Bowdoin College
Bowdoin Digital Commons

Honors Projects | Student Scholarship and Creative Work

2023

Effects of Picrotoxin Application on the Cardiac Ganglion of the American Lobster, *Homarus americanus*

John T. Woolley
Bowdoin College

Follow this and additional works at: https://digitalcommons.bowdoin.edu/honorsprojects

 Part of the Cellular and Molecular Physiology Commons, Molecular and Cellular Neuroscience Commons, and the Systems Neuroscience Commons

Recommended Citation

Woolley, John T., "Effects of Picrotoxin Application on the Cardiac Ganglion of the American Lobster, *Homarus americanus*" (2023). *Honors Projects*. 452.
https://digitalcommons.bowdoin.edu/honorsprojects/452

This Open Access Thesis is brought to you for free and open access by the Student Scholarship and Creative Work at Bowdoin Digital Commons. It has been accepted for inclusion in Honors Projects by an authorized administrator of Bowdoin Digital Commons. For more information, please contact mdoyle@bowdoin.edu, a.sauer@bowdoin.edu.

Effects of Picrotoxin Application on the Cardiac Ganglion of the American Lobster, *Homarus americanus*

An Honors Paper for the Program of Neuroscience

By John T. Woolley

Bowdoin College, 2023

©2023 John T. Woolley

Table of Contents

Acknowledgements

To Patsy Dickinson, thank you for being such an incredible advisor. Your knowledge of crustaceans and skills in teaching know no bounds and will never cease to amaze me. From long nights with a prep to huge conferences across the country, your reassurance and preparedness kept me and everyone else in the lab going and loving every moment. Thank you for welcoming me into your lab and mentoring me all the way through my research. I'm so glad that I got to learn from you in class and the lab. You have been so helpful, kind, and fun to have worked with on this research for the past year and I could not have enjoyed my time more with anyone else heading the project.

To Dan Powell, thank you for being my advisor along the whole journey of this project. You were the person who helped me to start the project from your Lab in Neurophysiology class and the one who helped it grow into the research that it is today. I can say for certain that I would have never started or continued this research without your neurophysiology expertise and enthusiasm for helping us all with our research.

To Jenny Wadhwa, thank you for starting the process of designing this research topic with me and testing preliminary concentrations of PTX and GABA in Lab in Neurophysiology with me. This project would have never happened without you helping start it.

To Miranda Maung, thank you for helping me with the research all summer. I would not have gotten nearly as much data without your assistance.

To the whole Dickinson Lab, thank you for being such amazing people to work with and be around, you made this research so incredible, and all provided so much feedback and support throughout it.

To my family and friends, thank you for supporting me while doing this research and letting me practice presenting and explaining it to you countless numbers of times. You all have enabled my love of neuroscience and made me able to do this research in the first place.

Abstract

Picrotoxin (PTX) has been employed extensively as a tool within the crustacean stomatogastric nervous system (STNS) for its efficacy in blocking K^+ and Cl^+ currents gated by both GABA and glutamate. Through blocking some currents in the STNS, PTX allows for examination of other components without their presence. However, effects of PTX are relatively unknown within the lobster's cardiac ganglion (CG). As an incredibly small nervous system of only nine neurons, the lobster CG presents an excellent model system for studying neural circuits. Given that the chemical synapses in the CG are mediated by glutamate, the present study aimed to investigate the action of PTX in the lobster CG with the intent of better understanding its pharmacological impacts as a potential tool for studying the system. Therefore, this study aimed to establish the effects of PTX on CG responses to the application of exogenous GABA or glutamate. When data from both modulators were pooled, PTX applied at a concentration of 10^{-5}M had significant effects on burst duration but not duty cycle or burst frequency of the CG. PTX did suppress GABA ($5x10^{-5}$M) mediated inhibition of burst duration and duty cycle. PTX did not have any significant effects on burst duration, duty cycle, or frequency compared to exogenous glutamate application. These results indicate that glutamatergic inhibitory synapses are not present in the CG and PTX partially suppresses only GABAergic responses in this system.

Introduction

Central pattern generators (CPGs) are neural circuits that can continually produce rhythmic outputs without the need for sustained rhythmic inputs or sensory feedback (Katz, 2016). CPGs drive a wide range of rhythmic motor behaviors including heartbeat, chewing, swimming, and walking. Many CPGs are relatively simple networks that can be isolated from the animal for experimentation while still retaining fictive rhythmic output (Hartline, 1979; Cooke, 2002; Katz, 2016). One such CPG is the cardiac ganglion (CG) of the American Lobster, *Homarus americanus*, which requires no input to sustain rhythmic output (Cooke, 2002). CPG systems have been studied in great depth and can provide means for investigating specific aspects of neural systems. Some of the most widely studied CPGs are found in crustaceans which rely on multiple patterned motor behaviors (Dando, 1969). The principles governing these systems are common across all neuronal systems, including circuit dynamics and synaptic properties (Marder and Bucher, 2007).

Unlike humans who have myogenic hearts controlled by specialized muscle cells, lobsters have neurogenic hearts controlled by the CG (Alexandrowicz, 1932; Hartline, 1979). The CG works as a CPG to generate a consistent rhythmic output and maintain the beating of the heart of the lobster (Cooke, 2002). This system, like many other CPGs, is used to understand the properties of nervous systems involved in rhythmic pattern generation with minimal components (Cooke, 2002). The CG of the lobster is a relatively simple neural network consisting of only 9 cells (Hartline, 1979). The 9 neurons of the CG are surrounded by glial and connective tissue. Five are large motor neurons that cause heart muscle to contract while four are small neurons that act as pacemakers (Hartline, 1979; Cooke, 2002). These pacemakers and motor neurons are both electrically coupled and synaptically connected to one another, meaning feedback from

each group affects output from the entire system. Together, these 9 cells generate the consistent, spontaneous output coordinating the contraction of muscle fibers to cause the heartbeat. The CG can be maintained *in vitro* where it will continue to spontaneously produce rhythmic output for at least a day. Despite the fact that the cardiac nervous system in the American lobster is one of the most well characterized nervous systems, there is still much to understand regarding the presence and properties of receptor channels expressed by the neurons (Cooke, 2002).

Neurotransmitters involved in the CG include glutamate, which is known to have depolarizing effects on the motor neurons and understood to be the main intraganglionic transmitter, and *y*-Aminobutyric acid (GABA), which drives inhibitory responses (Cooke, 2002; Delgado et al., 2000). The presence of GABA has not been identified as making inhibitory connections between neurons of the CG, but has been identified through immunoreactivity as a neurotransmitter used by inhibitory dorsal nerves which synapse onto the motor neurons of the CG (Delgado et al., 2000).

