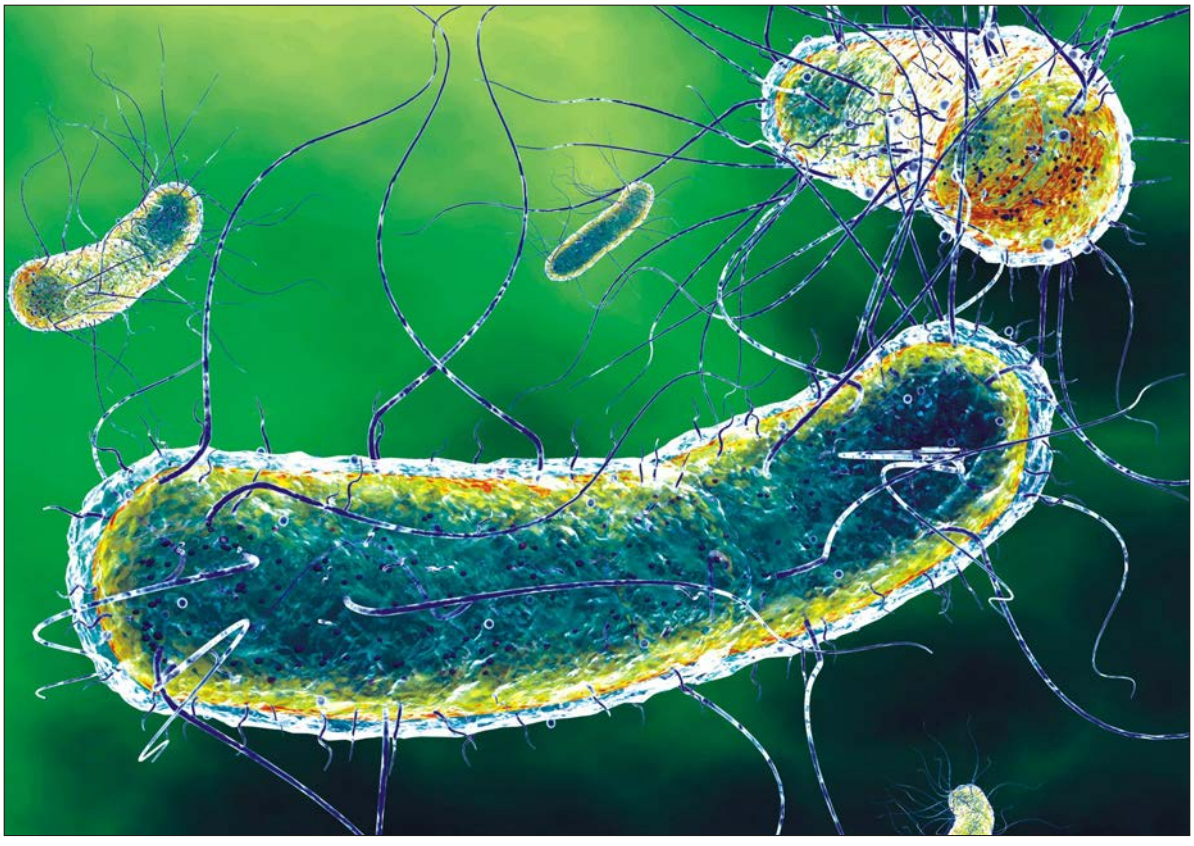

Current Research in Bacteriology

ISSN 1994-5426

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Current Research in Bacteriology

ISSN 1994-5426
DOI: 10.3923/crb.2020.10.21

Research Article
Assessment of Bacterial Endotoxin Lipopolysaccharide (LPS) Potential Interaction and TRPA1 Thermal Receptors on Synaptic Transmission

Jate Bernard, Nicole Marguerite, Morgan Inks and Robin L. Cooper

Department of Biology and Center of Muscle Biology, University of Kentucky, Kentucky, USA

Abstract

Background and Objective: An initial action of bacterial sepsis from gram-negative bacterial is a result due to the presence of Lipopolysaccharide (LPS), a bacterial endotoxin, which triggers the release of proinflammatory cytokines. There is suggestive evidence from neuronal responses in *Drosophila* and mammals that the gram-negative bacterial endotoxin LPS binds to TRPA1 receptors, one type of thermal detectors. We examined if LPS activates or blocks TRPA1 receptors in motor neurons and muscle fibers. **Materials and Methods:** The TRPA1 receptors were overexpressed and blocked in expression, by RNAi expression, in muscle and motor neurons. The effect on synaptic transmission and direct effects on neurons and muscle fibers were examined electrophysiologically. **Results:** The responses of blocking glutamatergic postsynaptic receptors by LPS were preserved with activation of TRPA1. Activation of TRPA1 in muscle depolarized the muscle in the presence of LPS but less so than without LPS due to the hyperpolarizing effect by LPS. Expression of RNAi for TRPA1 blocked responses to thermal activation but not actions by LPS. **Conclusion:** LPS does not activate or block TRPA1 receptors in these studies. This study has implications in the mechanisms by which LPS functions in its direct action on cells for potentially mediating the action of LPS and the downstream activation of proinflammatory cytokines.

Key words: TRPA1, lipopolysaccharide, neuron, muscle, synaptic transmission, proinflammatory cytokines, motor neurons

Citation: Bernard, J., N. Marguerite, M. Inks and R.L. Cooper, 2020. Assessment of bacterial endotoxin lipopolysaccharide (LPS) potential interaction and TRPA1 thermal receptors on synaptic transmission. Curr. Res. Bacteriol., 13: 10-21.

Corresponding Author: Robin. L. Cooper, Department of Biology and Center of Muscle Biology, University of Kentucky, Kentucky, USA

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

In the United States, an estimated 1.7 million adult cases of sepsis occur annually, contributing to 265,000 deaths each year[1,2]. The muscular system is greatly affected after contracting sepsis and is key in the body's response to bacterial endotoxins by releasing proinflammatory cytokines (TNF-α, IL-1, or IL-6)[3]. There is mounting evidence from neuronal responses in *Drosophila* and mammals that the gram-negative bacterial endotoxin lipopolysaccharide (LPS) binds to TRPA1 receptors[4,5]. The TRPA1 receptors are one form of thermal detectors.

Thermal receptors, such as TRPA receptors also known as TRP-ankyrin receptors, are ion channels (i.e, Ca^{2+} permeable nonselective cation channel) that alter the ionic flux, leading to smooth muscle contraction, electrical activity, or a cascade of various second messenger cascades depending on the cell type[6-8]. There is a family of TRPA receptors (i.e., Painless, Pyrexia and dTRPA1) known in *Drosophila* to be sensitive to temperature in the range of 25-45°C[9]. Various types of TRP channels are known to be expressed in cardiac muscle[10]. One of which, TRPA1 receptor, is known to be altered in expression in cardiac conditions and may serve to enhance contractility[11,12]. LPS itself has been found to cause septic myocardial dysfunction in mammals due to its effect of a sarcoplasmic leak, which decreases the ability of the heart muscles to contract[13]. This myocardial dysfunction is a key factor in the severity and survival of patients with septicemia[14-16]. In rodents, LPS infusion induced bradycardia within a minute[17], but it was not established if the effect was on neurons or the muscle and through what type of receptors. Exposure of rodent cardiomyocytes to LPS results in a decrease of systolic Ca^{2+} transients and myocyte contraction as well as overall sarcoplasmic reticulum Ca^{2+} content[18].

