# International Journal of Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Zoological Research

ISSN 1811-9778
DOI: 10.3923/ijzr.2019.38.42

# Editorial
# Effects of Bacterial Endotoxin Lipopolysaccharides (LPS) on Synaptic Transmission at Neuromuscular Junction in an Amphibian

Micaiah C. McNabb, Christa Marie Saelinger, Melody Danley and Robin Lewis Cooper

Department of Biology, University of Kentucky, 675 Rose Street, Lexington, 40506-0225 KY, USA

## Abstract

Lipopolysaccharides (LPS) are found in the outer membrane of gram-negative bacteria and can elicit direct cellular responses in addition to inflammatory immune responses in an infected organism. The purpose of this study was to investigate the effect of LPS on synaptic transmission at the neuromuscular junction (NMJ) of a model amphibian preparation. The cutaneous pectoris muscle of *Lithobates pipiens* was dissected and maintained in a physiological saline to acutely expose the preparation to LPS from *Serratia marcescens*. The evoked excitatory junction potentials (EJPs) completely diminished after 10 min ($n = 6$, $p<0.05$ paired t-test). These EJPs were able to be partially recovered after removal of LPS. The frequency and amplitude of the spontaneous miniature excitatory junction potentials (mEJPs) did not change with LPS exposure ($n = 6$, $p>0.05$ paired t-test). These findings suggest that LPS acts in frogs by inhibiting the activity of the voltage-gated $Ca^{2+}$ channels in presynaptic motor neurons and not by blocking the acetylcholine receptors on the skeletal muscle fibers. These findings imply the acute action of LPS in mammals is presynaptic at the NMJs and can suppress synaptic transmission independent of initiating a systemic immune response.

Key words: Lipopolysaccharides, frog, peptidoglycans, nicotinic receptors

Citation: Micaiah C. McNabb, Christa Marie Saelinger, Melody Danley and Robin Lewis Cooper, 2019. Effects of bacterial endotoxin lipopolysaccharides (LPS) on synaptic transmission at neuromuscular junction in an amphibian. Int. J. Zool. Res., 15: 38-42.

Corresponding Author: Robin Lewis Cooper, Department of Biology, University of Kentucky, 675 Rose Street, Lexington, 40506-0225 KY, USA
Tel: 859-559-7600



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Bacterial septicemia can be life threatening and is capable of infecting invertebrates as well as humans. A common gram-negative bacterial strain detected in septicemic mammals is from *Serratia marcescens*[1-3]. Gram-negative bacteria secrete the endotoxin lipopolysaccharide (LPS). The LPS can further induce the release of cytokines from many cell types as a systemic defense immune response. The various cytokines have a multitude of effects on target tissues. A common focus of the research and treatment of bacterial septicemia is on the downstream induced actions by cytokines but not necessarily on the direct cellular effects of LPS.

By examining the direct effects of LPS independent from the induced immune response, one may provide targeted treatment options accordingly for septicemic patients. Few studies have investigated the actions of LPS on neural tissue and synaptic transmission in the central nervous system of rodent models[4-6]. However, due to the synaptic complexity in brain slice models, it has been difficult to separate indirect actions of microglia and differential actions on excitatory and inhibitory neural circuits to determine the direct actions of LPS on a defined synaptic site. The neuromuscular junction (NMJ) of mammals and amphibians offers an approach to address the direct action of LPS on the function of cholinergic synapses. Past studies using the frog cholinergic NMJs indicated an increase in the occurrence of spontaneous quantal responses but irreversibly blocked the evoked amplitude of the excitatory junction potentials (EJPs) with only 10 μg $mL^{-1}$ of lyophilized LPS from *Salmonella typhimurium*[7,8]. The inability to reverse the effects of LPS is surprising since the effects are reversible in mammalian brain slices, at glutamatergic synapses at the NMJs of crayfish[9,10], at the NMJs of larval *Drosophila melanogaster*[11] and in the heart of larval *Drosophila*[12]. The motivation behind this current study was to re-examine the effects of LPS on the amphibian NMJ and to bring awareness to the fact that LPS can either enhance or depress synaptic transmission depending on the NMJ model used for experimentation.