Pharmacological agents such as drugs or neurotransmitters themselves are used widely in studying CPGs to alter states of the system replicating both natural changes within the organism and unnatural changes which isolate properties of the system (Bidaut, 1980). As a pharmacological tool for dissecting CPGs, drugs can be used to target and block neurotransmitter function at specific locations on the neuron. It is important to be able to independently agonize or antagonize neurotransmitters in the CG, like GABA and glutamate, to understand how the system may function in the presence of one and not another. One potential candidate which has been used to target GABAergic, glutamatergic, or both types of synapses in different animal models is picrotoxin (Bidaut, 1980).

Picrotoxin (PTX) is a drug known largely for its ability to block inhibitory conductances activated by GABA in systems of both vertebrates and invertebrates (Albert et al., 2012). It is derived from the plant *Anamirta cocculus* and was once used in medicine as a central nervous system stimulant to cure barbiturate poisoning, but is no longer used because of the dangers it poses as a lethal toxin (National Center for Biotechnology Information, 2022). In research, PTX is still used to block GABA receptors in vertebrate systems, and there are many invertebrate systems where PTX has been observed to alter neuronal responses to both GABA and glutamate (Marder and Paupardin-Trisch, 1978; Cleland, 1995). There are competing theories of competitive or noncompetitive antagonistic properties of PTX in different systems, suggesting either species specific differences or a need for further investigation into its mechanisms (Shank et al., 1974; Constanti, 1978; Olsen, 2006). Various crustacean systems have shown inconsistent sensitivity of GABA-activated chloride channels to PTX, and as such it is important to investigate the sensitivity of each system individually (Albert et al., 2012). This sensitivity ranges from a full blockage of GABA-induced responses by PTX 10^{-6}M to an insensitivity at concentrations up to 10^{-4}M in other decapod systems (Albert et al., 2012).

The decapod crustacean model has used PTX extensively as both a subject of research and a tool for isolating individual aspects of nervous systems within it. The primary CPG of study in the crustacean is the stomatogastric nervous system (STNS), a much more complex circuit made up of around 30 neurons and containing multiple CPGs located in the stomatogastric ganglion (STG) (Bucher et al., 2007). While PTX has not been thoroughly examined in the lobster cardiac system, is has been used extensively in the crab STNS to pharmacologically block inhibitory synapses (Cleland, 1995; Selverston et al., 1998; Selverston, 2009; Bidaut 1980). Early studies of the decapod crustacean STG in the crab *Cancer pagurus*

identified L-glutamate, GABA, and acetylcholine as inhibitory neurotransmitters at synapses in the STNS (Marder and Paupardin-Trisch, 1978). Later studies examining inhibitory synapses within the lobster STNS identified the presence of inhibitory glutamate receptors (IGluRs) which are PTX-sensitive in that organism as well (Cleland et al., 1995, 1996, 1997, 1998). This finding established that in the lobster STNS there are both GABA-gated and glutamate-gated inhibitory channels sensitive to PTX (Cleland et al., 1998). In STG research, PTX is used specifically to block these synapses, and application of PTX at $5x10^{-5}$M blocks all synaptic connections in the system apart from those stemming from the pyloric dilator neurons (Bidaut, 1980). In uses like this one, PTX isolates the function of specific STG components for study of their role in the workings of the system. However, prior research using PTX in the lobster CG has focused only on its ability to attenuate the GABA response and has lacked proper investigation of interactions between PTX and glutamate receptors (Kerrison and Freschi, 1992). Despite the presence of these two systems within the same organism, comparisons between them are relatively few, and their differing responses to PTX has not been a topic of focus.

In the cardiac neuromuscular system of the lobster, PTX has been investigated with application of GABA alone (Kerrison & Freschi, 1992). GABA is the primary inhibitory neurotransmitter synaptically released in the CG, and there appears to be no literature identifying IGluRs in the CG (Kerrison & Freschi, 1992). However, inhibitory GABA receptors and IGluRs are both present in the lobster STG, opening the possibility for their expression in the CG as well. Previous studies have not examined application of PTX in conjunction with glutamate.

Further isolation of the components of the components and deeper experimentation using the CG allows for an increased understanding of its properties. The need for deep characterization demands a thorough investigation into all components, regardless of assumed

properties. This hole in previous research is important to fill as it may determine the role of PTX in blocking purely GABA receptors or also additional inhibitory channels in the CG of the lobster. PTX has been acknowledged to have suppressant effects on the GABA response in the CG, yet glutamate and IGluRs have not been thoroughly investigated in the lobster CG (Kerrison & Freschi, 1992). Past literature examining PTX and GABA application to cardiac muscle fibers showed that PTX at modest ($2x10^{-6}$) concentrations did not affect CG output (Shank et al., 1974). The present study first aimed to apply PTX at concentrations that have no effect on baseline function but suppress GABAergic inhibition of bursting at the level of the isolated CG. This study aimed to confirm expected suppressant effects of PTX on inhibitory GABAergic responses and to examine the effect of PTX when coapplied to the CG with glutamate. I hypothesized that PTX would suppress the response of the CG to GABA application but would not affect the response of the CG to glutamate application. Initially, I applied PTX in the absence of exogenous modulators and found no effect on baseline firing of the isolated CG. Then I applied either GABA or glutamate independently to examine their baseline effects on the CG before applying each in conjunction with PTX.

Methods

Animals

Experiments were performed using adult male and female lobsters (*Homarus americanus)*, which were purchased from local seafood retailers in Brunswick, ME and housed at Bowdoin College. Lobsters were kept in filtered and aerated seawater tanks between 50 and 53^{o}F on a 12 hour light-dark cycle and fed scallops and shrimp.

Preparation

Prior to dissection, lobsters were covered in ice for 30 minutes to anesthetize. After anesthetization, the heart was removed with the posterior artery intact. Dissection of the cardiac ganglion was conducted in chilled physiological saline (all concentrations in mM/L: NaCl 493, KCl 13, $CaCl_2 \cdot 2H_2O$ 14, $MgSO_4 \cdot 7H_2O$ 20, Na_2SO_4 4, Trizma base 11, and maleic acid 5, pH 7.45±0.01; Sigma-Aldrich).