If LPS results in Ca^{2+} influx through TRPA1 receptors, then over or under expressing TRPA1 in cells while exposing the cells to LPS can help detail potential mechanisms of action by LPS. Besides, by overexpressing or reducing the expression of TRPA1 receptors in cells that are known to respond to LPS, it will be possible to determine if the responses are mediated through TRPA1 receptors. Using larval *Drosophila* body wall muscle which responds to LPS in an opposing manner to heat activation of TRPA1 addresses if the two are independent. Also, evoked neural stimulation is blocked by LPS; however, expression and activation of TPRA1 in the presynaptic motor neuron promote synaptic transmission. The ability to monitor synaptic responses in the muscle fibers due to evoked or spontaneous activity allows one to indirectly access if Ca^{2+} is altered within the nerve terminal. To address these topics, we overexpressed and reduced expression of TRPA1 using RNAi in body wall muscle as well as motor neurons while examining the effects of exposure of LPS.

Given that gustatory sensory neurons respond to LPS through a TRPA1 receptor and result in *Drosophila* avoiding food or an environment laced with LPS[19] and that it has been shown that body wall muscles of larval *Drosophila* respond directly to exposure of LPS, we set out to examine if the body wall muscles response to LPS is also mediated via a TRPA1 receptor. The rapid response in hyperpolarizing body wall muscle and blocking glutamate receptors by LPS in larval *Drosophila* has already been shown not to be due to immune deficiency (Imd) signaling pathway[20] despite the IMD receptors (peptidoglycan recognition proteins PGRP-LC and PGRP-LE) revealing to be key in the immune response in the whole animal to gram-negative bacterial exposure[21-25]. It was established that only the PGRP-LC and PGRP-LE responded to the exposure of gram-negative bacteria of the three peptidoglycan recognition proteins (ie. PGRP-SA, PGRP-LC and PGRP-LE) known to be present in *Drosophila* tissues[26,27]. However, RNAi suppression of PGRP-LC and PGRP-LE in body wall muscles did not alter the rapid response to LPS exposure[20] which supports that LPS is likely not mediating the Imd pathway directly but that other associated peptidoglycans of gram-negative bacteria are doing so[28].

The rapid (<1 sec) LPS induced transient hyperpolarization of the body wall muscles remains elusive. The effect does not appear to be due to activated Nitric Oxide Synthase (NOS) or the opening of Cl^- channels[29]. It was postulated that if the sodium-potassium-ATP pumps are transiently hyper-activated responsible for the large hyperpolarization phase of the LPA response[29]. However, no experimental evidence has been forthcoming to substantiate this suggestion. If a calcium-activated potassium channel were to be activated, this may explain the hyperpolarization. Potentially, if Ca^{2+} were to enter the muscle cell through a TRPA1 ionotropic channel, this could activate a calcium-activated potassium channel. Larval *Drosophila* muscle does express calcium-activated potassium channel and is blocked by TEA (20 mM)[30], but TEA (20 mM) did not block the LPS hyperpolarization induced response[29] and thus is not likely the mechanism present.

To demonstrate that TRPA1 can be functionally expressed in muscle, heated saline at 30 and 37°C was used[31]. Since it is suggested that LPS binds to TRPA1 receptors in both mammals and *Drosophila* receptors[4,5], used the genetically amenable model of *Drosophila* to overexpress and to block expression of the TRPA1 receptors, via RNAi expression, to examine the effect of LPS exposure.

MATERIALS AND METHODS

Study area: This study was carried out at the University of Kentucky, USA during February-June, 2020.

Protocols: The overall protocol was to stimulate the segmental nerve at 0.5 Hz while recording the EJPs and mEJPs as well as the resting membrane potential before and during exposure to LPS in the TRPA1, RNAi TRPA1 and UAS-parental TRPA lines. The TRPA1 and RNAi TRPA1 lines were targeted in body wall muscles as well as in motor neurons. In the second set of experiments, while exposing the preparation to LPS, the saline bath was exchanged with saline warmed to 30°C containing the same concentration of LPS. Also, the lines solely exposed to the change in temperature and not exposed to LPS were examined. The lines utilized are illustrated in Table 1 to determine the effects of temperature to examine the expression of TRPA1 and the effects of LPS with and without activation of TRPA1.

Fly lines: All *Drosophila* were obtained from the Bloomington Drosophila Stock Center (BDSC). The overexpression in the muscles of the TRPA1 receptor was performed by crossing non-stubble 24B-Gal4 (III) (BDSC stock # 1767) with female virgins of UAS-TRPA1 (BDSC stock # 26263). For targeting motor neurons males of D42-GAL4 (BDSC stock#8816) were used. The filial 1 (F1) generations were used for measures in the overexpression of TRPA1 receptors. The background UAS-TRPA1 was used as a control for these over expressers. The RNAi of the TRPA1 was obtained by virgin females of y[1] v[1]; P{y[+t7.7] v[+t1.8] = TRiP.HMS05348}attP2 (BDSC stock # 66905) crossed with males of non-stubble 24B-Gal4 (III) for targeting muscle and for targeting motor neurons males of D42-GAL4 (BDSC stock#8816) were used.

Only early 3rd instar *Drosophila* larvae were used (50-70 hrs) post-hatching. All larvae were maintained at room temperature ~20°C in vials partially filled with cornmeal-agar-dextrose-yeast medium.

Saline and compounds: Fly saline modified haemolymph-like 3 (HL3) was used: (in mmol L^{-1}) 70 NaCl, 5 KCl, 20 $MgCl_2$, 10 $NaHCO_3$, 1 $CaCl_2$, 5 trehalose, 115 sucrose, 25 N,N-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid (BES) and pH at 7.1[32]. LPS was dissolved in saline before use and was readily exchanged over the dissected preparations during the recording of evoked EJPs and mEJPs. The total volume of the chamber is only 1 mL, which is fully exchanged when switching the media. One form of LPS used was (*Serratia*

Table 1: Lines used to examine TRPA1 activation and to examine the effects of LPS with and without activation of TRPA1