No other reports besides these earlier reports[7,8] were forthcoming on the effects of LPS from different strains of bacteria at the cholinergic NMJs, so this study was designed to examine the direct effect of LPS from *S. marcescens* in order to provide additional insight to the effects of LPS on nicotinic cholinergic synapses, which are similar in function among most mammals including humans.

## MATERIALS AND METHODS

Adult, unsexed Northern leopard frogs (*Lithobates pipiens*) were obtained from a commercial supplier (Carolina Biological, Burlington, NC) in August, 2018. These studies were conducted during September and October, 2019. Frog animal care and use were approved by the Institutional Animal Care and Use Committee (IACUC Protocol No. 2014-1295). The in-depth details on the dissection of the cutaneous pectoris muscle are presented in Saelinger *et al.*[13]. The muscles were maintained during dissection and during recordings using a modified standard frog saline (Ringer's solution) composed of (mM): NaCl 0.11, KCl 0.33, $MgCl_2$ 8.0, $CaCl_2$-$2H_2O$ 0.105, Glucose 0.11 and HEPES 1.0. Saline was adjusted to a pH of 7.4 using NaOH. This saline composition was necessary to reduce twitching of the muscle fibers when the nerve was electrically stimulated. All saline chemicals and LPS were obtained from Sigma-Aldrich (St. Louis, MO, USA). The LPS was dissolved in saline prior to use and was readily exchanged over the preparations at a concentration of 500 μg $mL^{-1}$ while recording evoked EJPs and mEJPs.

Evoked EJPs and mEJPs were continuously monitored as exposure to LPS-containing saline was exchanged with normal saline 3 or 4 times. The evoked and spontaneous synaptic responses of the cutaneous pectoris muscle were measured using glass microelectrodes filled with 3 M KCl (30-40 mΩ resistance). Recordings were collected and analyzed using LabChart and LabScope software (AD Instruments) and a 1×LU head stage and an Axoclamp 2A amplifier (Molecular Devices, Sunnyvale, CA, USA). The motor nerve was stimulated with the use of suction electrodes[14]. The transected end of the nerve was suctioned into the tip of the electrode after prefilling the tip with saline. After the nerve ending was suctioned into the tip, a small amount of clear petroleum jelly was placed around the tip of the electrode to provide a tight fit for the nerve and lumen of the electrode. The tight fit allowed for a lower voltage to be used to stimulate the nerve. The motor nerve was then stimulated through the suction electrode via an S88 Stimulator (Astro-Med, Inc., USA) at a frequency of once every 10 sec. This allowed for both evoked EJPs and mEJPs to be recorded before and during LPS application. The acquisition rate was 20 kHz.

## RESULTS

The evoked EJPs were relatively small in the reduced $Ca^{2+}$ and elevated $Mg^{2+}$ containing saline as compared to the action potential, which would normally be induced in the

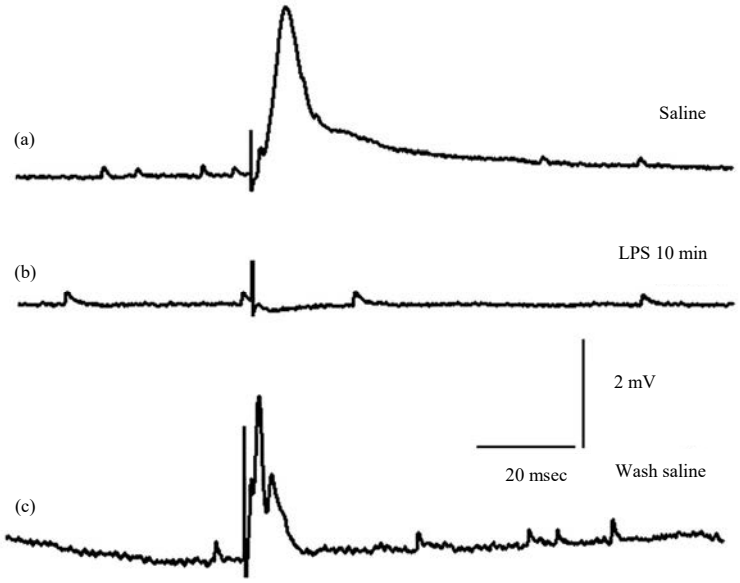


Fig. 1: Intracellular recording of evoked excitatory junction potentials (EJPs) and spontaneous miniature EJPs (mEJPs) in the cutaneous pectoris muscle before, during and after exposure to 500 μg $mL^{-1}$ lipopolysaccharides (LPS) endotoxin. (a) The evoked EJP and mEJPs are readily observed before application of LPS. The rapid stimulus artifact precedes the EJP. (b) The evoked EJP does not occur with exposure to LPS after 10 min, however, the mEJPs are still present at a low frequency. The stimulus artifact is still present. (c) After exchanging the saline and removing LPS the evoked EJPs partially return and mEJPs are still present