The heart was pinned out in a Sylgard lined dissecting dish with the ventral side facing upwards. Using a microscope, an incision was made into the posterior artery and cut along the ventral side of the heart, exposing the cardiac ganglion inside without damaging it. The cardiac ganglion was then dissected out with all five motor neurons and the region containing the four premotor neurons intact. A portion large enough to create a vaseline well was kept lateral to motor neurons 1 and 2 on each rostral branch of the anterior lateral nerves to be recorded from (Fig. 1). This isolated cardiac ganglion was then moved to a Sylgard dish filled with chilled physiological saline (~10^{o}C) and pinned out securely by the posterior artery and the end of each anterior lateral nerve branch. A water-tight vaseline well was made around the cardiac ganglion past the motor neuron on each anterior lateral nerve branch (Fig. 1). Two extracellular electrodes were inserted into the Sylgard dish, each with one wire within a vaseline well and the other

outside of the well (Fig. 1). An A-M Systems Model 1700 Differential AC Amplifier was used to record bursts and extracellular electrodes simultaneously recorded voltage differential data from activity of the whole CG in Spike2 (version 7) software.

Superfusion

A cooling superfusion setup (Warner Instruments temperature controller CL-100 single channel bipolar temperature controller; Harvard Apparatus SC-20; Warner Intstruments Koolance LCS-1 Heat Exchanger; GILSON Minipuls 2 peristaltic pump) was used to maintain a consistent temperature of around 10±1ºC within the Sylgard dish at all times. Physiological saline was superfused across the cardiac ganglion continuously and each preparation was given at least 30 minutes to acclimate to the consistent temperature before introducing GABA or glutamate. After the acclimation period, the superfusion procedure was conducted identically with either γ-Aminobutyric acid (GABA) or glutamate. GABA and glutamate were examined separately from one another, and never coapplied, and each cardiac ganglion was only exposed to either GABA or glutamate to prevent variation from any potential residual effects. In either setup, the procedure was as followed: saline for 30+ min, GABA ($5x10^{-5}$ M) or glutamate ($4x10^{-5}$ M) for 15 min, saline for 50+ min, PTX (10^{-5} M) for 15 min, GABA+PTX or glutamate+PTX for 15 min, and saline 50+ min.

Data analysis

Extracellular recordings were processed in Spike7 using scripts written by Dirk Bucher at the New Jersey Institute of Technology. Data were organized and peak effect windows were evaluated using Microsoft Excel. Recordings were examined for burst duration (s), burst duty cycle, and burst frequency (Hz). These three variables were analyzed and graphed using GraphPad Prism 9. Data were used only from within the peak effect of each application period or

just before the next application for saline and wash measurements, so as not to include the period of acclimation to each condition from the previous one. Data were compared across saline, neurotransmitter, PTX, and PTX+neurotransmitter conditions for both GABA and glutamate preparations using a repeated measures (RM) ANOVA for each burst parameter. This was followed with Tukey's within-subjects multiple-comparisons analysis. Each RM-ANOVA with multiple comparisons analysis was performed individually for GABA and glutamate preparations for burst duration, duty cycle, and burst frequency.

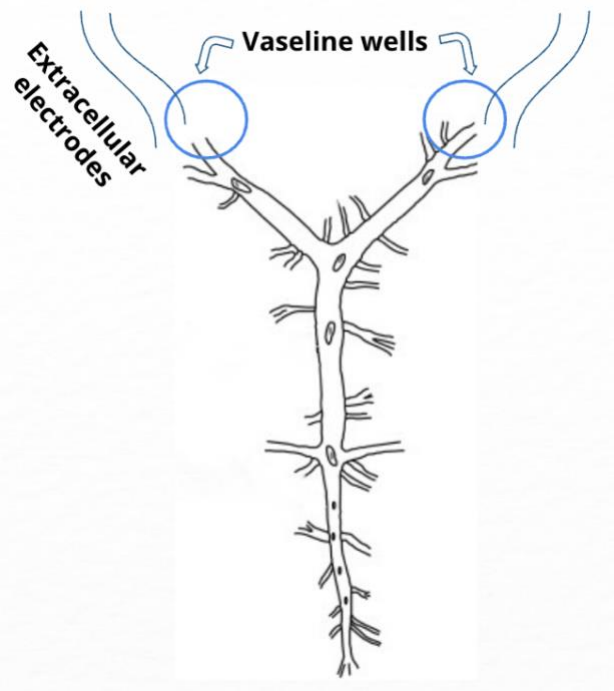

Figure 1. Cardiac ganglion extracellular recording setup. Action potentials from the whole CG were recorded from each site. Recordings were taken simultaneously from each set of extracellular electrodes but only the recording with the least disruptive artifacts was analyzed.

Results

PTX

Extracellular recordings were analyzed for burst duration, duty cycle, and burst frequency. These burst parameters were examined to first determine the effect of PTX application to the CG, and second, to examine the effects of PTX on the response of the CG to GABA or glutamate application. Wash and PTX data from all subjects–both GABA and glutamate experiments–were compiled and analyzed using a within-subjects paired t-test. Paired t-test indicated a significant difference between wash and PTX conditions by burst duration (p = 0.021), but not by duty cycle (p = 0.15), or burst frequency (p = 0.937, n = 16; Fig. 2). Tukey's post-hoc tests in analysis of data from all GABA conditions indicated that PTX increased duty cycle from wash conditions (p = 0.004), and potentially increased burst duration (p = 0.095) from wash conditions (n = 6; supplemental Fig. 7). However, Tukey's post-hoc tests in analysis of data from all glutamate conditions indicated no significant differences between wash and PTX conditions for burst duration (p = 0.677) or duty cycle (p = 0.997; n = 10; supplemental Fig. 8). Further data collection for GABA conditions is required to understand why this increase was present in only GABA and not glutamate conditions.

GABA

Data from saline, GABA, and GABA+PTX applications were analyzed using one-way within-subjects repeated measures ANOVAs (methods) to determine the effects of PTX on the observed GABA response. GABA application induced a consistent inhibitory response which decreased burst duration and duty cycle but not frequency (Fig. 3, 4). PTX+GABA application resulted in increases to burst duration and duty cycle from just GABA application for every preparation (Fig. 4). I observed a significant main effect of subject for each of burst duration (p <

0.0001), duty cycle ($p < 0.0001$), and burst frequency ($p < 0.0001$), and a significant main effect of application on condition for burst duration ($p = 0.0011$), duty cycle ($p = 0.0008$), and burst frequency ($p < 0.0365$; n=6: Fig. 4).

Tukey's post-hoc tests for burst duration indicated differences in saline and GABA application ($p = 0.041$), wash and GABA application ($p = 0.037$), and GABA application and PTX application ($p = 0.015$; n = 6; Fig. 4). Multiple comparisons for burst duration also indicated a potential difference between GABA application and PTX+GABA application ($p = 0.062$) but no difference ($p < 0.1$) for any other conditions (Fig. 4). This draws attention to the need to increase the number of experiments to clarify this potential difference.