	Conditions		
Lines	20°C	30°C	20°C
Saline only			
UAS TRPA1	√	√	√
TRPA1×24b (muscle)	√	√	√
TRPA1×D42 (motor nerve)	√	√	√
UAS RNAi	√	√	√
RNAi-TRPA1×24b (muscle)	√	√	√
RNAi-TRPA1×D42 (motor nerve)	√	√	√
LPS	20°C	20°C (LPS)	20°C
UAS TRPA1	√	√	√
TRPA1×24b (muscle)	√	√	√
TRPA1×D42 (motor nerve)	√	√	√
UAS RNAi	√	√	√
RNAi-TRPA1×24b (muscle)	√	√	√
RNAi-TRPA1×D42 (motor nerve)	√	√	√
LPS	20°C	30°C (LPS)	20°C
UAS TRPA1	√	√	√
TRPA1×24b (muscle)	√	√	√
TRPA1×D42 (motor nerve)	√	√	√
UAS RNAi	√	√	√
RNAi-TRPA1×24b (muscle)	√	√	√
RNAi-TRPA1×D42 (motor nerve)	√	√	√

	Conditions			
LPS	20°C	20°C (LPS)	30°C (LPS)	20°C
TRPA1×24b (muscle)	√	√	√	√

marcescens -S.m.). LPS and the chemicals used for saline were obtained from Sigma-Aldrich (St. Louis, MO, USA). LPS concentration was used at 500 μg mL^{-1} to compare with previous studies using LPS on the larval *Drosophila* muscles as well as frog and crayfish muscles and rodent CNS[20,29,33,34]. The LD50 in rodents for LPS from S.m. is 650 μg mL^{-1} (10) (6×10^6) CFU- colony-forming units[35]. This was another reason to use a relatively high concentration for *D. melanogaster* since they are likely exposed to gram-negative bacterial strains in their native environment.

Measures of membrane potential in body wall muscles: The technique to dissect larvae is described[36,37]. In brief, a longitudinal dorsal midline cut was made in 3rd instar larvae to expose the CNS. The segmental nerves were cut and sucked into a suction electrode, which is filled with saline and stimulated. The segmental nerves were stimulated at 0.5 Hz (S88 Stimulator, Astro-Med, Inc., Grass Co., West Warwick, RI, USA). To monitor the transmembrane potentials of the body wall muscle (m6) of 3rd instar larvae, a sharp intracellular electrode (30-40 M resistance) filled with 3M KCl impaled the fiber. An Axoclamp 2B (Molecular Devices, Sunnyvale, CA, USA) amplifier and 1XLU head stage was used.

The bathing saline was initially 20°C and exchanged for 30°C saline while recording the membrane potential for the temperature experiments. The recordings were made for the background parental lines and the TRPA1 overexpression or RNAi lines. The pH was monitored in the 30°C and was maintained at a pH of 7.1, which is likely because of the high concentration of BES buffer used in this HL3 modified saline.

Statistical analysis: Some data are expressed as raw values. A Sign pairwise test was used to analyze changes in membrane potential and amplitudes of evoked transmission after changing bathing conditions. Since some data sets are not normally distributed, (several zeroes in some groups) the non-parametric Sign test was used. When appropriate, paired and unpaired t-tests were used. A significant difference is considered $p<0.05$. Different symbols were used in the graphs to isolate individual preparations from each other.

RESULTS

The effect on the muscle membrane potential and evoked synaptic transmission upon exposure to LPS was consistent for the various *Drosophila* lines at 20°C without activation of the TRPA1 receptors. Exposure to LPS resulted in rapid hyperpolarization on the body wall muscle and reduction in the evoked EJP. As quantified in earlier studies, the quantal responses from spontaneous vesicle fusion also decreased in amplitude, supporting the proposed antagonistic action of LPS on the glutamate receptors. Since the amplitudes of the spontaneous quantal responses were measured in the earlier reports, an emphasis was placed on the amplitude of evoked responses.

A representative response is illustrated in Fig. 1a of the rapid hyperpolarization and dampening of evoked EJP, as well as the spontaneous quantal events (mEJPs) over the acute 3 min exposure. Upon exchanging the bathing media without LPS allowed a slow but incomplete recovery of the membrane potential and in the amplitude in evoked EJP and quantal responses within the 3 min of the acute removal of LPS exposure. Even after 10 min after the removal of LPS, the membrane potential and amplitude of quantal responses did not recover in the Canton-S *Drosophila* strain (Fig. 1b-c). Since each preparation has a varied initial resting membrane potential and amplitude of the EJP, a percent change in the responses was measured for the background control UAS-TRPA1 line (Fig. 1d) for comparisons. All 6 out of 6 preparations showed the same trend in hyperpolarization and reduced amplitude of evoked EJP ($p<0.05$, Sign-test). The most negative membrane potential and smallest EJP amplitude reached within the three minutes of LPS exposure was used for measures. The membrane potential commonly showed the largest decrease initially and then started to depolarize during the three minutes while the amplitude of the EJP was still reduced in amplitude (Fig. 1a).

For comparisons, the F1generation of the crosses TRPA1-24B and TRPA1-D42, the background UAS-RNAi and RNAi-TRPA1-24B and the RNAi-TRPA1-D42, percent changes in the EJP amplitude and muscle membrane potential to exposure of LPS at 20°C are shown (Fig. 2). All the lines examined showed statistically significant effects ($p<0.05$, Sign-test, $N = 6$ for each line)

To illustrate the effect of increased temperature (30°C) on the membrane potential as well as the amplitude of the evoked EJP response for the background control (UAS-TRPA1, Fig. 3a) and expressing TRPA1 in the muscle (TRPA1-24B, Fig. 3b, $p<0.05$, non-parametric Sign-test; $N = 6$) or in motor neurons (TRPA1-D42, Fig. 3c), representative traces are shown. Exposure to saline with increased temperature (20-30°C) led to the hyperpolarization of the membrane potential as expected for the background control (UAS-TRPA1) as compared to muscle expressing TRPA1 (TRPA1-24B) which depolarized. The effects of the increased temperature for the lines expressing TRPA1 in motor neurons (TRPA1-D42) also