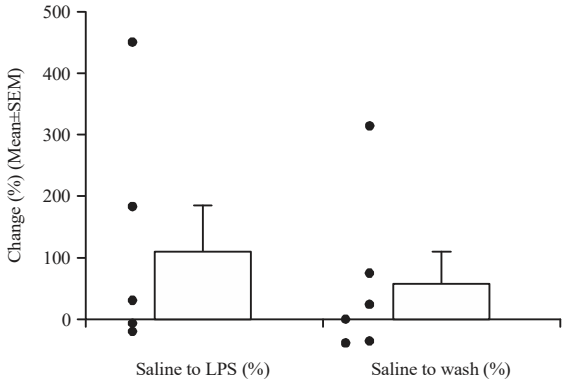


Fig. 2: Frequency in occurrences of spontaneous miniature EJPs with exposure to LPS. The percent change (relative to normal saline) in the frequency of spontaneous miniature EJPs in the cutaneous pectoris muscle during and after exposure to 500 μg $mL^{-1}$ lipopolysaccharides (LPS) endotoxin was not significantly different. Among the six preparations only two preparations showed a marked increase in the frequency of spontaneous quantal events. After removing the LPS by triplicate exchanges of the saline bath, only one preparation still had a large increase in frequency, as compared to the initial recording prior to LPS exposure (right side). The dots indicate the percent changes for individual preparations

muscle fibers with nerve stimulation. However, to reduce the twitching and obtain graded EJPs to assess the effects of LPS on synaptic transmission, this modified saline was optimal to be below the action potential threshold. As shown in Fig. 1 for a representative evoked EJP, the amplitude was blocked after 10 min of incubation in LPS while the spontaneous quantal events can still be observed (Fig. 1b, $n = 6$, $p<0.05$ paired t-test). Upon exchanging the bathing media three times with fresh saline without LPS, the evoked EJPs partially recovered. Even after 10 min and repetitive exchanging of the bathing media, the amplitudes of the EJPs did not fully recover. In contrast, the spontaneous quantal events occurred prior to and during exposure to LPS, as well as during the wash out with exchanging the bathing media (Fig. 1c). The rate in occurrences of the spontaneous events was not significantly increased when the evoked EJP was reduced during the 10 min of LPS incubation. A mean in the percent change of the number of spontaneous events over an average of three, 10 sec intervals in saline and with an average of three, 10 sec intervals after 10 min in LPS, did not reveal a consistent change in the occurrence of spontaneous quantal events (Fig. 2). In the six preparations, one preparation illustrated a high level in the percent increase in the frequency of spontaneous events after the 10 min of LPS exposure, however, the initial frequency was relatively low. Also, no

consistent trends occurred in comparing the percent change in the spontaneous events after washing the preparations with fresh saline to the initial saline (Fig. 2).

## DISCUSSION

Because the amplitude of the evoked EJP in this study at the NMJ gradually decreased over the exposure time, which was also reported in a previous study[7,8], it is not likely that LPS inhibited the induction of an action potential in the motor nerve or reduced the amplitude below the threshold; otherwise, a more abrupt reduction in the EJP amplitude would have been observed. Considering the effects in the evoked EJP amplitudes were not reversible for the LPS from *Salmonella typhimurium*[7,8], such observations support the idea that there are differences in the LPS from *S. marcescens* and *Salmonella typhimurium* in binding to targets on the neuron. Person[7,8] suggested LPS was sticky and could not be washed off the tissue; however, the effect of LPS (2 μg $mL^{-1}$) from *S. marcescens* on the crayfish glutamatergic NMJ induced an increase in evoked EJPs within 5 min during exposure and the effect of LPS exposure were readily reversed within 2 min of washing the preparation with fresh saline[9]. Since the spontaneous quantal responses were not blocked and were able to be detected on the postsynaptic muscle fibers for the nicotinic receptors of the frog NMJ for *S. marcescens* and *Salmonella typhimurium*, as well as the glutamatergic synapses of the crayfish NMJ, such findings indicate the vesicle fusion process remains intact and the receptors on the muscle remained responsive to the transmitters. As suggested by Person's [7,8], it is likely that the voltage-gated $Ca^{2+}$ channels may be compromised by LPS in the motor neurons of the frog. However, the $Ca^{2+}$ channels may be enhanced at the crayfish NMJ since evoked responses increased. The doubling in the frequency of spontaneous events at the crayfish NMJ during LPS exposure indeed may be a result of increases in residual $Ca^{2+}$ within the motor nerve terminals.