Tukey's post-hoc tests for duty cycle indicated differences in saline and GABA application ($p = 0.015$), wash and GABA application ($p = 0.04$), GABA application and PTX application ($p = 0.005$), GABA application and PTX+GABA application ($p = 0.038$), wash and PTX application ($p = 0.004$), and PTX application and PTX+GABA application ($p = 0.01$; n = 6; Fig. 4). A potential difference between saline and PTX+GABA application ($p = 0.061$) and saline and PTX application ($p = 0.055$) emerged, but there were no differences ($p < 0.1$) for any other conditions (n = 6; Fig. 4).

Tukey's post-hoc tests for burst frequency indicated a difference only between PTX application and PTX+GABA application ($p = 0.037$) but no other differences ($p < 0.1$) for any other conditions (n = 6; Fig. 4).

Glutamate

Data from saline, glutamate, and glutamate+PTX applications were analyzed using one-way RM-ANOVAs to determine the effects of PTX on the observed GABA response. Glutamate application generally increased burst duration and duty cycle but not frequency (Fig. 5, 6). I

observed a significant main effect of subject for each of burst duration ($p < 0.0001$), duty cycle ($p < 0.0001$), and burst frequency ($p = 0.0001$), and a significant main effect of application condition for burst duration ($p = 0.0007$) and duty cycle ($p = 0.0006$) but not burst frequency, which has a potential main effect ($p < 0.0598$; n=10; Fig. 6).

Tukey's post-hoc tests for burst duration indicated significant differences in saline and glutamate application ($p = 0.024$), wash and glutamate application ($p = 0.026$), glutamate application and PTX application ($p = 0.015$), wash and PTX+glutamate application ($p = 0.015$), and PTX application and PTX+glutamate application ($p = 0.007$; n = 10; Fig. 6). There were no other differences ($p < 0.1$) for any other conditions (Fig. 6).

Tukey's post-hoc tests for duty cycle indicated differences in saline and glutamate application ($p = 0.008$), wash and glutamate application conditions ($p = 0.012$), glutamate application and PTX application conditions ($p = 0.009$), wash and PTX+glutamate application conditions ($p = 0.013$), and PTX application and PTX+GABA application conditions ($p = 0.008$; n = 10; Fig. 6). Multiple comparisons for burst duration indicated no other significant or potentially significant comparisons ($p < 0.1$) for any other conditions (see Figure 6).

Tukey's post-hoc tests for burst frequency indicated no differences ($p < 0.01$) for any conditions (n = 10; Fig. 6).

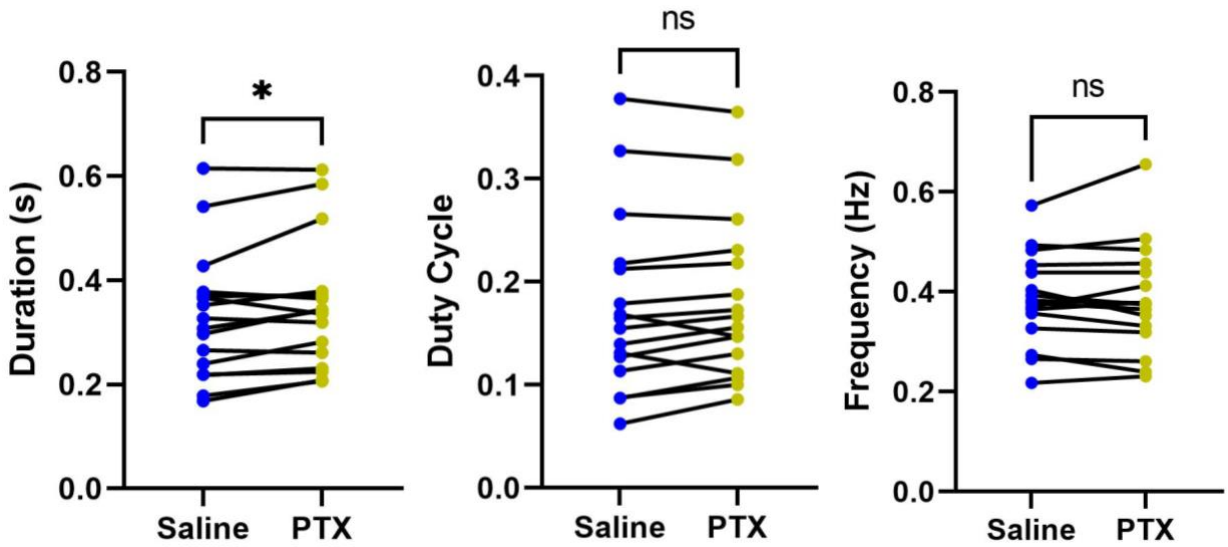

Figure 2. Within-subject CG burst measurements during PTX application compared with saline control combined for all GABA and glutamate exposed subjects. PTX and saline groups were compared using within-subjects paired t-test. PTX caused a change to burst duration when applied to the CG but not to duty cycle or burst frequency. This change in burst duration was only significant in GABA-exposed subjects (Fig. 4) and not those exposed to glutamate (Fig. 6), but still emerged when analyzed as a collective. Burst duration $p = 0.0208$, duty cycle $p = 0.1495$, burst frequency $p = 0.9374$, $n = 16$.

Figure 3. Example trace of extracellularly recorded bursts before and during GABA application. Burst duration and duty cycle decreased during application of GABA at $5x10^{-5}$M. Burst frequency also appeared to decrease during application of glutamate in this recording, however that was not consistent between preparations, and some increased in frequency or did not change.