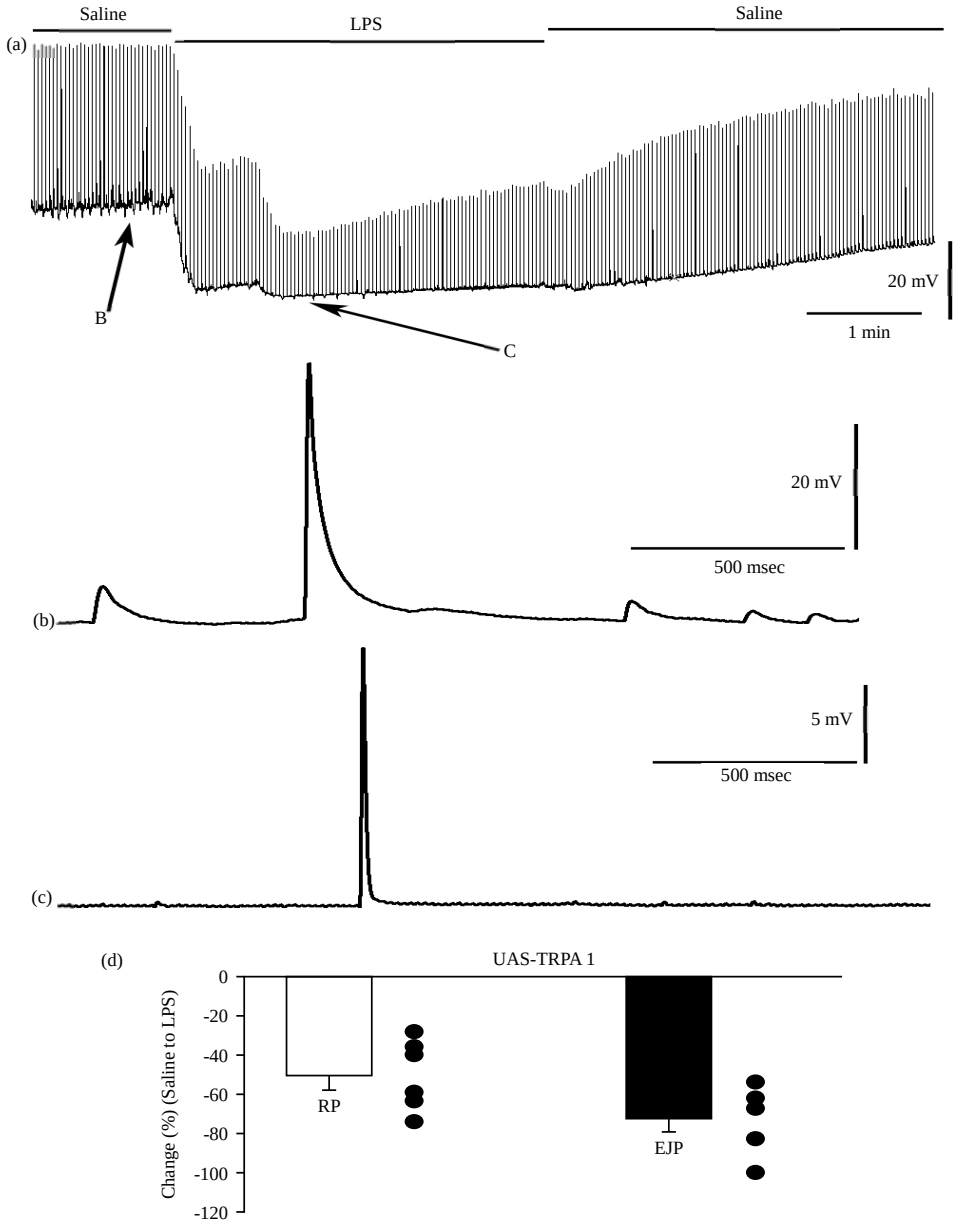

Fig. 1(a-b): LPS effect on evoked synaptic transmission and resting membrane potential for preparations at 20°C

(a) The background control UAS-TRPA1 larvae response to LPS (500 ug mL^{-1}) resulted in muscle hyperpolarization and reduced evoked excitatory junction potential (EJP) and quantal mEJP amplitudes. Evoked transmission occurred at 0.5 Hz. Enlarged views in sections of the trace are shown, (b) Enlarged view during the saline only exposure, (c) Enlarged view during the lowest membrane potential reached during the LPS exposure. Arrows indicate where the enlarged views were obtained, (d) The percent change of the evoked EJP and the resting membrane (RP) for all the preparations (dots) along with the mean (+/- SEM) are shown ($p<0.05$, non-parametric Sign test, N = 6)

resulted in membrane potential hyperpolarizing, as expected, but with a substantial increase in spontaneous quantal events occurring. The increased number of quantal events with exposure to the higher temperature for the TRPA1-D42 line is obvious and was not quantified as the increase was so substantial. Many quantal events superimposed upon others making a precise count unrealistic. The burst in spontaneous quantal events subsided, as well as the hyperpolarization over the following 3 min of the initial increased temperature exposure. During the hyperpolarization of the muscle membrane for UAS-TRPA1 and TRPA1-D42 lines, the amplitude of the evoked EJPs increased (Fig. 3a,c) likely due to the increased sodium ion driving gradient for the EJPs as compared to the TRPA1-24B line, which had a decreased driving gradient in the depolarized state. Note that the amplitude of the evoked EJPs increased as the depolarized

membrane potential recovered to a more negative potential (Fig. 3b). The enlarged section of "3c" showed in Fig. 3d. The percent change and individual responses for the membrane potential of the muscle and amplitude of the evoked EJPs for each line are indicated in Fig. 4.

To examine how the TRPA1 responded in the presence of LPS, the saline bath at 20°C was exchanged to saline containing LPS at 30°C. The backgrounds UAS-TRPA1 and UAS-RNAi, as well as RNAi-TRPA1-24B (muscle) and RNAi-TRPA1-D42

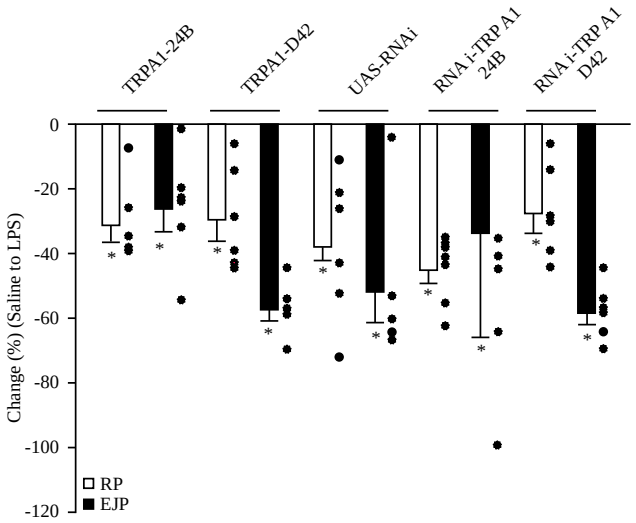

Fig. 2: LPS effects on evoked synaptic transmission and the resting membrane potential for lines expressing TRPA1 and reducing TRPA1 by RNAi examined at 20°C

Evoked transmission occurred at 0.5 Hz. Responses for the TRPA1×24B, TRPA1×D42, UAS-RNAi, RNAi×24B and RNAi×D42 lines are indicated, The percent change of the evoked EJP and the resting membrane (RP) for all the preparations along with the mean (+/- SEM) are shown for each line. All lines show a significant effect in a reduction in the EJP amplitude and hyperpolarization of the muscle membrane, (*p<0.05, non-parametric Sign-test; N = 6 for each line)

