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EFFECTS OF GABAB LIGANDS ON THE GSH-INDUCED ELECTRICAL ACTIVITY OF THE HYPOSTOME IN HYDRA

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EFFECTS OF $GABA_B$ LIGANDS ON THE GSH-INDUCED ELECTRICAL ACTIVITY OF THE HYPOSTOME IN HYDRA

BY

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ABSTRACT

Reduced glutathione, GSH, artificially induces the signature feeding behavior in the early-evolved metazoan, Hydra *vulgaris*. Evidence has shown that the mouth opening response is prolonged by the inhibitory neurotransmitter, GABA. By making extracellular recordings of a detached reduced-tentacle hypostome, it is possible to record the electrical activity produced by GSH and to observe the effects of the inhibitory neurotransmitter, gamma-amino-butyric acid (GABA), the $GABA_B$ agonist (baclofen) and the $GABA_B$ antagonist, (phaclofen).

When an electrode is placed on the mouth of the hypostome, thus blocking the mouth opening, and the ligands are placed in the bath surrounding the base of the hypostome, the following effects are observed: GSH increased small-uncorrelated hypostomal pulses (SUHPs), medium-uncorrelated hypostomal pulses (MUHPs), pacemaker bursting pulses (PBPs) and pulses per pacemaker bursting pulse (P/PBPs). Although GABA per se produced no effect when administered with GSH, baclofen caused an increase in SUHPs, while phaclofen per se caused a decrease; coadministration of baclofen and phaclofen mutually cancelled their individual effects. This suggests that at least some of the SUHPs might be GSH neuronal impulses having metabotropic ($GABA_B$) receptor involvement. GSH coadministered with baclofen and phaclofen caused a decrease in MUHPs and rhythmic potentials (RPs); GABA administered with GSH produced no effect on MUHPs and RPs.

When the ligands were placed within the pipette at the mouth (exposing the mouth opening to ligands and blocking the proximal portion of the hypostome), the following effects were observed: GSH increased MUHPs and decreased extra-large

uncorrelated hypostomal pulses (XLUHPs) and P/PBPs; this comports with the previously observed GSH induced cone-formation of the hypostome, now hypothesized to be reflected in the increase MUHPs (which may be muscle pulses) and the concurrent inhibition of body contraction (considered to be mediated by XLUHPs and PBPs). This effect was abolished by GABA, which increased the frequency of the large pulses, but not mimicked by baclofen nor counteracted by phaclofen, both of which also decreased in the large pulses. This suggests that GABA inhibition of GSH activity might also involve the action of GABA on its ionotropic receptors and that $GABA_B$ receptors exist on the excitatory effector circuits. GSH administered with baclofen caused a decrease in SUHPs.

In general, GSH administered alone, GSH and GABA, GSH and phaclofen, GSH and baclofen and GSH coadministered with baclofen and phaclofen caused significantly increased activity when applied directly to the apex of the hypostome, indicating that both GSH and $GABA_B$ receptors are concentrated in or around the hypostomal apex.

Although GABA combined with GSH produced no significant differences in the frequency of any of the parameters measured in the bath-applied method, coadministration increased LUHPs, XLUHPs, PBPs and RPs in the pipette-applied method—suggesting prolongation of mouth opening. The results support the behavioral observations that GABA inhibits the cessation of the GSH-induced feeding response and indicates that GSH and GABA receptors are differentially distributed in the hypostome.

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PREFACE

This thesis is being submitted in manuscript format. It is composed of one manuscript and one appendix. The title of the manuscript is "Effect of $GABA_B$ ligands on the GSH-induced electrical activity of the hypostome in hydra." The manuscript is prepared for submission to Comparative Biochemistry and Physiology A.

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"EFFECTS OF $GABA_B$ RECEPTOR LIGANDS ON THE GSH-INDUCED ELECTRICAL ACTIVITY OF THE HYPOSTOME IN HYDRA"

BY

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EFFECTS OF $GABA_B$ RECEPTOR LIGANDS ON THE GSH-INDUCED ELECTRICAL ACTIVITY OF THE HYPOSTOME IN HYDRA

INTRODUCTION

Hydra is an early-evolved metazoan found in small lakes and ponds, and is considered the quintessential example of an animal with a simple nervous system. Hydra's long, cylindrical body column has two main body layers consisting of an ectoderm and endoderm separated by a gel-like mesoglea. Distributed along the ectoderm lays a simple nervous system composed of interconnecting, synapsing neurons (Hadzi, 1909; Koizumi, 2007; Kinnamon and Westfall, 1981). The two body layers meet at the apex of the mouth surrounded by a whorl of tentacles amid specialized stinging cells called cnidocytes—used for capturing prey. Its feeding behavior consists of tentacle writhing, longitudinal body contractions, and mouth opening/closing. Nonetheless, the neuronal mechanisms controlling the patterned behavior have not been fully described.

Numerous sensory cells are involved in hydra's feeding behavior. One of the most intriguing physiological phenomena is the chemical induction of a complex feeding pattern of behavior in the fresh water polyp, Hydra *vulgaris* by GSH (Loomis, 1955). The artificially induced GSH feeding behavior of hydra is a well-defined quantifiable mechanism and is one of the most familiar chemosensory behaviors to date. Specifically, used to study the dynamics of receptor binding (Lenhoff and Bovaird, 1961) and the behavioral physiology of a ligand-induced feeding behavior. After piercing its prey (with cnidocytes on hydra's tentacles), the

captured releases the tripeptide glutathione (GSH). Tentacle writhing, mouth (hypostome) opening, and body contractions result and are the key features of this synchronized behavior (Loomis and Lenhoff, 1956; Lenhoff et al., 1961; Bellis et al., 1992; Grosvenor et al., 1996; Pierobon et al., 1995; Kass-Simon et al., 2003). The hypostome maximally expands to accompany the size of homogenate and the prey is ingested along the endoderm-lined gut. Eventually, hydra regurgitates the quarry and closes the mouth; the feeding behavior lasts approximately 30 minutes. The signature role and specific function of receptors and organelles involved during a centrally correlated behavior (such as the ability to capture, ingest, and regurgitate prey) has yet to be understood; it is important to identify the existence and the behaviorally-correlated output of these receptors and organelles in hydra's feeding.

Experiments to localize the GSH receptors have been carried out by many investigators. After approximately one-two minutes of GSH exposure, the mouth will rapidly open and remain open until an inhibitory stimulus is initiated. The feeding response is quickly terminated by the removal of GSH and application of KCl and veratridine (Pierobon et al., 2004). The GSH-induced feeding behavior is also antagonized by L-glutamic acid (Lenhoff and Bovaird, 1961). Homogenized cnidocyte-fractions of hydra tentacles with radiolabeled glutamate inhibited GSH binding (Venturini, 1987) and it was believed that glutamate was a competitive inhibitor of GSH binding at the GSH receptor site. However, other studies showed that glutamate had bound to its own receptors and that GSH was still binding to its receptor site (Bellis et al., 1991; Grosvenor et al., 1992). Thus, there may be a site on the glutamate receptor, specifically for GSH binding.

Neuronal gap junctions indicated the first ultrastructural evidence of electrical synapses in Hydra's nervous system and more frequently occurring, chemical synapses produced by the same neuron in the hypostome (Westfall et al., 1980). These chemical and electrical synapses are similar to indirect and direct, interneuronal communication between neurons in the brain (Meier and Dermietzel, 2006). Synaptic connectivity between the hypostome and the tentacles is due in part to multiple neuronal clusters found between the hypostome-tentacle junction (Kinnamon and Westfall, 1982)—similar to ganglia found in the mammalian nervous system. Chemical synapses and gap junctions between neurons of the hypostome and tentacle junction may be involved in eliciting the feeding behavior from mouth opening to tentacle writhing (Kinnamon and Westfall, 1982). The simultaneous opening of the mouth and tentacle writhing is a signature behavior that may be under specific neuronal control. Kass-Simon (1972) placed electrodes just near the tentacles and the original electrical findings indicated that there were impulse initiation sites at the base of the tentacles. Thus, the newly observed proximal nerve net at the base of the hypostome and the distal nerve net at the apex of the hypostome may be involved in coordinating hydra's feeding response (Hufnagel and Kass-Simon, unpublished).