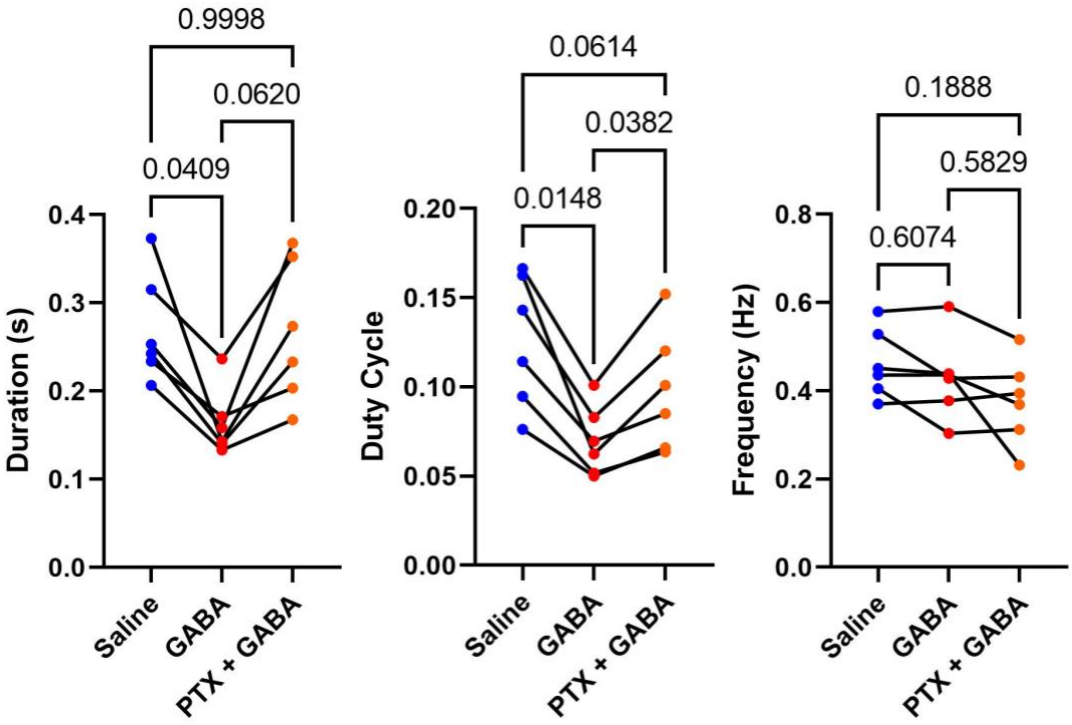

Figure 4. Inhibitory GABA responses in the CG compared with saline control and PTX application with GABA. Data were analyzed using within-subjects one-way repeated measures ANOVA followed by within-subjects Tukey's multiple-comparisons test. Graphs show within-subject changes in burst duration, duty cycle, and frequency for applications of GABA and PTX+GABA compared to saline and include *p* values from the multiple-comparisons test comparing each condition. n=6.

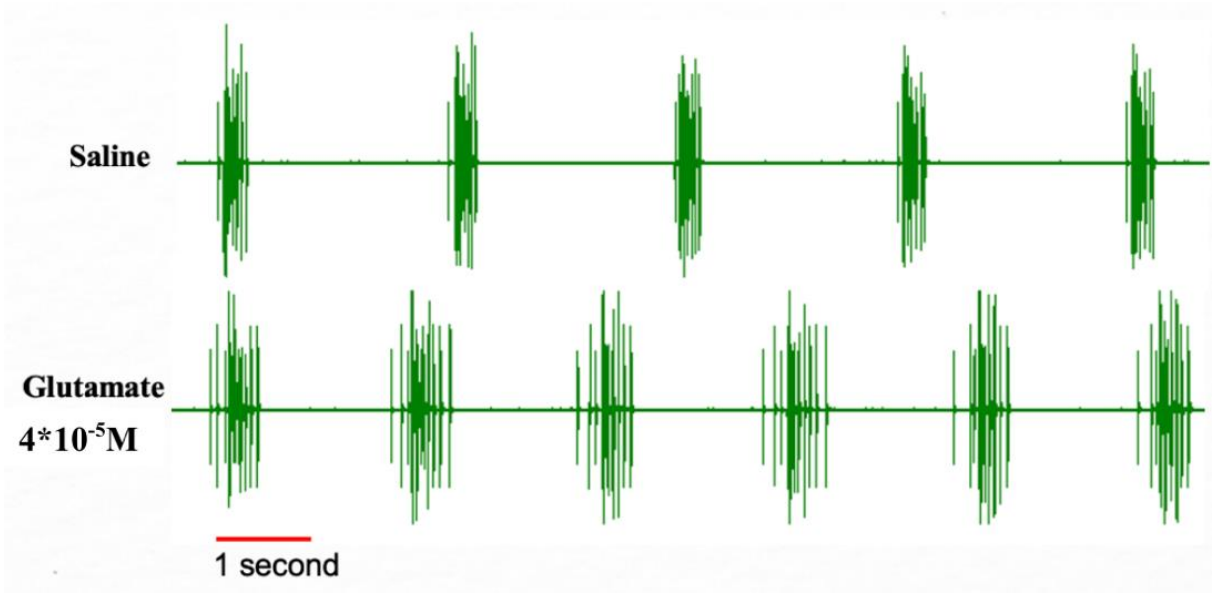

Figure 5. Example trace of extracellularly recorded bursts before and during glutamate application. Burst duration and duty cycle increased during application of glutamate at $4x10^{-5}$M. Burst frequency also appeared to increase during application of glutamate in this recording, however, that was not consistent between preparations, and some decreased in frequency or did not change.