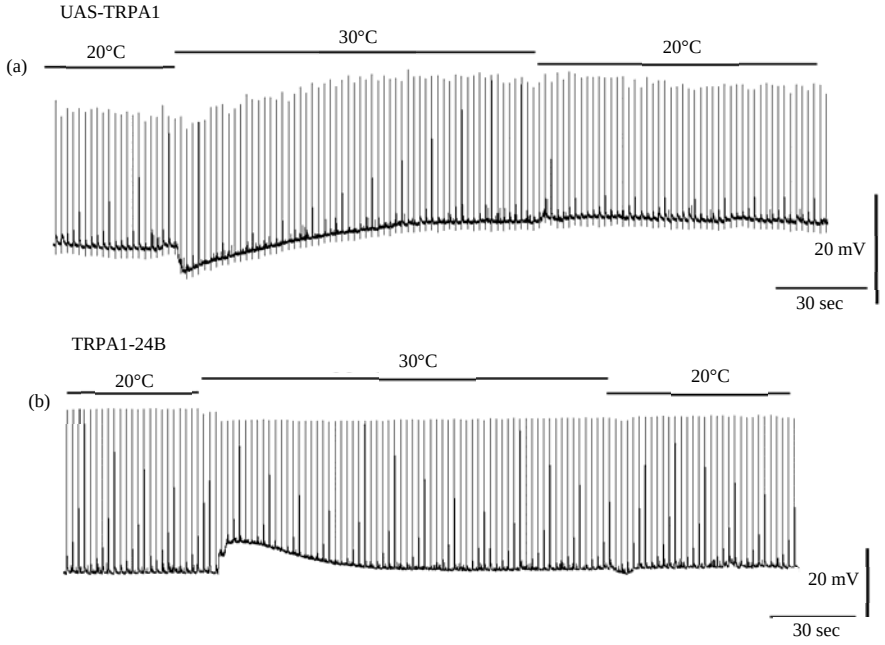

Fig. 3(a-d): Continue

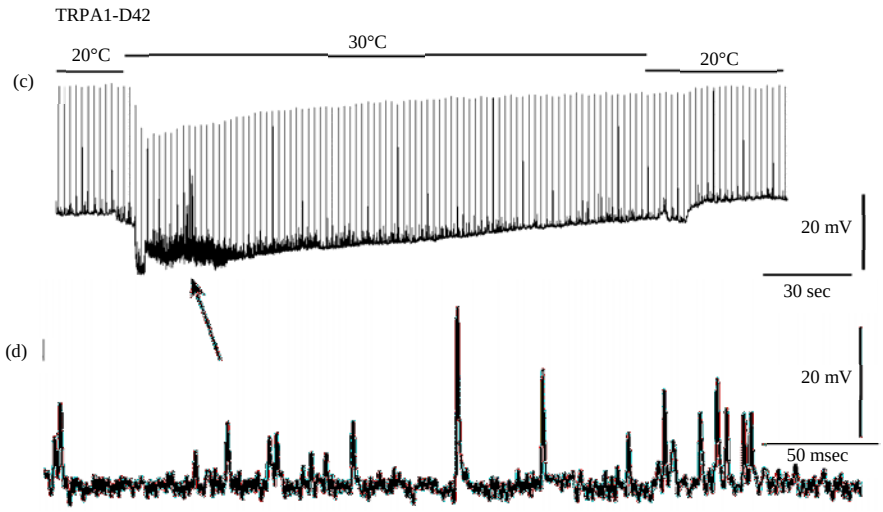

Fig. 3(a-d): The effect of activating the TRPA1 channels expressed with heat

(a) Background control UAS-TRPA1 showed hyperpolarization when exposed to saline at 30°C along with an increased driving force for the evoked EJPs, (b) Expressing TRPA1 in the muscle (24B) resulted in a large depolarization of the muscle upon exposure to 30°C saline, (c) Expressing TRPA1 in motor nerves (D42) resulted in a hyperpolarization of the muscle and a burst of spontaneous quantal EJPs upon exposure to 30°C saline, (d) The enlarged section of c shown, ($p<0.05$, non-parametric Sign-test; N = 6 for each line)

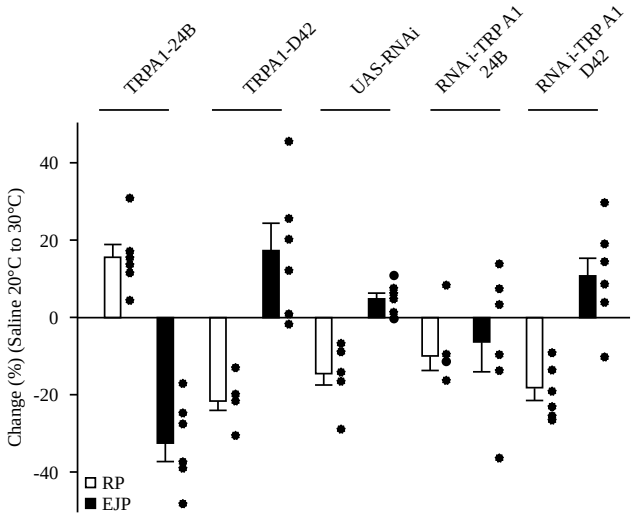

Fig. 4: The effect of increased temperature on evoked synaptic transmission and resting membrane potential for lines expressing TRPA1 and reduced TRPA1 by RNAi was examined at 20-30°C

Evoked transmission occurred at 0.5 Hz. Responses for the TRPA1×24B, TRPA1×D42, UAS-RNAi, RNAi×24B and RNAi×D42 lines are indicated. The percent change of the evoked EJP and the resting membrane (RP) for all the preparations along with the mean (+/- SEM) are shown for each line. Note activation of TRPA1 in the muscle (TRPA1-24B) results in depolarization of muscle and depression in the amplitude of the EJP ($p<0.05$, non-parametric Sign-test; N = 6)

(motor neurons) all responded similarly with a hyperpolarizing and dampening of the evoked EJP and mEJPs (Fig. 5a, $p<0.05$, non-parametric Sign-test; N = 6 for each line). The expression of TRPA1-D42 (motor neurons) response to LPS was similar to saline without LPS at 30°C with hyperpolarizing and a burst of spontaneous events (mEJPs), but the EJPs and mEJPs decreased in amplitude as compared to exposure to saline at 30°C without LPS (Fig. 5b, $p<0.05$, non-parametric Sign-test;