Evidence of chemoreception, elicited by hydra's response to GSH, can be found when hydra is exposed to concentrations ranging from high nanomolar to low micromolar of GSH (Lenhoff, 1961; Bellis et al., 1992). A quantitative assay of mouth opening duration (Lenhoff, 1961) led to characterization of the glutathione chemoreceptor; the GSH-induced feeding response (Pierobon et al., 1995) was quantified by duration of mouth opening that lasted 10 minutes with1 μm GSH.

Maximal duration of mouth opening occurs at 5 μM GSH with a 50% response at 1 μM GSH (Grosvenor et al., 1996). After the 30-minute time lapse of hydra's feeding behavior, the mouth will slowly close. However, evidence has shown that the major invertebrate inhibitory neurotransmitter, gamma-immuno butyric acid (GABA) at 100 μM, prolonged the duration of the response in which the time for the mouth to close was increased (Pierobon et al., 1995). In addition, the major excitatory neurotransmitter in the mammalian nervous system, glutamate has been shown to be involved in this coordinated effect by increasing tentacle activity in the tentacle pulse pacemaker system (TPs) (Kay and Kass-Simon, 2008); the GSH-induced feeding behavior is dose dependent, saturable, and antagonized by L-glutamic acid (Lenhoff and Bovaird, 1961; Bellis et al., 1991).

The hypostome (mouth) plays a signature role in executing this behavior. Numerous sensory nerve cells surround the dome of the hypostome and the question that has yet to be answered is what do these nerves do to open and close the mouth? Labeling with L96+ antibody has indicated a specialized endodermal tissue type separating the ectoderm from the endoderm in this specialized structure (Technau et al., 1995). The hypostome's ability to extend considerably during feeding behavior without tearing is due to this one-cell thick ring of endodermal tissue between the ectodermal and endodermal lining of the mouth (Technau et al., 1995). Scanning electron microscopy of the internal lining of the hypostome has revealed that it has endodermal cylindrical microvilli along the inside of the hypostome with protruding flagella and microvilli extending towards the hypostomal, tentacle region (Wood, 1979). The microvilli in addition to the mucous producing endoderm along the inside

lining of the mouth may be chemoreceptive sites that initiate chemically mediated behaviors (Kass-Simon and Hufnagel, 1992; Slautterback, 1967). In addition, an even distribution of multiple synpases between epitheliomuscular cells and neurons were found in the region between the hypostome and the tentacle area in the oral epidermis (ectoderm)—suggesting delicate muscular control of the mouth opening/closing behavior and its ability to engulf prey (Kinnamon and Westfall, 1982).

Previous studies identified a circular nerve ring surrounding the hypostome (Westfall et al., 1974; Grimmelikhuijzen et al., 1985; Koizumi et al., 1992). However, recent evidence has identified two centralized nerve rings found within the hypostome—the proximal and distal nerve rings of the ectodermal layer representing a simplified model of the mammalian brain; they are connected to one another by radially anastomosing neurons (Hufnagel and Kass-Simon, unpublished). The proximal nerve ring has been identified to run between, and slightly below the tentacles (Hufnagel and Kass-Simon, unpublished) and is presumed responsible for the body-contraction pacemaker impulses (Passano and McCullough, 1964; Kass-Simon, 1972, 1973). The proximal nerve ring receives neuronal and behaviorally-correlated input from impulses arising in the tentacle pacemaker conducting system (Rushforth and Burke, 1971; Kass-Simon, 1972, 1973; Hufnagel et al., 2009). There is also recent evidence of an anti-$GABA_B$ receptor antibody labeling of the proximal nerve ring suggesting the existence of $GABA_B$ receptor proteins occurring in Hydra (Kass-Simon and Hufnagel, unpublished). Although the newly observed distal nerve ring, located at the tip of the hypostome, is a loosely organized ring of interconnecting neurons and is hypothesized to be responsible for coordinating hydra's feeding

response—has not been found to label with anti-$GABA_B$ receptor antibody (Hufnagel and Kass-Simon, unpublished). However, labeling of the endodermal layer of the hypostome with anti-$GABA_B$ receptor antibody suggests possible involvement in hydra's mouth opening and closing behavior during feeding (Hufnagel and Kass-Simon, unpublished).

Three main endogenous pacemaker systems work together to control the behavior of Hydra—the ectodermal contraction burst system (CBs)—located in between and just below the tentacles (Passano and McCullough, 1963, 1964), the tentacle pulse system (TP)—located in the proximal part of each tentacle (Rushforth and Burke, 1971; Kass-Simon, 1972, 1973), and the endodermal rhythmic potential system—located near the base of the hydra (Passano and McCullough, 1962; Kass-Simon and Passano, 1978).

During the initial stages of feeding behavior, Hydra's tentacles writhe together. In the presence of 10μM GSH in whole tentacle preparations, recordings from the tentacles revealed that GSH inhibits the tentacle contraction pulse (TCP) system and induces monophasic pulses. These pulses are suggestive of the characteristic writhing movement of tentacles observable during feeding behavior (Rushforth and Burke, 1971). The TCP system produces bursts similar to that of the contraction burst system and sometimes precede contraction burst pulses; the interpulse interval within a burst of pulses decreases and then slowly increases. GABA and glutamate receptors are also involved in modulating pacemaker activity in hydra (Kass-Simon et al., 2003). Initial post-feeding behavior results in an increased frequency of tentacle pulses and contraction bursts (Grosvenor et al., 1996). However, GABA alone decreases the

number of contraction bursts (CBs) and pulses per pacemaker burst (P/PBP) among the ectoderm and rhythmic potentials (RPs) among the endoderm; GABA does not affect the tentacle pacemaker system. The contraction burst system is conducted through the body column and around the hypostome—resulting in a burst of pulses parallel with a shortening of the body column and tentacular contractions (Kass-Simon, 1972, 1973). The rhythmic potential system produces pulses that are frequently not identifiable with any overt behavior of hydra although they increase in frequency when the animal elongates. They are conducted in a regular pattern, on the endoderm (Kass-Simon and Passano, 1978; Kass-Simon et al., 2003).

Multiple endogenous neurotransmitters have been discovered in hydra and may be involved in the modulation of such an effect. Strychnine-sensitive glycine receptors (glyRs) occur in hydra's tissues and activation of these glyRs cause increased prolongation to the GSH-induced feeding response. Glutamate, the major excitatory neurotransmitter in the mammalian nervous system, has also been reported in hydra's tissues. In particular, biochemical and immunohistochemical studies have identified the existence of GABA in hydra's tissues. Pierobon *et al.* (1995) and Concas *et al.* (1998), report high affinity specific binding of radiolabeled GABA to hydra membranes—binding was displaced by the $GABA_A$ agonist, muscimol. Specifically, co-application of 1 μM GABA and 100 nM pentobarbital ($GABA_A$-receptor modulator) to hydras caused a significant increase in the response to feeding behavior (Pierobon et al., 2004)—suggesting that $GABA_A$ receptors may be involved in the prolongation of hydra's feeding behavior.