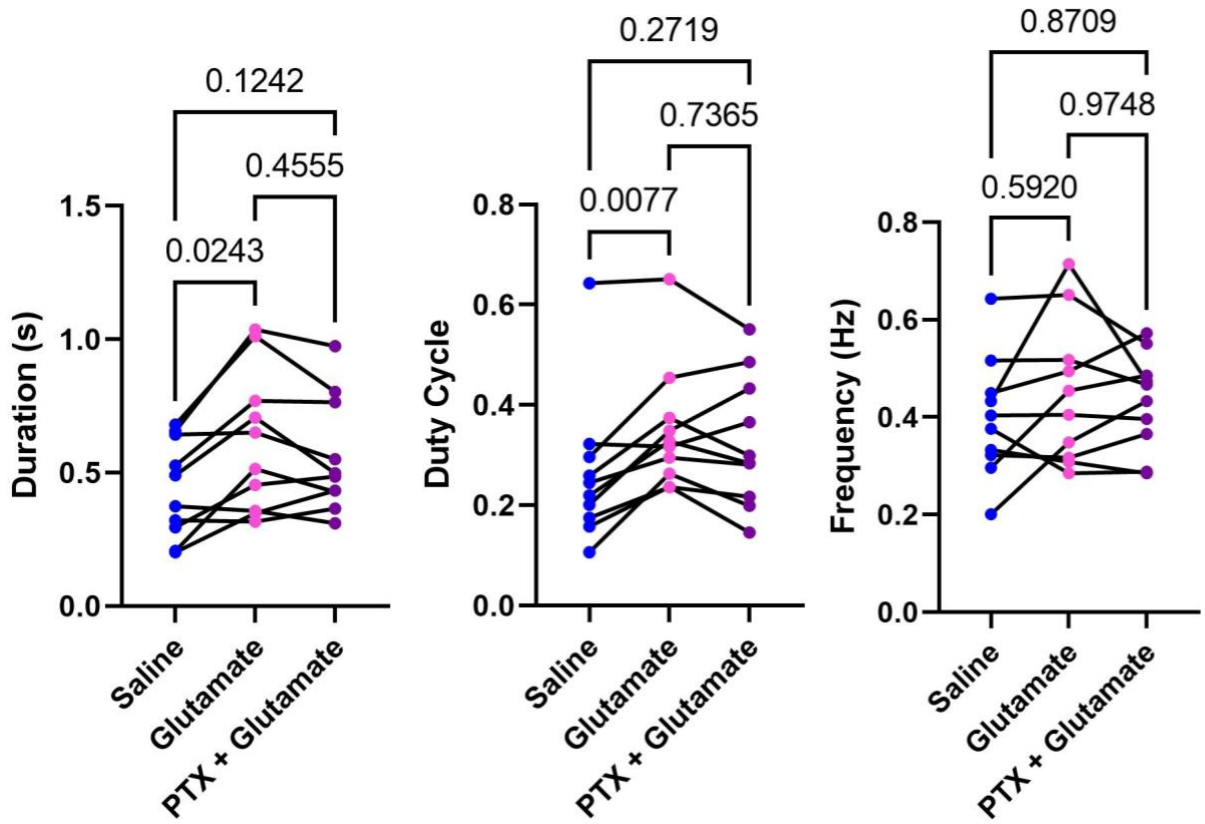

Figure 6. The glutamate response with and without PTX application. Data were analyzed using within-subjects one-way repeated measures ANOVA followed by within-subjects Tukey's multiple-comparisons test. Graphs show within-subject changes in burst duration, duty cycle, and frequency for applications of glutamate and PTX+glutamate compared to saline and include *p* values from the multiple-comparisons test comparing each condition. n=10.

Discussion

The current study examined the effects of PTX on both the GABA and glutamate responses of the isolated lobster CG. This study aimed to expand the investigation in which effects of PTX were found on the GABA response by Kerrison & Freschi (1992) to also include co-application of PTX with glutamate. The first aim of the study was to apply PTX at concentrations which did not alter CG bursting from baseline values in burst duration, burst frequency, and duty cycle at the level of the isolated CG. This aim was not successful. For GABA application experiments, PTX increased duty cycle from wash conditions and potentially increased burst duration (Fig. 4). When analyzed together, GABA and glutamate experiment data indicated significant effects of PTX on altering burst duration (Fig. 2). However, for glutamate experiments examined alone, PTX had no effect on CG bursting by any measure (Fig. 6). Differences in PTX action between GABA and glutamate experiments without either neurotransmitter present may be explained by a prolonged effect of GABA application which may have failed to wash out. This difference may also be explained by a desensitization during glutamate application which extended into PTX application periods and prevented any effects of PTX from being observed during that condition. This aim was predicted to be achieved, as it done in a past study on cardiac muscle fibers, another component of the cardiac system (Shank et al., 1974). The gap between the previous studies examining the function of PTX at the muscle fibers of the cardiac system and at the isolated CG remains, as this study was unable to conclude that PTX has no effects on the CG (Shank et al., 1974; Kerrison & Freschi, 1992). In the study of muscle fibers which succeeded in this aim, Shank et al. (1974) utilized intracellular recording, indicating a potential difference which may also have resulted in this difference. While the aim was not achieved as expected, PTX still had effects to suppress GABAergic inhibition of CG

burst duration and duty cycle which were substantial enough to be distinct from GABA application without PTX.

The second aim of the present study was to reaffirm the effects of PTX application in conjunction with GABA that were established by past studies in the isolated CG (Kerrison & Freschi, 1992). I hypothesized that PTX would suppress the inhibitory response of the CG to application of GABA. The results of the present study supported this hypothesis (Fig. 4). PTX application with GABA significantly increased both burst duration and duty cycle as compared to application of GABA alone (Fig. 4). PTX application appeared to entirely block GABAergic decreasing of burst duration and return it to levels in saline (Fig. 4). However, PTX only appeared to partially block the GABAergic inhibition in duty cycle, as there was a potential difference between saline and PTX+GABA (p=0.061; Fig. 4).

The second aim also involved introducing the application of PTX with glutamate. In line with the hypothesis, the results showed no significant effects of PTX in suppressing or enhancing the response of the CG to application of glutamate (Fig. 6). This result suggests that there may not be PTX-sensitive glutamate receptors in the CG, or they may be few enough to have no visible effects. Had there been a moderate concentration of PTX-sensitive inhibitory glutamate channels, burst duration and duty cycle would have likely increased further during PTX application. We know that excitatory glutamatergic channels which are not PTX sensitive are present in the CG (Cooke, 2002). Had there also been inhibitory glutamatergic channels present that are PTX sensitive, then any inhibitory effect they contribute during glutamate application would be blocked with co-application of PTX. This would appear as a consistent increase in burst duration and duty cycle during the PTX+glutamate, even greater than that during glutamate application without PTX.

One must also consider that the present study has several limitations that may be addressed by future studies or continuations of the present study. First, PTX was not able to be established as having no effects at the level of the isolated CG when applied without exogenous GABA. This study also examined the CG only through extracellular recordings. Implementation of intracellular recording techniques could prove greatly beneficial to understanding more specific dimensions of the changes caused by GABA, glutamate, and PTX along with in combination, to the action of motor neurons in the CG. Further, a characterization of gene expression specifically from the neurons in the CG would allow for a much better understanding of the presence of IGluRs in this system and whether they are absent or present at low densities. Past literature examining general heart tissue–which included the CG and surrounding muscle and connective tissue–found an upregulation in the expression of GluK3, a kainite family glutamate receptor which has been characterized as functionally part of the IGluR family, in heart tissues as compared to abdominal ganglia (McGrath et al., 2016; Kumari et al., 2019). In this study however, GluK3 was not identified as a glutamate-gated Cl^- channel, which were separately identified as having upregulated expression in abdominal ganglia (McGrath et al., 2016). This study suggests the strong possibility that if IGluRs are present their broad effects may be less visible due to the primary role of glutamate as excitatory in the CG.

To conclude, the current study examined the effects of PTX on altering responses of the lobster CG to GABA and glutamate. The results of the present study supported understandings of PTX as suppressing the CG's inhibitory responses to GABA application and did not indicate any significant effects of PTX on altering glutamate's action at the CG. These suggest potential for PTX use in examining the CG through blocking GABA, differing from its use in the STG.