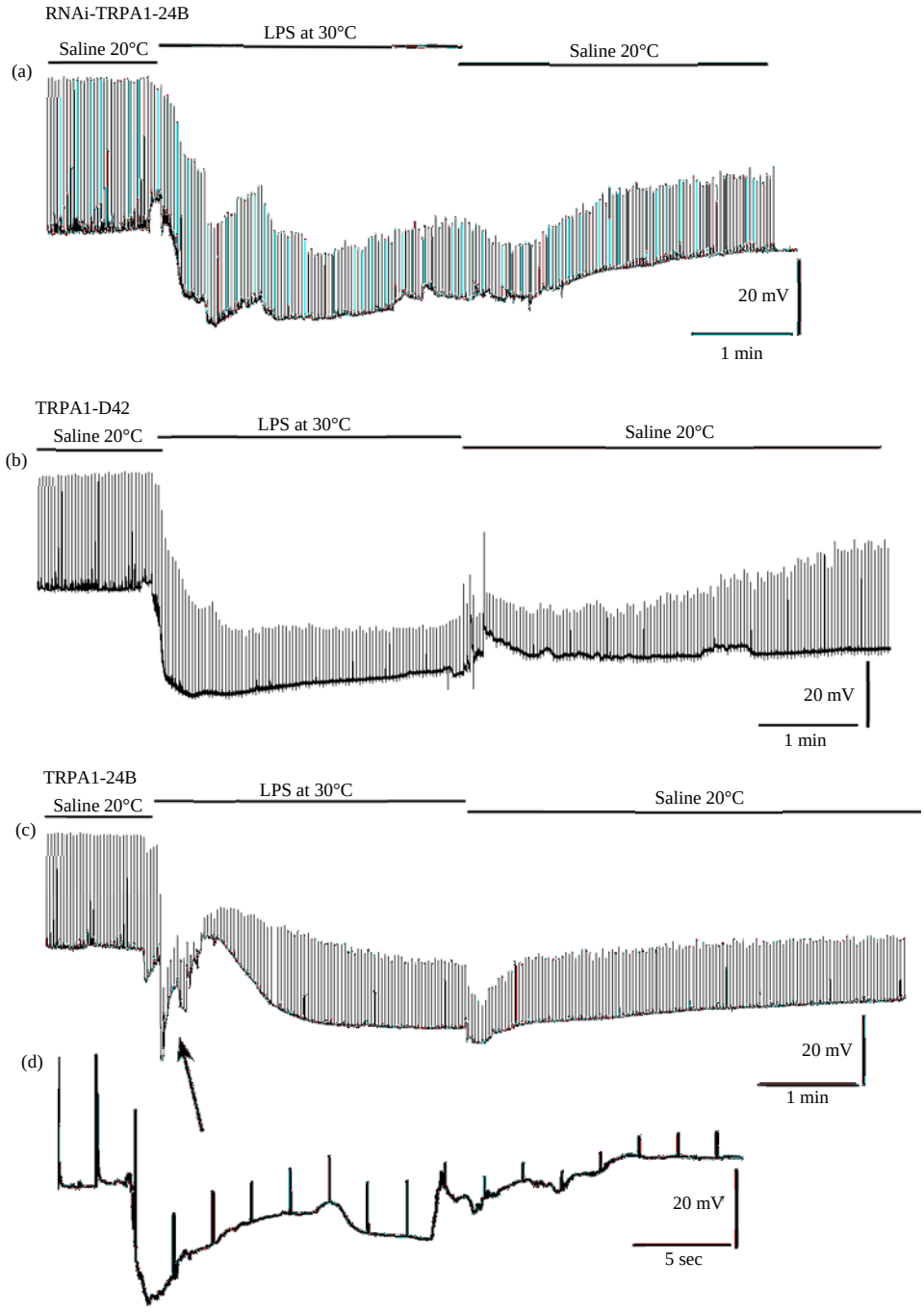

Fig. 5(a-d): The effect of LPS and heat on synaptic transmission and resting membrane potential

(a) Background control UAS-RNAi-TRPA1 showed hyperpolarization with LPS presented at 30°C and reduction of the EJP amplitude, (b) The overexpression of TRPA1 in motor neurons of the TRPA1 (c) The effects of exposure of LPS at 30°C illustrated the initial hyperpolarization followed by a reduction in the amplitude of the evoked EJPs (d) Enlarged section of C shown ($p<0.05$, non-parametric Sign-test; N = 6 for each line)

N = 6). However, the TRPA1-24B line showed a response to the LPS with quick hyperpolarizing and then a depolarization. Thus, the TRPA1 receptors responded and were not blocked by LPS but interestingly the response to LPS was quicker than the response to TRPA1 (Fig. 5c, $p<0.05$, non-parametric Sign-test; N = 6, the enlarged section shown in Fig. 5d. Each preparation responded differently in the degree of altering the amplitudes of the evoked EJPs and changes in the membrane

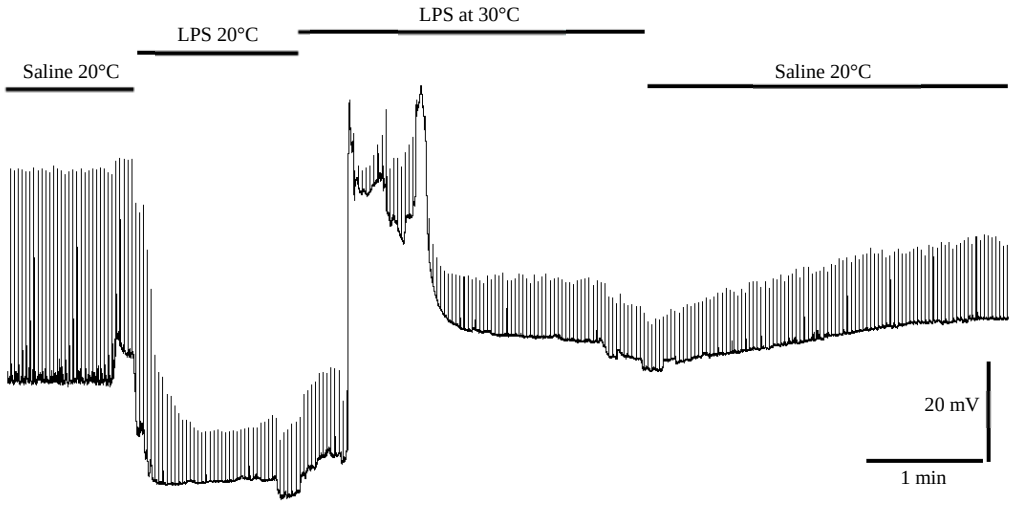

Fig. 6: The effects of LPS on the activation of the TRPA1 channels.
The line expressing TRPA1 in the muscle (24B) hyperpolarizes with exposure to LPS and depolarizes with exposure to LPS at 30°C, LPS exposure promotes the hyperpolarization and reduces evoked EJP but upon changing the bath to LPS at 30°C, the membrane depolarizes, Upon removal of LPS and returning to 20°C the evoked EJP starts to recover, ($p<0.05$, non-parametric Sign-test, $N = 6$)

potentials with LPS and heated saline; however, the same trends were present in all six out of six of preparations.

To examine the effect of activating TRPA1 after exposure to LPS, the preparations were bathed in LPS at 20°C and then the TRPA1 receptors were activated by heated saline (30°C) containing LPS. The TRPA1 still responded during the hyperpolarizing action of the LPS (Fig. 6, $p<0.05$, non-parametric Sign-test; $N = 6$). This supports the concept that the TRPA1 receptors are not blocked by LPS and that LPS is not activating the TRPA1 receptors.