Widely expressed in the human body, GABA is involved in numerous neurological and psychiatric functions. Studies on membrane preparation from rat brain using selective drugs in pharmacology have identified at least two distinct classes of GABA receptor—$GABA_A$ and $GABA_B$—differing substantially in electrophysiological properties (Olsen et al., 1999). The $GABA_A$ receptor complex contains an integral Cl^- ionophore, whereas $GABA_B$ receptors couple to Ca^{2+} and K^+ channels via GTP-binding proteins (Bormann, 1988).

If GABA is involved in prolonging the duration of the response in which the time for the mouth to close was increased, the question that needs to be answered is what are the specific receptors involved in controlling this behavior? Electrophysiological evidence demonstrates that GABA and glutamate differentially affect hydra's pacemaker systems and appear to do so by acting upon their respective ionotropic receptors. Kass-Simon *et al*. (2003) report strong evidence that GABA's effects on the endodermal pacemaker systems are inhibitory, while glutamate's effects are excitatory; this evidence is consistent with the assigned roles of glutamate and GABA in other systems—giving support for classical receptor-mediated amino-acid transmission. Evidence exists supporting the inhibitory effect of GABA by prolonging the GSH-induced mouth opening during feeding behavior (Pierobon et al., 1995). Electrophysiological studies have shown that agonists and antagonists to GABA affect the electrical activity in hydra— $GABA_A$ agonists decreased the number of contraction bursts and rhythmic potentials; GABA antagonists caused an increase in the frequency of rhythmic potentials and the number of pulses per contraction burst (Kass-Simon and Pannaccione, unpublished;

Kass-Simon et al., 2003). There is also electrophysiology evidence showing the role of NMDA and $GABA_B$ receptors involved in controlling nematocyst discharge in hydra (Scappaticci and Kass-Simon, 2008). Nematocyst discharge was increased with application of baclofen ($GABA_B$ agonist) and counteracted with phaclofen ($GABA_B$ antagonist)—suggesting possible modulation of other chemosensory behaviors within hydra.

A central problem concerning hydra's feeding response is the question of whether $GABA_B$ receptors might be involved in orchestrating the GSH induced feeding behavior. The main question addressed in the present study is what is the role of $GABA_B$ receptors in modulating the GSH electrical activity. In order to determine the role of $GABA_B$ on the GSH induced impulses, $GABA_B$ agonists and antagonists combined with GSH were used during electrical recording exploiting the proximal and distal nerve rings of hydra—the bath applied method and the pipette filled method, respectively. The experiments were carried out on isolated, reduced-tentacle hypostomes.

MATERIALS AND METHODS

I. Animals

Hydra vulgaris, raised at 18 ± 1.0°C in bicarbonate versene culture solution (BVC) consisting of $1x10^{-7}$M $NaHCO_3$, $1x10^{-6}$M $CaCl_2$, $1x10^{-8}$M EDTA (Loomis and Lenhoff, 1956) at a pH between 6.8-7.2 were selected at random, 24 ± 2 hours after having been fed with brine shrimp *ad liberatum*. Hydra exhibit increased contractile behaviors after having been fed (Passano and McCullough, 1964; Grosvenor et al., 1996) and thus were consistently selected, prepped and used for recordings at the allotted time. Hydra heads and tentacles were ablated from the body of the experimental animals; tentacles were allowed to fully relax to maximal expansion and were carefully cut below the tentacle insertion region, taking care to leave intact the contraction burst pacemaker region located at the origin of the tentacle insertion site; the excised heads were allowed to heal for 24 ± 2 hours before electrical recordings (Figure 1)—small regenerated tentacle buds (not exceeding 1 mm) were evident at time of recording (Figure 2).

II. Recording Methods

Electrical recordings were conducted at 22 ± 2.0°C, under red light on a low setting (Dolan-Jenner Industries, Inc. Fiber-Lite 190 Lamp with a red filter). The light was turned on before the start of recording. Earlier work had indicated that red light did not affect the pacemaker-controlled behavior of hydra (Passano and McCullough, 1962; 1964) and that hydras were unresponsive to red light (Wilson, 1891; Haug, 1933). However, recent evidence in our laboratory indicated that tentacles are sensitive to red light—increasing the frequency of their contractions relative to

darkness (Guertin and Kass-Simon, 2015). Nonetheless, since all of the present experiments were conducted in constant red light, light exposure would not have affected our experimental results.

The electrical recording protocol was modified from the procedures of Passano and McCullough, 1964, Kass-Simon et al., 2003, Ruggieri et al., 2004, Kay and Kass-Simon, 2009. Extracellular recordings were made with a suction electrode attached directly at the mouth opening of the hypostome of the hydra. Recordings were begun as soon as the hypostome was attached. Impulses from the suction electrode were delivered to the head stage of an AM systems, Model 3000 AC/DC differential amplifier, converted to digital output with Power Lab and visualized using LabChart 7 software (AD Instruments) on a MacBook Pro. During recording, the preparations were observed through a dissecting microscope at 100X magnification.

III. Ligands. The following ligands were used: reduced glutathione (GSH), GABA, and the $GABA_B$ agonists and antagonists, baclofen and phaclofen. Test substances were made fresh at 10-fold their final concentration and were subsequently diluted. Two methods were used to apply ligands to the hypostome.

a) **Bath-applied Ligand:** One tentacle-free hypostome was placed in a 10 mL petri dish with 7 mL BVC. A suction electrode was attached over the apex of the mouth. The recording protocol was as follows: a ten-minute BVC control period followed by a ten minute treatment period at the beginning of which 1 mL GSH at $4x10^{-6}$ M and/or neuro-transmitter ligand was added to the bath with a 1.0 mL syringe (Figure 3). Each ten-minute period was subdivided into two periods, control period 1

(C1) and control period 2 (C2), treatment period 1 (T1) and treatment period 2 for statistical analysis. The first thirty seconds of each experimental sub-period was omitted in the analysis to allow the preparation to adapt. C1 (acclimation period) was eliminated from statistical analysis. Thus, comparisons were made for 4.5 minutes in C2, T1, and T2 (Figure 4).

b) **Pipette-filled Ligand:** One tentacle-free hypostome was placed in a 10 mL petri dish containing 7 mL BVC. The stopcock on the electrode holder was opened and a test substance was drawn into the pipette tip under slight negative pressure prior to hypostome attachment. The stopcock was then closed, so that no liquid leaked from the pipette. Visual examination of the pipette tip ensured that the fluid level within the pipette tip remained unchanged as the tip was placed onto a hypostome in the BVC-containing dish. By opening the stopcock, the slight negative pressure in the pipette allowed a hypostome to be attached to the pipette tip. The stopcock was then closed preventing further leakage and/or suctioning of BVC into the pipette tip (Figure 5). Recordings began as soon as the hypostome was attached and lasted for 10 minutes with the thirty seconds (acclimation) omitted from analysis. The remaining recording time was divided into two treatment periods (T1, T2) for analysis with the first 30 seconds from treatment period (T1) omitted. The BVC control period, C1 and C2- The C1 and C2 of the bath-applied ligand experiments, at 30 sec after attachment (above), were used as the controls for T1 and T2, respectively. Thus, comparisons were made for 4.5 minutes in C1, C2, T1, and T2 (Figure 6).

The following agonists and antagonists were used: L-glutathione reduced (GSH), gamma-amino butyric acid (GABA), baclofen, and phaclofen. All substances

were purchased from Tocris Cookson Inc. (Ballwin, MO, USA), except GABA, and GSH, which were purchased from Sigma-Aldrich (St Louis, MO, USA). The following single treatment and combination experiments in final concentrations were performed: GSH ($1x10^{-6}$M), GSH ($5x10^{-7}$M), GSH ($1x10^{-8}$M), GSH ($5x10^{-7}$M)+ GABA ($1x10^{-6}$M), GSH ($5x10^{-7}$M) + Phaclofen ($1x10^{-8}$M), GSH ($5x10^{-7}$M) + Baclofen ($1x10^{-8}$M), GSH ($5x10^{-7}$M) + Phaclofen ($1x10^{-8}$M) + Baclofen ($1x10^{-8}$M). Doses of Phaclofen, Baclofen, and GABA used in combination experiments were chosen from previous electrophysiology experiments (Nandivada and Kass-Simon, unpublished; Pierobon et al., 2003, Scappaticci et al., 2004).