References

Albert, J., Lingle, C. J., Marder, E., & O'Neil, M. B. (1986). A GABA-activated chloride-conductance not blocked by picrotoxin on spiny lobster neuromuscular preparations. *British Journal of Pharmacology*, *87*(4), 771–779. doi: 10.1111/j.1476-5381.1986.tb14596.x

Alexandrowicz, J. S. (1932). Memoirs: The Innervation of the heart of the crustacea. I. decapoda. *Journal of Cell Science*, *s2-75*(298), 181–249. doi: 10.1242/jcs.s2-75.298.181

Bidaut, M. (1980). Pharmacological dissection of pyloric network of the lobster stomatogastric ganglion using picrotoxin. *Journal of Neurophysiology*, *44*(6), 1089–1101. doi: 10.1152/jn.1980.44.6.1089

Bucher, D., Johnson, C. D., & Marder, E. (2007). Neuronal morphology and neuropil structure in the stomatogastric ganglion of the lobster, *Homarus americanus*. *The Journal of Comparative Neurology*, *501*(2), 185–205. doi: 10.1002/cne.21169

Cleland, T. A. (1996). Inhibitory glutamate receptor channels. *Molecular Neurobiology*, *13*(2), 97–136. doi: 10.1007/bf02740637

Cleland, T. A. (1997). Inhibitory glutamate receptor channels: Characterization and modulation in cultured lobster stomatogastric neurons. *Dissertation at University of California, San Diego*.

Cleland, T. A., & Selverston, A. I. (1995). Glutamate-gated inhibitory currents of central pattern generator neurons in the lobster stomatogastric ganglion. *The Journal of Neuroscience*, *15*(10), 6631–6639. doi: 10.1523/jneurosci.15-10-06631.1995

Cleland, T. A., & Selverston, A. I. (1998). Inhibitory glutamate receptor channels in cultured lobster stomatogastric neurons. *Journal of Neurophysiology*, *79*(6), 3189–3196. doi: 10.1152/jn.1998.79.6.3189

Constanti, A. (1978). The "mixed" effect of picrotoxin on the GABA dose/conductance relation recorded from lobster muscle. *Neuropharmacology*, *17*(3), 159–167. doi: 10.1016/0028-3908(78)90095-3

Cooke, I. M. (2002). Reliable, responsive pacemaking and pattern generation with minimal cell numbers: The crustacean cardiac ganglion. *The Biological Bulletin*, *202*(2), 108–136. doi: 10.2307/1543649

Dando, M. R. (1969). Studies on the structure and function of mechanoreceptors in the stomatogastric nervous system of some decapoda crustacea. *Dissertation at Gatty Marine Laboratory and Department of Natural History. University of St. Andrews*.

Delgado, J. Y., Oyola, E., & Miller, M. W. (2000). Localization of GABA- and glutamate-like immunoreactivity in the cardiac ganglion of the lobster *Panulirus argus*. *Journal of Neurocytology*, *29*(8), 605–619. doi: 10.1023/a:1011080304472

Hartline, D. K. (1979). Integrative neurophysiology of the lobster cardiac ganglion. *American Zoologist*, *19*(1), 53–65. doi: 10.1093/icb/19.1.53

Katz, P. S. (2016). Evolution of central pattern generators and rhythmic behaviours. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1685), 20150057. doi: 10.1098/rstb.2015.0057

Kerrison, J., & Freschi, J. E. (1992). The effects of γ-aminobutyric acid on voltage-clamped motoneurons of the lobster cardiac ganglion. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, *101*(2), 227–233. doi: 10.1016/0742-8413(92)90265-9

Kumari, J., Vinnakota, R., & Kumar, J. (2019). Structural and functional insights into GLUK3-kainate receptor desensitization and recovery. *Scientific Reports*, *9*(1). doi: 10.1038/s41598-019-46770-z

Marder, E., & Bucher, D. (2007). Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. *Annual Review of Physiology*, *69*(1), 291–316. doi: 10.1146/annurev.physiol.69.031905.161516

Marder, E., & Paupardin-Tritsch, D. (1978). The pharmacological properties of some crustacean neuronal acetylcholine, gamma-aminobutyric acid, and L-glutamate responses. *The Journal of Physiology*, *280*(1), 213–236. doi: 10.1113/jphysiol.1978.sp012381

McGrath, L. L., Vollmer, S. V., Kaluziak, S. T., & Ayers, J. (2016). De novo transcriptome assembly for the lobster *Homarus americanus* and characterization of differential gene expression across nervous system tissues. *BMC Genomics*, *17*(1). doi: 10.1186/s12864-016-2373-3

National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 31304, Picrotoxin. Retrieved December 1, 2022 from https://pubchem.ncbi.nlm.nih.gov/compound/Picrotoxin.

Olsen, R. W. (2006). Picrotoxin-like channel blockers of GABA a receptors. *Proceedings of the National Academy of Sciences*, *103*(16), 6081–6082. doi: 10.1073/pnas.0601121103

Selverston, A. (2009). Neural mechanisms underlying the generation of the Lobster Gastric Mill Motor Pattern. *Frontiers in Neural Circuits*, *3*. doi: 10.3389/neuro.04.012.2009

Selverston, A., Elson, R., Rabinovich, M., Huerta, R., & Abarbanel, H. (1998). Basic principles for generating motor output in the stomatogastric ganglion. *Annals of the New York Academy of Sciences*, *860*(1), 35–50. doi: 10.1111/j.1749-6632.1998.tb09037.x

Shank, R. P., Pong, S. F., Freeman, A. R., & Graham, L. T. (1974). Bicuculline and picrotoxin as antagonists of γ-aminobutyrate and neuromuscular inhibition in the lobster. *Brain Research*, *72*(1), 71–78. doi: 10.1016/0006-8993(74)90651-9