DISCUSSION

In this study, it was shown that LPS is not activating or blocking TRPA1 receptors expressed in body wall muscles or motor neurons. The activation of TRPA1 receptors overexpressed in muscle or motor neurons as well as reduced in expression by RNAi was documented by changing the temperature from 20-30°C. TRPA1 receptors were able to be activated after and during the cellular responses to LPS. Upon simultaneous exposure to LPS and higher temperature (30°C), the hyperpolarization of the muscle and reduced EJP amplitudes were rapid to the effect of activating TRPA1 receptors, but the activation of TRPA1 was no different than without prior exposure to LPS.

The background UAS-RNAi, UAS-TRPA1 as well as RNAi-TRPA1-24B and RNAi-TRPA-D42 did not show differences between the lines for LPS or to the change in temperature (20-30°C) with or without LPS. Therefore, it is likely that the TRPA1 is not expressed inherently in the larval body wall muscles and that the putative mediator of LPS is the same in all the lines. Perhaps raising the larvae at 20°C did not fully block expression of the RNAi in the lines due to the low temperature since the UAS-Gal4 regulating expression is temperature-dependent. However, raising the TRPA1-24B and TRPA1-D42 lines, also under a UAS-Gal4 driver, at 20°C did give a heightened response to higher temperatures. Thus, suggesting the RNAi lines would be sufficiently functioning as well as at 20°C.

The results indicate that TRPA1 receptors do not appear to be receptors for LPS from *Serratia marcescens*; however, the receptors might be for other forms of LPS or higher concentrations of LPS. It is known that overexpression of TRP receptors sensitive to capsaicin alters larval behaviour[38]. A high concentration of LPS was used in this study (500 μg mL^{-1}) to compare with previous studies using LPS on the larval *Drosophila* muscles as well as the frog, crayfish muscles and rodent CNS[20,29,33,34,39,40]. Since *Drosophila* larvae can be exposed to relatively high concentrations of gram-negative bacterial strains in their native environment, the digestive properties may be well-conditioned to resist infection as well as a prominent innate immune response[23,41-43].

The mechanism to account for the rapid hyperpolarization of the body wall muscle in *Drosophila*

larvae as well as crayfish muscle[40] to LPS remains elusive. Potential mechanisms were addressed in recent reports demonstrating that the potential PGRP-LC and PGRP-LE receptors, known to be present in *Drosophila* tissues[26,27], did not account for the effect. These receptors might be activated by other associated peptidoglycans of gram-negative bacteria[28]. The ECl–rev for body wall muscle of *Drosophila* larvae is more depolarized than the resting membrane potential[44,45]. Thus, the response is not due to a chloride ion flux. As suggested in recent reports, enhanced transient activation of the sodium-potassium pump or an ion exchanger seems plausible, but at present, this is the only speculation without experimentation[20,29,33,34,39,40]. Since the hyperpolarization is transient in the presence of LPS, the response is either desensitized, inactivated, or compensated. Repeated acute exposures to LPS are now being investigated to examine this possibility in our research group[46]. The implications of the study suggest that possible TRPA1 receptors are not directly involved in the cellular action of LPS; however, the factors induced by LPS could be indirectly in other studies.

CONCLUSION

LPS acts independently from the TRPA1 channels in cellular responses and does not appear to block or activate TRPA1 receptors. Activating TRPA1 responses masks the membrane potential responses induced by LPS. Potentially TRPA1 receptors in other cell types are interactive with downstream cascades of LPS induced responses. No known mechanism yet to account for the hyperpolarization in muscle induced by LPS.

SIGNIFICANCE STATEMENT

This study will help the researcher to uncover the critical areas of receptors involved and not involved in the direct actions of LPS. Thus, a focus can be aimed toward secondary responses induced by LPS maybe responsible for the observed animal behaviors and cellular responses assumed to be directly related to the action of LPS.

REFERENCES

1. Rhee, C., R. Dantes, L. Epstein, D.J. Murphy and C.W. Seymour *et al*., 2017. Incidence and trends of sepsis in US hospitals using clinical vs claims data, 2009-2014. Jama, 318: 1241-1249.
2. Dantes, R.B. and L. Epstein, 2018. Combatting Sepsis: A public health perspective. Clin. Infect. Dis., 67: 1300-1302.
3. Costamagna, D., P. Costelli, M. Sampaolesi and F. Penna, 2015. Role of inflammation in muscle homeostasis and myogenesis. Mediators Inflammation, 2015: 1-14.
4. Boonen, B., Y. Alpizar, V. Meseguer and K. Talavera, 2018. TRP channels as sensors of bacterial endotoxins. Toxins, Vol. 10. 10.3390/toxins10080326
5. Meseguer, V., Y.A. Alpizar, E. Luis, S. Tajada and B. Denlinger *et al*., 2014. TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. Nat. Commun., 5: 18-14.
6. Clapham, D.E., 2003. TRP channels as cellular sensors. Nature, 426: 517-524.
7. Ramsey, I.S., M. Delling and D.E. Clapham, 2006. An introduction to TRP channels. Annu. Rev. Physiol., 68: 619-647.
8. Paulsen, C.E., J.P. Armache, Y. Gao, Y. Cheng and D. Julius, 2015. Structure of the TRPA1 ion channel suggests regulatory mechanisms. Nature, 520: 511-517.
9. Wang, H., M. Schupp, S. Zurborg and P.A. Heppenstall, 2012. Residues in the pore region of *Drosophila* transient receptor potential A1 dictate sensitivity to thermal stimuli. J. Physiol., 591: 185-201.
10. Hof, T., S. Chaigne, A. Récalde, L. Sallé, F. Brette and R. Guinamard, 2019. Transient receptor potential channels in cardiac health and disease. Nat. Rev. Cardiol., 16: 344-360.
11. Bodkin, J.V., P. Thakore, A.A. Aubdool, L. Liang and E.S. Fernandes *et al*., 2014. Investigating the potential role of TRPA1 in locomotion and cardiovascular control during hypertension. Pharmacol. Res. Perspect., Vol. 2. 10.1002/prp2.52
12. Andrei, S.R., M. Ghosh, P. Sinharoy, S. Dey, I.N. Bratz and D.S. Damron, 2017. TRPA1 ion channel stimulation enhances cardiomyocyte contractile function via a CaMKII-dependent pathway. Channels, 11: 587-603.
13. Celes, M.R.N., C.M. Prado and M.A. Rossi, 2013. Sepsis: going to the heart of the matter. Pathobiology, 80: 70-86.
14. Shankar-Hari, M., G.S. Phillips, M.L. Levy, C.W. Seymour and V.X. Liu *et al*., 2016. Developing a new definition and assessing new clinical criteria for septic shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). J. Am. Med. Assoc., 315: 775-787.
15. Singer, M., C.S. Deutschman, C.W. Seymour, M.S. Hari and D. Annane *et al*., 2016. The third international consensus definitions for sepsis and septic shock (Sepsis-3). J. Am. Med. Assoc., 315: 801-810.
16. Jayaprakash, N., O. Gajic, R.D. Frank and N. Smischney, 2018. Elevated modified shock index in early sepsis is associated with myocardial dysfunction and mortality. J. Crit. Care, 43: 30-35.