IV. Data Analysis

As stated above, because the prolonged ten-minute treatment could have resulted in either desensitization, or have been necessary for the substances to take effect and/or reach their site of action, each ten-minute period was subdivided into two 4.5-minute periods for data analysis. In the bath-applied method, the first 30 seconds was eliminated in each sub-period to allow for acclimation—treatment 1 (T1) and treatment 2 (T2).

For each ligand series, at least seven animals were used. The following comparisons were made in the bath-applied method: C2 vs. T1, C2 vs. T2, T2 vs. T1. The following comparisons were made in the pipette-applied method: C1 vs. T1, C1 vs. T2, C2 vs. T1, C2 vs. T2 and T2 vs. T1. In the bath-applied series, each set of animals (in the testing periods T1 and T2) was compared against its own BVC control period BVC (C2). In the pipette-filled series, each set of test periods- (T1, T2) for 7

preparations was respectively compared to the set of 7 (C1) and (C2) control periods of the bath-applied series as described above.

The following parameters were measured for each 4.5-min period: frequency of small uncorrelated hypostomal pulses (SUHPs, 30-300 μV), medium uncorrelated hypostomal pulses (MUHPs, 301-570 μV), large uncorrelated hypostomal pulses (LUHPs, 571-800 μV), extra-large uncorrelated hypostomal pulses (≥ 801 μV), rhythmic potentials (RPs), pacemaker bursting pulses (PBPs) and pulses per pacemaker bursts pulse (P/PBP). Pulses were measured from peak to peak. PBPs and P/PBPs (subset of MUHPs, LUHPs and XLUHPs) were visually identified by their characteristic bursting pattern. RP's (subset of SUHPs) were identified by their regular recurrence pattern (Passano and McCullough, 1962; Guertin and Kass-Simon, 2015).

Pulses were binned using the Spike Histogram module on Lab Chart 7 (AD Instruments). The sub-period being analyzed (C1, C2, T1, T2) was highlighted and selected for analysis. Using the spike train-setup prompt, a train parameter was created. The pulses were binned using arbitrary size categories. The pulse, spike detector was set to 80.1 mV, 57.1 mV, 30.1 mV, and 3.0 mV to identify the number of pulses including and greater than the set voltage for the selected 4.5-min period. To identify the number of pulses between 30-300 μV (SUHPs), the number of pulses generated for 30.1 mV was subtracted from 3.0 mV. To identify the number of pulses between 301-570 μV (MUHPs), the number of pulses generated for 57.1 mV was subtracted from 30.1 mV. To identify the number of pulses between 571-800 μV (LUHPs), the number of pulses generated for 80.1 mV was subtracted from 57.1 mV.

To identify the number of pulses equal and greater to 801 μV (XLUHPs), the number of pulses generated for 80.1 mV was reported.

Data analysis was similar to that used in previous electrophysiology studies (Kay and Kass-Simon, 2009; Ruggeri et al., 2004; Guertin and Kass-Simon, 2015). A Friedman Two-Way Analysis of Variance (FANOVA) for each parameter was used in R (Revolution Analytics) to determine differences among the designated recording periods in each class of treatments in the bath-applied method and in the pipette-applied method. Significant differences were further analyzed using the Friedman-test-with-post-hoc command for multiple comparisons.

In order to determine the effect of GSH concentrations ($5x10^{-8}$, $5x10^{-7}$, $5x10^{-6}$) on the parameters measured, T1 + T2 were added together in the bath-applied method and in the pipette-applied method. The treatment periods for each concentration were compared with FANOVAs for each parameter measured. Significant differences were analyzed with post-hoc analysis. Thus, comparisons were made between (GSH $5x10^{-8}$, GSH $5x10^{-7}$, GSH $5x10^{-6}$) in the bath-applied method and in the pipette-applied method.

In post-hoc analysis, to determine whether the treatments in the bath-applied method were significantly different from those in the pipette-applied method, comparisons were made as follows: For each set of trials in which T1 and T2 were not significantly different from each other either in the bath-applied or pipette-applied method, T1+T2 were added to create the parameter Tb (bath applied) and Tp (pipette applied) which were compared with the Welch two-sample t-tests for SUHPs, MUHPs LUHPs, XLUHPs, PBPs, P/PBPs and RPs. In those cases where T1 and T2 were

significantly different from each other in either method, T1 of the bath-applied method was compared to T1 of the pipette-applied method and T2 of the bath-applied was compared to T2 of the pipette-applied method. SUHPs, MUHPs, LUHPs, XLUHPs and PBPs are presented as medians ± inter-quartile ranges (m ± i.q.r.) and as means ± standard deviations (μ ± s.d.). P/PBP are the average number of pulses per pacemaker burst and are calculated by taking the total number of pulses in each PBP and dividing the total by the number of PBPs in that period. RPs and P/PBP are reported as medians ± inter-quartile ranges (m ± i.q.r) and as means ± standard error (μ ± s.e.). Values were considered to be significantly different at $P< 0.5$, with a potentially significant trend at $0.05<P<0.1$ (Guertin and Kass-Simon, 2015).

RESULTS

I. Effect of GSH Concentration

a) *Bath-applied*

GSH at $5x10^{-6}$M (Fig. 4) caused significant increases in SUHPs in treatment period (T1) and (T2) relative to BVC control period (C2) (SUHPs: Table 1a, T1>C2*, p≤0.0426, T1>C2*, p≤0.0428). GSH at $5x10^{-6}$M caused significant increases in MUHPs in treatment period (T1) relative to BVC control period (C2) (MUHPs: Table 2a, T1>C2*, p≤0.00505). GSH at $5x10^{-6}$M caused significant increases in PBPs and P/PBPs in treatment period (T1) relative to BVC control period (C2) and potentially significant increases in treatment period (T2) relative to (C2) (PBPs: Table 5a, T1>C2*, p≤0.0152, T2>C2^, p≤0.0537; P/PBPs: Table 6a, T1>C2*, p≤0.0151, T2>C2^, p≤0.0538). GSH at $5x10^{-7}$M caused significant increases in SUHPs in treatment period (T1) relative to BVC control period (C2) (SUHPs: Figure 7, Table 1a, T1>C2*, p≤0.00984). GSH at $5x10^{-7}$M caused significant decreases in XLUHPs in treatment period (T2) relative to BVC control period (C2) and potentially significant decreases in treatment period (T2) relative to treatment period (T1) (XLUHPs, Figure 8, Table 4a, T2<C2*, p≤0.0428, T2<T1^, p≤0.0612). GSH at $5x10^{-8}$M caused potentially significant decreases in LUHPs and RPs in treatment period (T2) relative to BVC control period (C2) (LUHPs, Table 3a, T2<C2^, p≤0.0693; RPs, Table 7a, T2<C2^, p≤0.0751). GSH at $5x10^{-8}$M caused significant decreases in MUHPs in treatment period (T2) relative to treatment period (T1) (MUHPs, Table 2a, T2<T1*, p≤0.0266). GSH at $5x10^{-8}$M caused significant increases in PBPs in treatment period (T2) relative to BVC control period (C2) (PBPs: Table 5a, T2>C2*, p≤0.0327).

