 | 27 |
| | 1.00 | 25 |
| condition | .00 | 27 |
| | 1.00 | 25 |

***Descriptive Statistics***

Dependent Variable: hmaxmmax

| sex | condition | Mean | Std. Deviation | N |
|---|---|---|---|---|
| .00 | .00 | .29923431 | .174305162 | 14 |
| | 1.00 | .35687163 | .123646709 | 13 |
| | Total | .32698561 | .152015310 | 27 |
| 1.00 | .00 | .32254488 | .126768192 | 13 |
| | 1.00 | .37006054 | .175698854 | 12 |
| | Total | .34535240 | .150900262 | 25 |
| Total | .00 | .31045792 | .150827801 | 27 |
| | 1.00 | .36320231 | .147777774 | 25 |
| | Total | .33581580 | .150274603 | 52 |

***Tests of Between-Subjects Effects***

Dependent Variable: hmaxmmax

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | .041[a] | 3 | .014 | .589 | .625 |
| Intercept | 5.894 | 1 | 5.894 | 254.694 | .000 |
| sex | .004 | 1 | .004 | .187 | .668 |
| condition | .036 | 1 | .036 | 1.548 | .219 |
| sex * condition | .000 | 1 | .000 | .014 | .905 |
| Error | 1.111 | 48 | .023 | | |
| Total | 7.016 | 52 | | | |
| Corrected Total | 1.152 | 51 | | | |

a. R Squared = .035 (Adjusted R Squared = -.025)

**M Latency**

***Between-Subjects Factors***

| | | N |
|---|---|---|
| sex | .00 | 27 |
| | 1.00 | 25 |
| condition | .00 | 27 |
| | 1.00 | 25 |

***Descriptive Statistics***

Dependent Variable: mlatency

| sex | condition | Mean | Std. Deviation | N |
|---|---|---|---|---|
| .00 | .00 | .00304640 | .000595303 | 14 |
| | 1.00 | .00298842 | .000489088 | 13 |
| | Total | .00301849 | .000537091 | 27 |
| 1.00 | .00 | .00336920 | .000548676 | 13 |



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| | | | | |
|---|---|---|---|---|
| | 1.00 | .00326658 | .000409717 | 12 |
| | Total | .00331994 | .000479792 | 25 |
| Total | .00 | .00320182 | .000585792 | 27 |
| | 1.00 | .00312194 | .000465467 | 25 |
| | Total | .00316342 | .000527752 | 52 |

***Tests of Between-Subjects Effects***

Dependent Variable: mlatency

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 1.268E-6[a] | 3 | 4.227E-7 | 1.568 | .209 |
| Intercept | .001 | 1 | .001 | 1930.232 | .000 |
| sex | 1.170E-6 | 1 | 1.170E-6 | 4.342 | .043 |
| condition | 8.357E-8 | 1 | 8.357E-8 | .310 | .580 |
| sex * condition | 6.459E-9 | 1 | 6.459E-9 | .024 | .878 |
| Error | 1.294E-5 | 48 | 2.695E-7 | | |
| Total | .001 | 52 | | | |
| Corrected Total | 1.420E-5 | 51 | | | |

a. R Squared = .089 (Adjusted R Squared = .032)

**H Latency**

***Between-Subjects Factors***

| | | N |
|---|---|---|
| sex | .00 | 27 |

| | | |
|---|---|---|
| | 1.00 | 25 |
| condition | .00 | 27 |
| | 1.00 | 25 |

***Descriptive Statistics***

Dependent Variable: hlatency

| Sex | condition | Mean | Std. Deviation | N |
|---|---|---|---|---|
| .00 | .00 | .00662500 | .000455191 | 14 |
| | 1.00 | .00625769 | .000614006 | 13 |
| | Total | .00644815 | .000559088 | 27 |
| 1.00 | .00 | .00683845 | .000950918 | 13 |
| | 1.00 | .00659999 | .000503182 | 12 |
| | Total | .00672399 | .000763514 | 25 |
| Total | .00 | .00672777 | .000729901 | 27 |
| | 1.00 | .00642199 | .000578802 | 25 |
| | Total | .00658076 | .000673091 | 52 |

***Tests of Between-Subjects Effects***

Dependent Variable: hlatency

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 2.252E-6[a] | 3 | 7.507E-7 | 1.728 | .174 |
| Intercept | .002 | 1 | .002 | 5167.261 | .000 |
| Sex | 1.001E-6 | 1 | 1.001E-6 | 2.304 | .136 |
| condition | 1.189E-6 | 1 | 1.189E-6 | 2.737 | .105 |



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| | | | | | |
|---|---|---|---|---|---|
| sex * condition | 5.380E-8 | 1 | 5.380E-8 | .124 | .726 |
| Error | 2.085E-5 | 48 | 4.345E-7 | | |
| Total | .002 | 52 | | | |
| Corrected Total | 2.311E-5 | 51 | | | |

a. R Squared = .097 (Adjusted R Squared = .041)

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