17. Rameshrad M., N. Maleki-Dizaji, H. Vaez, H. Soraya, A. Nakhlband and A.A. Garjani, 2015. Lipopolysaccharide induced activation of toll like receptor 4 in isolated rat heart suggests a local immune response in myocardium. Iran. J. Immunol., 12: 104-116.
18. Yang, J., R. Zhang, X. Jiang, J. Lv and Y. Li *et al*., 2017. Toll-like receptor 4-induced ryanodine receptor 2 oxidation and sarcoplasmic reticulum Ca2+leakage promote cardiac contractile dysfunction in sepsis. J. Biol. Chem., 293: 794-807.
19. Soldano, A., Y.A. Alpizar, B. Boonen, L. Franco and A. López-Requena *et al*., 2016. Gustatory-mediated avoidance of bacterial lipopolysaccharides via TRPA1 activation in *Drosophila*. Elife, Vol. 5. 10.7554/eLife.13133
20. Ballinger-Boone, C., O. Anyagaligbo, J. Bernard, S.M. Bierbower and E.E. Dupont-Versteegden *et al*., 2019. The effects of bacterial endotoxin (LPS) on cardiac and synaptic function in various animal models: Larval *Drosophila*, crayfish, crab and rodent. Int. J. Zool. Res., 16: 33-62.
21. Nie, L., S.Y. Cai, J.Z. Shao and J. Chen, 2011. Toll-like receptors, associated biological roles and signaling networks in non-mammals. Front. Immunol., 9: 210-226.
22. Loker, E.S., C.M. Adema, S.M. Zhang and T.B. Kepler, 2004. Invertebrate immune systems-not homogeneous, not simple, not well understood. Immunol. Rev., 198: 10-24.
23. Kleino, A. and N. Silverman, 2014. The *Drosophila* IMD pathway in the activation of the humoral immune response. Dev. Compa. Immunol., 42: 25-35.
24. Leclerc, V. and J.M. Reichhart, 2004. The immune response of *Drosophila melanogaster*. Immunol. Rev., 198: 59-71.
25. Werner, T., G. Liu, D. Kang, S. Ekengren, H. Steiner and D. Hultmark, 2000. A family of peptidoglycan recognition proteins in the fruit fly *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA., 97: 13772-13777.
26. Gottar, M., V. Gobert, T. Michel, M. Belvin and G. Duyk *et al*., 2002. The *Drosophila* immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature, 416: 640-644.
27. Takehana, A., T. Katsuyama, T. Yano, Y. Oshima, H. Takada, T. Aigaki and S. Kurata, 2002. Overexpression of a pattern-recognition receptor, peptidoglycan-recognition protein-LE, activates imd/relish-mediated antibacterial defense and the prophenoloxidase cascade in *Drosophila* larvae. Proc. Natl. Acad. Sci. USA., 99: 13705-13710.
28. 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.
29. 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
30. Gho, M. and A. Mallart, 1986. Two distinct calcium-activated potassium currents in larval muscle fibres of *Drosophila melanogaster*. Pflugers Arch., 407: 526-533.
31. Marguerite, N.T., D. Harrison, D. Harris and R.L. Cooper, 2019. Behavioral effects to heat in the blow fly (Phaenicia sericata). Annual meeting of the Kentucky Academy of Sciences. Nov. 1-2, 2019 at Berea College. Berea, KY.
32. De Castro, C., J. Titlow, Z.R. Majeed and R.L. Cooper, 2014. Analysis of various physiological salines for heart rate, CNS function and synaptic transmission at neuromuscular junctions in *Drosophila melanogaster* larvae. J. Comp. Physiol. A, 200: 83-92.
33. 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.
34. Istas, O., A. Greenhalgh and R. Cooper, 2019. The effects of a bacterial endotoxin on behavior and sensory-CNS-motor circuits in *Drosophila melanogaster*. Insects, Vol. 10, No. 4. 10.3390/insects10040115
35. Iwaya, A., S. Nakagawa, N. Iwakura, I. Taneike and M. Kurihara *et al*., 2005. Rapid and quantitative detection of blood *Serratia marcescens* by a real-time PCR assay: Its clinical application and evaluation in a mouse infection model. FEMS Microbiol. Lett., 248: 163-170.
36. Stewart, B.A., H.L. Atwood, J.J. Renger, J. Wang and C.F. Wu, 2004. Improved stability of *Drosophila* larval neuromuscular preparations in haemolymph-like physiological solutions. J. Comp. Physiol. A., 175: 179-191.
37. Dasari, S. and R.L. Cooper, 2004. Modulation of sensory-CNS-motor circuits by serotonin, octopamine and dopamine in semi-intact *Drosophila* larva. Neurosci. Res., 48: 221-227.
38. Cooper, R.L., A.S.O. Neil and C. Kim, 2020. Learning and memory retention in larval *Drosophila*. J. Entomol., 17: 36-47.
39. McNabb, M.C., C.M. Saelinger, M. Danley and R.L. Cooper, 2019. Effects of bacterial endotoxin lipopolysaccharides (LPS) on synaptic transmission at neuromuscular junction in an amphibian. Int. J. Zool. Res., 15: 38-42.
40. Saelinger, C.M., M.C. McNabb, R. McNair, S. Bierbower and R.L. Cooper, 2019. Effects of bacterial endotoxin on regulation of the heart, a sensory-CNS-motor nerve circuit and neuromuscular junctions: Crustacean model. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., Vol. 237. 10.1016/j.cbpa.2019.110557
41. Capo, F., A. Wilson and F.D. Cara, 2019. The intestine of *Drosophila melanogaster*: an emerging versatile model system to study intestinal epithelial homeostasis and host-microbial interactions in humans. Microorganisms, Vol. 7. 10.3390/microorganisms7090336

42. Yuzhen Lu, Fanghua Su, Qilin Li, Jie Zhang, Yanjun Li, Ting Tang, Qihao Hu, Xiao-Qiang Yu 2020. Pattern recognition receptors in *Drosophila* immune responses. Dev. Comp. Immunol. Vol. 102. 10.1016/j.dci.2019.103468
43. Myllymäki, H., S. Valanne and M. Rämet, 2014. The *Drosophila* IMD signaling pathway. J. Immunol., 192: 3455-3462.
44. Rose, U., C. Derst, M. Wanischeck, C. Marinc and C. Walther, 2007. Properties and possible function of a hyperpolarisation-activated chloride current in *Drosophila*. J. Exp. Biol., 210: 2489-2500.
45. Stanley, C.E., A.S. Mauss, A. Borst and R.L. Cooper, 2019. The effects of chloride flux on *Drosophila* heart rate. Methods Protocols, Vol. 2, No. 3. 10.3390/mps2030073
46. Istas, O., A. Greenhalgh and R.L. Cooper, 2020. Repetitive exposure to bacterial endotoxin LPS alters synaptic transmission. J. Pharmacol. Toxicol., 15: 65-72.