There was a significant increase in frequency of SUHPs in GSH $5x10^{-6}$ relative to GSH $5x10^{-7}$ (Figure 9, Table 16a, $p \leq 0.00195$*) and a potentially significant increase in SUHPs in GSH $5x10^{-8}$ relative GSH $5x10^{-7}$ (Figure, 9, Table 16a, $p \leq 0.0604$^)—suggesting that the higher and lower concentrations of GSH were able to induce smaller, neuronal pulses. There were no significant differences in LUHPs or XLUHPs at these concentrations (Figure 10, Table 16a). There was a significant increase in PBPs and P/PBPs in GSH 5x10-8 relative to GSH 5x10-6 (Figure 11, Table 16a, PBPs: $p \leq 0.0444$*, P/PBPs: $p \leq 0.0184$*)—suggesting that the stronger concentration of GSH may saturated receptors, decreasing larger muscle pulses associated with contraction bursts. There was a significant increase in RPs in GSH 5x10-6 relative to GSH 5x10-7 (Figure 9, Table 16a, RPs: $p \leq 0.0104$*)—suggesting that the stronger concentration of GSH induced small, RPs.

b) *Pipette-Applied*

GSH at $5x10^{-6}$M caused significant decreases in XLUHPs in treatment period (T2) and (T1) relative to BVC control periods (C1) and (C2) (XLUHPS: Table 4b, T1<C1*, $p \leq 0.0323$; T2<C1*, $p \leq 0.0323$; T1<C2*, $p \leq 0.0171$; T2<C2*, $p \leq 0.0169$). GSH at $5x10^{-7}$M caused potentially significant increases in MUHPs in treatment period (T1) relative to BVC control period (C2) (MUHPs: Figure 12, Table 2b, T1>C2^, $p \leq 0.0741$). GSH at $5x10^{-8}$M caused significant decreases in P/PBPs in treatment period (T1) relative to BVC control period (C1) (P/PBPs: Table 6b, T1<C1*, $p \leq 0.0157$).

There was a significant decrease in LUHPs, XLUHPs, PBPs and P/PBPs in GSH $5x10^{-6}$M relative to GSH $5x10^{-7}$M (LUHPs: Figure 13, Table 16b, $p \leq 0.0324$*;

XLUHPs: Figure 13, Table 16b, p≤0.0140*; PBPs: Figure 14, Table 16b, p≤0.0483*; P/PBPs: Figure 14, Table 16b, p≤0.0379*). There were no significant differences in SUHPs, MUHPs or RPs at these concentrations (Figure 15, Table 16b).

II. Effect of GABA on GSH-elicited potentials

a). Bath-applied

GABA at $1x10^{-6}$M combined with GSH at $5x10^{-7}$M produced no significant differences in the rates of any of the six parameters being measured, compared to plain BVC control periods (p>0.1) (Figure 7, 8, 16-20, Table 1a-7a).

b). Pipette-applied

GABA at $1x10^{-6}$M combined with GSH at $5x10^{-7}$M caused significant increases in LUHPs and potentially significant increases in P/PBPs in treatment period (T2) relative to BVC control period (C2) (LUHPs: 21, Table 3b, T2>C2*, p≤0.0440; P/PBPs: Figure 22, Table 6b, T2>C2^, p≤0.0569). GABA at $1x10^{-6}$M combined with GSH at $5x10^{-7}$M caused significant increases in XLUHPs in treatment period (T1) relative to BVC control period (C1) (XLUHPs: Figure 23, Table 4b, T1>T1*, p≤0.0450). GABA at $1x10^{-6}$M combined with GSH at $5x10^{-7}$M caused significant increases in RPs in treatment period (T1) relative to BVC control period (C2) (RPs: Figure 24, Table 7b, T1>C2*, p≤0.03235).

Although GABA at $1x10^{-6}$M combined with GSH at $5x10^{-7}$M produced no significant differences in the rates of any of the parameters measured in the bath-applied method, GABA at $1x10^{-6}$M combined with GSH at $5x10^{-7}$M increased four of the seven parameters measured in the pipette-applied method—LUHPs, XLUHPs,

PBPs and RPs (Figure 25) (Table 3b, 4b, 5b, 7b). The number of pulses produced for the parameters MUHPS, LUHPs, XLUHPs, PBPs, P/PBPs and RPs in the pipette-applied method was also significantly greater relative to the pipette-applied method.

III. Effect of Baclofen on GSH-elicited potentials

a). Bath-applied

Baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH, caused a potentially significant increase in MUHPs in T2 relative to BVC control period (C2) (MUHPs: Figure 16, Table 2a T2>C2^ $p \leq 0.0798$).

b). Pipette-applied

Baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant decreases in SUHPs in treatment period (T2) relative to BVC control periods (C1) and (C2) and potentially significant decreases in treatment period (T1) relative to BVC control period (C1) (SUHPs: Figure 26, Table 1b, T1<C1^, $p \leq 0.06227$, T2<C1*, $p \leq 0.00236$, T2<C2*, $p \leq 0.00536$). Baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant decreases in XLUHPs in treatment period (T2) relative to BVC control period (C1) and (C2) and caused significant decreases in XLUHPs in treatment period (T1) relative to BVC control period (C2) (XLUHPs: Figure 23, Table 4b: T2<C1*, $p \leq 0.0413$; T2<C2*, $p \leq 0.0109$; T1<C2*, $p \leq 0.0414$). Baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant decreases in P/PBPs in treatment period (T1) and (T2) relative to BVC control period (C1) (P/PBPs: Figure 22, Table 6b, T1<C1*, $p \leq 0.00827$, T2<C1*, $p \leq 0.0439$).

Baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant decreases in SUHPs in the bath-applied method (Table 1a), however, baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant increases in SUHPs, XLUHPs, and P/PBPs in the pipette-applied method (Figure 27, Table 1b, 4b, 6b). In addition, the pipette-applied method produced significantly more SUHPs relative to the bath-applied method (Table 8).

IV. Effect of Phaclofen on GSH-elicited potentials

a). Bath-applied

Phaclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH, caused a potentially significant decrease in SUHPs in T2 relative to BVC control period (C2) (SUHPs: Figure 7, Table 1a, T2<C2^ $p \leq 0.0784$).

b). Pipette-applied

Phaclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused potentially significant increases in LUHPs in treatment period (T2) relative to BVC control period (C2) (LUHPs: Figure 21, Table 3b, T2>C2^, $p \leq 0.0734$). Phaclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant decreases in XLUHPs in (XLUHPs, Figure 23, Table 4b, T2<T1*, $p \leq 0.00821$) and potentially significant decreases in PBPs in treatment period (T2) relative to treatment period (T1) (PBPs: Figure 28, Table 5b, T2<T1^, $p \leq 0.0709$).

Phaclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused potentially significant decreases in SUHPs in the bath-applied method (Table 1a). Phaclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused increases

in LUHPs but decreases in XLUHPs and PBPs in the pipette-applied method (Table 3b, 4b, 5b). Phaclofen, inhibits, the inhibition produced by the GABA mechanism. In addition, Phaclofen administered along with GSH 5x10-7M caused significantly higher SUHPs, MUHPs and LUHPs in the pipette-applied method relative to the bath-applied method (Figure 29, Table 8, 9, 10). In the case where treatment period 1 (T1) was different from treatment period 2 (T2) for PBPs, the pipette-applied method caused potentially significant increases in PBPs relative to the bath-applied method (Table 15c).