Appendix A: Expanded Figures for GABA and Glutamate ANOVA Analysis

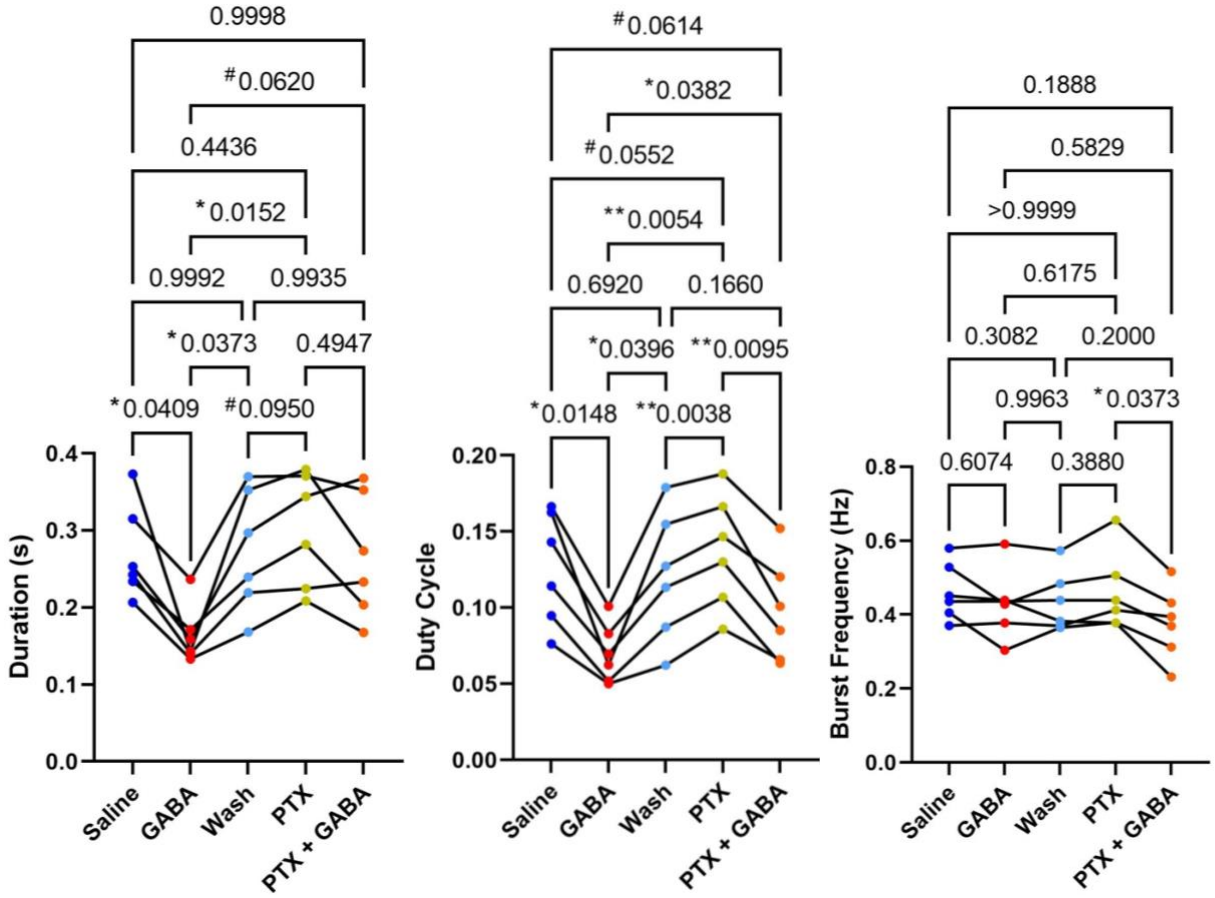

Figure 7. Expansion of Figure 4 to include all conditions. $p < 0.05$ indicated by *, $p < 0.01$ indicated by **, $p < 0.1$ indicated by #, n = 6.

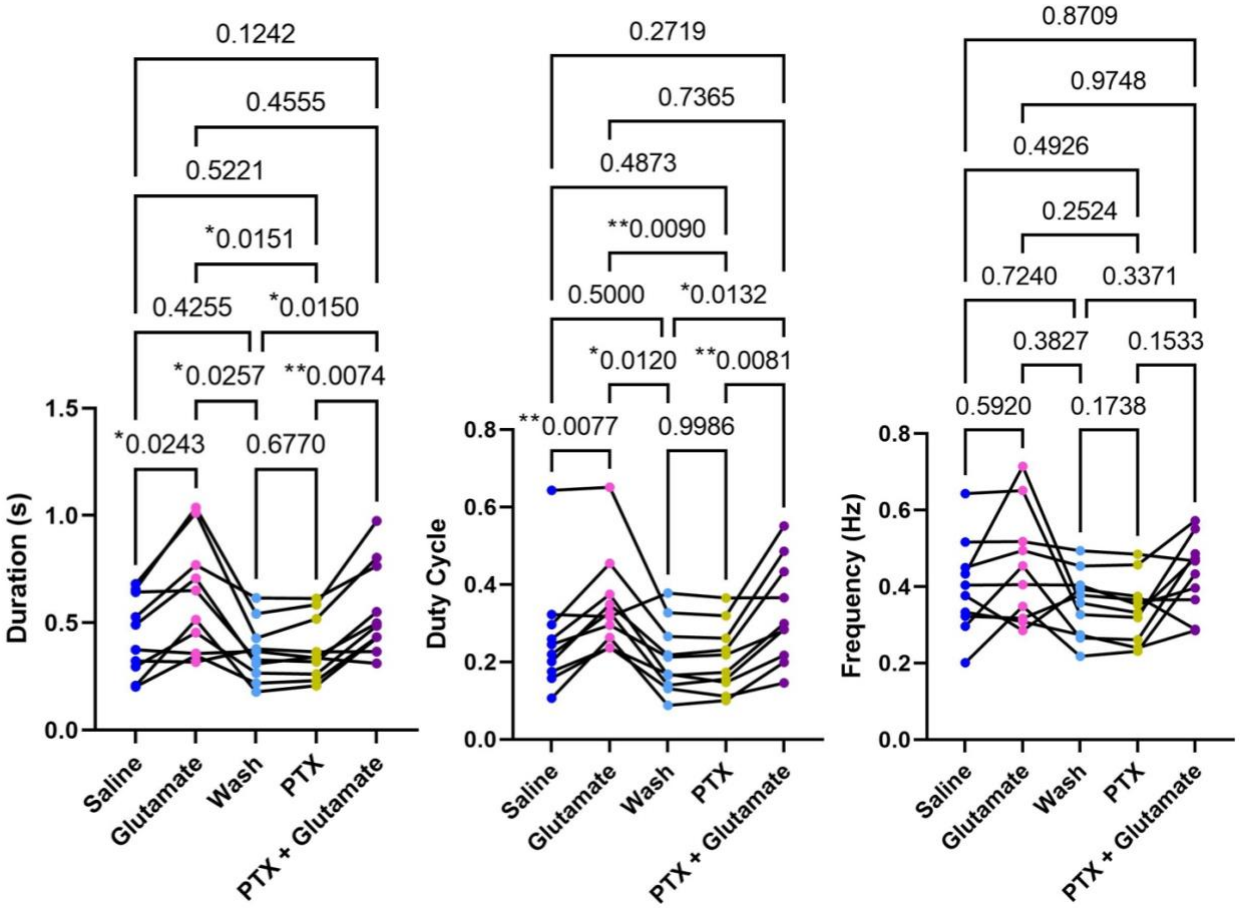

Figure 8. Expansion of Figure 6 to include all conditions. $p < 0.05$ indicated by *, $p < 0.01$ indicated by **, n = 10.