It is important to understand the effects LPS can impose on cells for potential therapeutic treatments. The structure of LPS is different in the various gram-negative bacterial strains. These structural differences are the cause of the range in virulence of the immunological reactions. Thus, each form of LPS may result in a range of direct effects on cells, as well as inducing varied secondary effects. It is important to note that commercially obtained LPS likely contains associated peptidoglycans from the same strain of gram-negative bacteria[15]. Thus, exposed preparations in the past or present with commercially obtained LPS is likely a mixture, but still represents the effects of what compounds the tissue or what the animal would be exposed to from gram-negative bacteria. To further complicate LPS's effects, such responses are highly dependent on the neurological model being examined. For example, opposing effects of LPS exposure have been noted at NMJs, including promotion of synaptic efficacy at the glutamatergic crayfish NMJ[9] but depression at the cholinergic frog NMJ[7,8] and depression at the larval *Drosophila* NMJ[16]. When considering the effects of LPS on neurons, one should consider sensory perception (i.e., mechanical, thermal, chemical), electrical excitation, conduction and presynaptic transmission and postsynaptic reception. This study focused on synaptic transmission at the nicotinic neuromuscular junction as a model preparation. The effects were rapid and are unlikely due to other cell types around the NMJ, as mostly perisynaptic Schwann cells, nerve and muscle are present.

The rapid effects of LPS may be related to acetylcholine (ACh) synaptic transmission. One study reported that tracheal gland acinar cells from swine responded rapidly to LPS through a TLR4 ligand by the production of nitric oxide, resulting in the activation of cyclic guanosine monophosphate (cGMP)-dependent protein kinase[17]. This resulted in an increase in secretion of electrolytes from the cells. However, the action is mimicked by ACh and does not block the action of ACh. In a recent study, LPS from *S. marcescens* (500 μg $mL^{-1}$) was used to determine if there were effects on mechanical transduction and initiation of neural activity in proprioceptors from blue crab and crayfish. No effect on mechanical transduction or in initiation of action potentials was observed[18].

The significance and general conclusion of this study is that LPS from *S. marcescens* depresses evoked synaptic transmission at a model nicotinic cholinergic synapse without altering responsiveness to Ach or frequency due to spontaneous quantal events. In addition, in contrast to earlier reports the effect of depressed synaptic transmission is partially reversible with exchange of the LPS containing bath to fresh saline.

The varied responses in altering evoked synaptic transmission and the effects on spontaneous vesicle fusion events with different forms of LPS on different preparations indicate that further investigations for future studies are needed to understand these differences in the direct effects of the various forms of LPS.

## ACKNOWLEDGMENTS

Funding by sustaining Excellence-2014 Howard Hughes Medical Institute (Grant #52008116) awarded to the University of KY (VM Cassone, PI). The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal; Department of Biology, University of KY bought the reagents for this study; Page charges (personal funds RLC).