V. Effect of Baclofen and Phaclofen on GSH-elicited potentials

a). Bath-applied

Baclofen, at $1x10^{-8}$M, added with $1x10^{-8}$M Phaclofen and $5x10^{-7}$M GSH caused a significant decrease in MUHPs and RPs in T2 relative to BVC control period (C2) (MUHPs: Figure 16, Table 2a, T2<C2*, $p \leq 0.00379$; RPs: Figure 20, Table 7a, T2<C2*, $p \leq 0.00969$).

b). Pipette-applied

Baclofen, at $1x10^{-8}$M, added with $1x10^{-8}$M Phaclofen and $5x10^{-7}$M GSH caused significant decreases in SUHPs in treatment period (T2) relative to BVC control periods (C1) and (C2) (SUHPs: Figure 26, Table 1b, T2<C1*, $p \leq 0.0106$, T2<C2*, $p \leq 0.0196$).

Baclofen, at $1x10^{-8}$M, added with $1x10^{-8}$M Phaclofen and $5x10^{-7}$M GSH caused decreases in MUHPs and RPs in the bath-applied method (Table 2a, 7a) and decreases in SUHPs in the pipette-applied method (Table 1b). In addition, Baclofen,

at $1x10^{-8}$M, added with $1x10^{-8}$M Phaclofen and $5x10^{-7}$M GSH caused significantly higher MUHPs, LUHPs, PBPs and P/PBPs in the pipette-applied method relative to the bath-applied method (Figure 30, Table 9, 10, 12, 13).

VI. Comparison of Responses in Bath-applied method vs. pipette applied method where treatment period 1 (T1) was the same relative to treatment period 2 (T2)

a). Effect of the bath-applied method vs. pipette-applied method on GSH Dose Response

GSH at 5x10-6M caused potentially significant increases in SUHPs and significantly increased the amount of XLUHPs in the bath-applied treatment (Tb) relative to the pipette-applied treatment (Tp) (SUHPS: Table 8, Tb>Tp^, $p \leq 0.0702$; XLUHPs: Table 11, Tb>Tp*, $p \leq 0.0265$). GSH at 5x10-6M caused significant decreases in PBPs and P/PBPs in the bath-applied treatment relative to the pipette-applied treatment (PBPs: Table 12, Tb<Tp*, $p \leq 0.00649$; P/PBPs: Table 13, Tb<Tp*, $p \leq 0.00256$). GSH at 5x10-7M caused significant decreases in SUHPs, LUHPs, PBPs, P/PBPs and RPs in the (Tb) relative to the (Tp) (SUHPs, Table 8, Tb<Tp*, $p \leq 0.0126$; LUHPs, Table 10, Tb<Tp*, $p \leq 0.0146$; PBPs, Table 12, Tb<Tp*, $p \leq 0.00369$; P/PBPs, Tb<Tp*, $p \leq 0.005567$; RPs, Table 14, Tb<Tp*, $p \leq 0.0254$). GSH at 5x10-8M caused potentially significant decreases in PBPs in (Tb) relative to (Tp) (PBPs: Table 12, Tb<Tp*, $p \leq 0.0982$).

Thus, increased level of electrical activity in the parameters measured was greater in the pipette-applied method relative to the bath-applied method.

b). Effect of the bath-applied method vs. pipette-applied method on GABA-elicited GSH-potentials

GABA at $1x10^{-6}$M combined with GSH at $5x10^{-7}$M caused significant decreases in XLUHPs, PBPs, P/PBPs and RPs in the treatment bath-applied (Tb) relative to the treatment pipette-applied (Tp) (XLUHPs: Table 11, Tb<Tp*, $p \leq 0.000182$; PBPs: Table 12, Tb<Tp*, $p \leq 0.0000499$; P/PBPs: Table 13, Tb<Tp*, $p \leq 0.0326$; RPs: Table 14, Tb<Tp*, $p \leq 0.00657$).

Thus, the increased level of activity in the parameters measured was greater in the pipette-applied method relative to the bath-applied method.

c). Effect of the bath-applied method vs. pipette-applied method on Baclofen-elicited GSH-potentials

Baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant decreases in SUHPs in the treatment bath-applied (Tb) relative to the treatment pipette-applied (Tp) (SUHPs: Table 8, Tb<Tp*, $p \leq 0.00364$).

Small pulses with no observable pattern in behavior may be produced by the endoderm located in the hypostome associated with elongation of the mouth during feeding. Baclofen caused significant increases in small pulses (SUHPs) in the pipette-applied method relative to the bath-applied method where GABA is also found to be working, suggesting that there are more GABA B receptors at the apex.

d). Effect of the bath-applied method vs. pipette applied method on Phaclofen-elicited GSH-potentials

Phaclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant decreases in SUHPs, MUHPs and LUHPs in the treatment bath-applied

(Tb) relative to the treatment pipette-applied (Tp) (SUHPs: Table 8, Tb<Tp*, $p \leq 0.00758$; MUHPs, Table 9, Tb<Tp*, $p \leq 0.00309$; LUHPs: Table 10, Tb<Tp*, $p \leq 0.0119$).

Thus, increased level of electrical activity in the parameters measured was greater in the pipette-applied method relative to the bath-applied method.

e). Effect of the bath-applied method vs. pipette-applied method on Baclofen and Phaclofen-elicited GSH-potentials

Baclofen, at $1x10^{-8}$M, added with $1x10^{-8}$M Phaclofen and $5x10^{-7}$M GSH caused potentially significant decreases in MUHPs and significant decreases in LUHPs, PBPs and P/PBPs in treatment bath-applied (Tb) relative to treatment pipette-applied (Tp) (MUHPs: Table 9, Tb<Tp^, $p \leq 0.0702$; LUHPs: Table 10, Tb<Tp*, $p \leq 0.00943$; PBPs: Table 12, Tb<Tp*, $p \leq 2.60x10^{-6}$; P/PBPs: Table 13, Tb<Tp*, $p \leq 0.00272$).

Baclofen, coadministered with Phaclofen and GSH caused significantly more medium, large, pacemaker bursting pulses and pacemaker per pacemaker bursting pulses in the pipette-applied method relative to the bath-applied method. GSH administered alone caused significant increases in medium and larger pulses. Larger pulses may be associated with mouth contractions observed prior to mouth opening, after mouth elongation.

VII. Comparison of Responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was different relative to treatment period 2 (T2)

a). Effect of XLUHP responses using GSH $5x10^{-7}$M in the bath-applied method vs. pipette-applied method

GSH $5x10^{-7}$M produced more XLUHPs in treatment period (T2p) for the pipette-applied method than the treatment period (T2b) for the bath-applied method (Table 15a, T2p>T2b*, $p \leq 0.0469$).

XLUHPs may be produced by the ectoderm, associated with mouth contractions. Thus, GSH increasing the amount of contractile activity in pipette-applied method relative to the bath-applied method as well as in the latter treatment period may indicate initial mouth elongation followed by secondary mouth contractions associated with hydra's feeding behavior prior to mouth opening.

b). Effect of MUHP responses using GSH $5x10^{-8}$M the bath-applied method vs. pipette-applied method

GSH at $5x10^{-8}$M produced no significant increases or decreases in MUHPs in the treatment period (T1p) for the pipette-applied method relative to the treatment period (T1b) for the bath-applied method. GSH at $5x10^{-8}$M produced no significant increases or decreases in MUHPs in the treatment period (T2p) for the pipette-applied method relative to the treatment period (T2b) for the bath-applied method (Table 15b).

c). Effect of PBP responses using GSH $5x10^{-7}$M administered with Phaclofen $1x10^{-8}$M in the bath-applied method vs. pipette-applied method

GSH $5x10^{-7}$M administered with Phaclofen $1x10^{-8}$M produced potentially more PBPs in the treatment period (T1p) for the pipette-applied method than the treatment period (T1b) for the bath-applied method (T1P>T1B, $p \leq 0.0617$). GSH $5x10^{-7}$M administered with Phaclofen $1x10^{-8}$M for PBPs is the same in the treatment period (T2p) for the pipette-applied method as the treatment period (T2b) for the bath-applied method (Table 15c).

DISCUSSION

In determining the electrical correlates associated with mouth opening and closing behavior, one must first consider the changing anatomical structure observable during this behavior. Of the many behaviors exhibited by hydra during feeding, one of the first is the elongation of the hypostome. As tentacle writhing is activated, the cone-shaped hypostome elongates as the tentacles begin to direct the prey homogenate towards the mouth opening. The mouth rapidly opens and contractile motions of the hypostome follow. It is hypothesized that the smaller pulses (SUHPS, RPs, MUHPs and LUHPs) may be involved in the initial opening of the hypostome and the larger bursting pulses (XLUHPs, PBPs and P/PBPs) may be involved in the observed contraction of the hypostome.

In studies on *Hydra,* the question of the role of neurotransmitters in modulating the GSH-induced feeding response has been raised. This study presents electrophysiology evidence of the GSH-induced feeding response in Hydra and evidence that in Hydra, GABA, acting through an inhibitory mechanism, inhibits cessation of the GSH-induced feeding response—prolonging hypostomal activity. Although it is not possible to specifically discern where the receptor ligands are affecting the pacemaker systems, the above findings support previous studies on GABA receptor ligands altering hydra's pacemaker activity (Concas et al., 1998; Kass-Simon et al, 2003; Kass-Simon and Scappaticci, 2004; Kass-Simon and Scappaticci, 2008).

In order to find out the exact role of GABA in modulating the GSH-induced feeding response, we recorded from reduced-tentacle hypostomes. At the apex of the

hypostome, there are sensory cells; distributed perpendicular to the apex of the hypostome, there are ganglion cells. The large putative hypostomal contraction pacemaker pulses may be produced by pacemaker neurons associated with the proximal nerve ring (Kass-simon, 1972, Hufnagel and Kass-Simon unpublished) and the epithelial muscular cells. The small, uncorrelated hypostomal pulses do not make patterns and appear to be neuronal and part of the hypostomal nerve net. The medium, uncorrelated hypostomal pulses may or may not be a subset of the large, uncorrelated hypostomal pulses because they do not fall into a bursting pattern. The rhythmic potentials have previously been found to be conducted on the endoderm and are associated with the contraction of the circular endodermal epithelial muscle cells (Kass-Simon and Passano, 1978). The small, uncorrelated hypostomal pulses binned in the present analysis include the frequency of rhythmic potentials.

We hypothesize that GSH induces the subtentacular pacemaker system located at or near the proximal nerve ring due to the increased level of extra-large uncorrelated hypostomal pulses (XLUHPs) in the bath-applied method—not observed during the pipette-applied method. Pacemaker activity, at the site of a loosely involved nerve ring under the tentacles can be GSH-induced. GABA, through its inhibitory mechanism, may be inhibiting some neuron that was previously inhibiting the GSH response.

I. Effect of GSH Concentration

In the bath-applied method, GSH increased small-uncorrelated pulses, (SUHPs), medium-uncorrelated pulses (MUHPs), pacemaker bursting pulses (PBPs)

and pulses per pacemaker bursting pulse (P/PBPs). In the pipette-applied method GSH did not affect SUHPs but increased MUHPs and decreased XLUHPs and P/PBPs; this supports previously observed GSH induced cone-formation of the hypostome, now hypothesized to be reflected in the increase in medium sized pulses (MUHPs) and the concurrent inhibition of body contraction (considered to be mediated by XLUHPs and PBPs which are presumed to include neuroeffector responses to the activity of the proximal nerve ring and pacemaker system. The absence of activity attributed to rhythmic potentials in this finding is supported by previous studies in that the contraction burst system (ectodermal pulses) may inhibit the RP system (Passano and McCullough, 1963; Taddei-Ferretti and Chillemi, 1987). Kass-Simon et al., 1975 showed a morphological basis for the communication between the endoderm and the ectoderm through gap junctions, and thus, the ectodermal contraction burst system communicates with the endodermal rhythmic potential system such that the CB system will contract and inhibit the RP system until the contraction is over and an RP results. It is our hypothesis that the PBP system is a subset of the CB system. That both PBPs and P/PBPs were affected suggests that an entire PBP system in the hypostome may exist and has been essentially activated. Comparison of responses in bath-applied method vs. pipette-applied method revealed that GSH at $5x10^{-7}$ caused significantly more pulses in the pipette-applied method where the base of the hypostome was blocked, and ligand administration was directly at the apex of the mouth. Whether the mouth opened during recording is unknown. Thus, the increased level of electrical activity in the parameters measured and compared in the pipette-applied method relative to the bath-applied method support

the hypothesis that the receptors for GSH may be located towards the distal portion of the mouth near the apex of the hypostome.

II. Effect of GABA on GSH-elicited potentials

The administration of GABA with GSH yielded no activity in the bath-applied method where the mouth of the hypostome was blocked. However, GSH administered with GABA in the pipette-applied method where ligand was in direct contact with the mouth opening produced increased larger pulse activity and rhythmic potentials. The prolonged GSH-induced electrical activity by GABA and subsequent increased larger bursting pulses suggests that GABA essentially inhibited the cessation of the GSH-induced pacemaker activity. Comparison of responses in the parameters measured yielded higher activity in the pipette-applied method relative to the bath-applied method. This supports the hypothesis that GABA is acting at the distal portion or apex of the hypostome.

III. Effect of Baclofen on GSH-elicited potentials

Baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused significant decreases in SUHPs in the bath-applied method. Baclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH where ligand was in direct contact with mouth opening caused significant increases in SUHPs, XLUHPs, and P/PBPs in the pipette-applied method. This suggests that there are metabotropic $GABA_B$ neuronal receptors on the hypostomal nerve net, which include neurons of the pacemaker systems that mediate cone formation and hypostomal and body contractions. Baclofen caused significant increases in small pulses (SUHPs) in the pipette-applied method

relative to the bath-applied method where GABA is also found to be working. These small pulses with no observable pattern in behavior may be produced by the endoderm located in the hypostome associated with elongation of the mouth during feeding. This outcome is supported by recent findings in which the endodermal layer of the hypostome was labeled with anti-$GABA_B$ receptor antibody (Hufnagel and Kass-Simon, unpublished).

IV. Effect of Phaclofen on GSH-elicited potentials

Phaclofen, administered at $1x10^{-8}$M in the presence of $5x10^{-7}$M GSH caused potentially significant decreases in SUHPs in the bath-applied method, supporting the idea that neuronal metabotropic $GABA_B$ receptors are distributed around the hypostome. Phaclofen caused significantly higher small, medium, and large pulses in the pipette-applied method relative to the bath-applied method, indicating that $GABA_B$ receptors at the mouth or the lining of the mouth inhibit mouth closure and that these pulses are inhibited by the $GABA_B$ antagonist, phaclofen. Thus, phaclofen may block the inhibitory mechanism caused by GABA and it's agonist, baclofen, by decreasing the amount of small, medium and large pulses associated with mouth elongation during hydra's feeding behavior.

V. Effect of Baclofen and Phaclofen on GSH-elicited potentials

Baclofen, coadministered with phaclofen and GSH caused significantly more medium, large, pacemaker bursting pulses and pacemaker per pacemaker bursting pulses in the pipette-applied method relative to the bath-applied method. GSH administered alone caused significant increases in medium and larger pulses. Larger

pulses may be associated with mouth contractions observed secondary to mouth elongation but prior to mouth opening. Thus, baclofen together with phaclofen may wipe out the inhibitory mechanism of GABA prolonging the cessation of the feeding behavior by producing more medium to larger pacemaker bursting pulses in the pipette-applied method relative to the bath-applied method where more pacemaker cells may be located relative to the newly observed distal nerve ring.