## REFERENCES

1. Carl, M.A., I.M. Ndao, A.C. Springman, S.D. Manning and J.R. Johnson *et al*., 2014. Sepsis from the gut: The enteric habitat of bacteria that cause late-onset neonatal bloodstream infections. Clin. Infect. Dis., 58: 1211-1218.
2. Kim, S.B., Y.D. Jeon, J.H. Kim, J.K. Kim and H.W. Ann *et al*., 2015. Risk factors for mortality in patients with *Serratia marcescens* bacteremia. Yonsei Med. J., 56: 348-354.
3. Pien, B.C., P. Sundaram, N. Raoof, S.F. Costa and S. Mirrett *et al*., 2010. The clinical and prognostic importance of positive blood cultures in adults. Am. J. Med., 123: 819-828.
4. Abudara, V., L. Roux, G. Dallérac, I. Matias and J. Dulong *et al*., 2014. Activated microglia impairs neuroglial interaction by opening Cx43 hemichannels in hippocampal astrocytes. Glia, 63: 795-811.
5. Gao, F., Z. Liu, W. Ren and W. Jiang, 2014. Acute lipopolysaccharide exposure facilitates epileptiform activity via enhanced excitatory synaptic transmission and neuronal excitability *in vitro*. Neuropsychiatr. Dis. Treat., 10: 1489-495.
6. Zhang, J., A. Malik, H.B. Choi, R.W. Ko, L. Dissing-Olesen and B.A. MacVicar, 2014. Microglial CR3 activation triggers long-term synaptic depression in the hippocampus via NADPH oxidase. Neuron, 82: 195-207.
7. Person, R.J., 1979. Bacterial lipopolysaccharide depresses spontaneous, evoked and ionophore-induced transmitter release at the neuromuscular junction. J. Neurosci. Res., 4: 105-114.
8. Person, R.J., 1979. Depression of spontaneous and ionophore-induced neurotransmitter release by *Salmonella*. Experientia, 35: 801-802.
9. Parnas, I., R. Reinhold and J. Fine, 1971. Synaptic transmission in the crayfish: increased release of transmitter substance by bacterial endotoxin. Science, 171: 1153-1155.
10. Saelinger, C.M., M.C. McNabb, R. McNair, S. Bierbower and R.L. Cooper, 2019. Effects of Bacterial Endotoxin (LPS) on the cardiac function, neuromuscular transmission and sensory-CNS-motor nerve circuit: A crustacean model. Comparative Biochemistry and Physiology, Part A., (In Press).
11. Istas, O., A. Greenhalgh and R.L. Cooper, 2019. The effects of a bacterial endotoxin (LPS) on behavior and sensory-CNS-Motor circuits in *Drosophila melanogaster*. Insects, Vol. 10, No. 4. 10.3390/insects10040115.
12. Anyagaligbo, O., J. Bernard, A. Greenhalgh and R.L. Cooper, 2019. The effects of bacterial endotoxin (LPS) on cardiac function in a medicinal blow fly (*Phaenicia sericata*) and a fruit fly (*Drosophila melanogaster*). Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 217: 15-24.
13. Saelinger, C.M., M.C. McNabb, S. Trent, M. Danley and R.L. Cooper, 2019. Investigating effects of eugenol on nerve conduction and synaptic transmission at neuromuscular junction in an amphibian. Impulse: Premier J. Undergraduate Publ. Neurosci., (In Press).
14. Baierlein, B., A.L. Thurow, H.L. Atwood and R.L. Cooper, 2011. Membrane potentials, synaptic responses, neuronal circuitry, neuromodulation and muscle histology using the crayfish: Student laboratory exercises. J. Visual. Exp., Vol. 18. 10.3791/2322.
15. Kaneko, T., W.E. Goldman, P. Mellroth, H. Steiner and K. Fukase *et al*., 2004. Monomeric and polymeric gram-negative peptidoglycan but not purified LPS stimulate the Drosophila IMD pathway. Immunity, 20: 637-649.
16. Cooper, R.L., M. McNabb and J. Nadolski, 2019. The effects of a bacterial endotoxin LPS on synaptic transmission at the neuromuscular junction. Heliyon, Vol. 5, No. 3. 10.1016/j.heliyon.2019.e01430.
17. Murakami, K., T. Tamada, M. Nara, S. Muramatsu and T. Kikuchi *et al*., 2001. Toll-like receptor 4 potentiates $Ca^{2+}$-dependent secretion of electrolytes from swine tracheal glands. Am. J. Respir. Cell. Mol. Biol., 45: 1101-1110.
18. Stanback, M., A.E. Stanback, S. Akhtar, R. Basham and B. Chithrala *et al*., 2019. The effect of lipopolysaccharides on primary sensory neurons in crustacean models. Impulse: Premier J. Undergraduate Publ. Neurosci., (In Press).