Although GABA combined with GSH produced no significant differences in the frequency of any of the parameters measured in the bath-applied method, coadministration of GSH and GABA alone increased LUHPs, XLUHPs, PBPs and RPs in the pipette-applied method (Figure 25). The results support the behavioral observations that GABA inhibits the cessation of the GSH-induced feeding response and indicates that GSH and GABA receptors are differentially distributed in the hypostome. It is also possible to conclude that GABA acting through its metabotropic receptors is inhibiting the GSH-induced feeding response by altering the underlying GSH-induced electrical activity. This is supported by our findings that the application of baclofen and phaclofen on the GSH-induced elicited potentials blocked $GABA_B$ electrical activity and its presumed contribution to GABA inhibition. GSH and GABA alone caused significant increases in LUHPs, XLUHPs, P/PBPs and RPs; the application of baclofen and phaclofen, together with GSH counteracted this effect and caused significant decreases in SUHPs, MUHPs and RPs. Increased levels of electrical activity in the parameters measured was greater in the pipette-applied method relative to the bath-applied method in all treatments—suggesting that most of the GSH receptors may be found in the distal nerve ring closer to the apex of the

hypostome relative to the proximal nerve ring located around the base of the hypostome (Figure 31).

Table 1. The effect of various treatments on the number of small, uncorrelated hypostomal pulses in the a) bath-applied method and b) pipette-applied method. Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range ($m \pm$ i.q.r). Significance was calculated with FANOVAs. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 1
a)

SUHPs								
Treatment	**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r		
GSH $5x10^{-6}$	122.86±94.77	118 ± 99	294.14 ± 48.95	306 ± 61.5	204.57 ± 77.49	225 ± 126.5	7	**T1>C2*, p≤0.0426 T2>C2*, p≤0.0428**
GSH $5x10^{-7}$	48.25 ± 37.58	47 ± 35.75	92.88 ± 53.44	81.5 ± 58.25	79.25 ± 52.64	67.5 ± 86	8	**T1>C2*, p≤0.00984**
GSH $5x10^{-8}$	162.88 ± 80.48	146.5 ± 95.5	173.5 ± 98.74	150.5 ± 158.75	146.13 ± 80.63	117.5 ± 113.75	8	None
GSH $5x10^{-7}$ + GABA 10^{-6}	157.63 ± 88.54	134 ± 154.75	186.13 ± 125.02	148 ± 144.75	185.38 ± 136.65	150.5 ± 148.5	8	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	140.5 ± 58.23	133.5 ± 52.25	132.25 ± 56.07	121.5 ± 45.5	85 ± 21.45	83.5 ± 13.5	8	**T2<C2^, p≤0.0783**
GSH $5x10^{-7}$ + Baclofen 10^{-8}	164.18 ± 98.89	162 ± 157	150 ± 97.35	127 ± 136	144.55 ± 102.99	141 ± 158.5	11	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	143.75 ± 58.93	121.5 ± 55.5	139.75 ± 37.83	157.5 ± 38	136.25 ± 102.11	101.5 ± 124	8	None

Table 1
b)

SUHPs										
	C1		C2		T1		T2			
Treatment	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	**n**	**Significant Differences**
GSH $5x10^{-6}$	205.71 ± 108.83	156 ± 69	184.86 ± 82.21	136 ± 71	155.57 ± 117.52	159 ± 202.5	177.43 ± 150.16	144 ± 262	7	None
GSH $5x10^{-7}$	205.71 ± 108.83	156 ± 69	184.86 ± 82.21	136 ± 71	156 ± 72.15	169 ± 124.5	143.57 ± 67.01	108 ± 101.5	7	None
GSH $5x10^{-8}$	205.71 ± 108.83	156 ± 69	184.86 ± 82.21	136 ± 71	130.17 ± 120.09	71 ± 137.25	71.67 ± 51.37	51.5 ± 61.75	6	None
GSH $5x10^{-7}$ + GABA $1x10^{-6}$	205.71 ± 108.83	156 ± 69	184.86 ± 82.21	136 ± 71	225 ± 77.04	235 ± 116	169.14 ± 39.87	178 ± 55	7	None
GSH $5x10^{-7}$ + Phaclofen $1x10^{-8}$	205.71 ± 108.83	156 ± 69	184.86 ± 82.21	136 ± 71	187.71 ± 77.75	200 ± 114.5	174.57 ± 65.38	194 ± 69	7	None
GSH $5x10^{-7}$ + Baclofen $1x10^{-8}$	205.71 ± 108.83	156 ± 69	184.86 ± 82.21	136 ± 71	68.14 ± 21.51	66 ± 37	43.71 ± 13.40	48 ± 22	7	**T1<C1^, p≤0.00623 T2<C1*, p≤0.00237, T2<C2*, p≤0.00537**
GSH $5x10^{-7}$ + Baclofen $1x10^{-8}$ + Phaclofen $1x10^{-8}$	205.71 ± 108.83	156 ± 69	184.86 ± 82.21	136 ± 71	109.86 ± 83.91	53 ± 124.5	53.43 ± 30.56	47 ± 33	7	**T2<C1*, p≤0.0106 T2<C2*, p≤0.0196**

Table 2. The effect of various treatments on the number of medium, uncorrelated hypostomal pulses in the a) bath-applied method and b) pipette-applied method. Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range ($m \pm$ i.q.r). Significance was calculated with FANOVAs. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 2
a)

MUHPs								
Treatment	**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r		
GSH $5x10^{-6}$	2.14 ± 3.14	0 ± 3.5	11.71 ± 7.57	13 ± 12.5	3.86 ± 3.14	4 ± 5	7	**T1>C2*, p≤0.00505**
GSH $5x10^{-7}$	1.5 ± 1	1 ± 1.25	3.25 ± 2.90	2.5 ± 4.75	6 ± 5.59	5.5 ± 6.25	8	None
GSH $5x10^{-8}$	6 ± 6.26	6 ± 4.75	7.5 ± 6.98	6 ± 8	4.63 ± 6.52	1 ± 6.25	8	**T2<T1*, p≤0.0266**
GSH $5x10^{-7}$ + GABA 10^{-6}	8.25 ± 9.35	4.5 ± 11	6.13 ± 4.91	5.5 ± 4.75	4.25 ± 7.14	1 ± 2	8	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	2.13 ± 2.09	1.5 ± 2	1.88 ± 2.09	1.5 ± 1.25	1.13 ± 1.36	0.5 ± 2	8	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	6.55 ± 9.51	3 ± 5.5	4.91 ± 6.27	3 ± 4	7.18 ± 13.97	2 ± 2	11	**T2>C2^, p≤0.0798**
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	3.88 ± 2.09	3 ± 2.75	4.88 ± 7.25	1 ± 6.25	0.38 ± 0.70	0 ± 0.25	8	**T2<C2*, p≤0.00379**

Table 2
b)

MUHPS										
Treatment	**C1**		**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r		
GSH $5x10^{-6}$	6.29 ± 3.88	6 ± 5	3 ± 3.66	2 ± 4	3.71 ± 3.49	2 ± 3.5	7.43 ± 6.99	5 ± 11	7	None
GSH $5x10^{-7}$	6.29 ± 3.88	6 ± 5	3 ± 3.66	2 ± 4	11.14 ± 5.33	11 ± 7.5	3.71 ± 4.06	2 ± 2.5	7	**T1>C2^, p≤0.0741**
GSH $5x10^{-8}$	6.29 ± 3.88	6 ± 5	3 ± 3.66	2 ± 4	7 ± 7.94	3 ± 6.75	3 ± 2.77	2 ± 3.5	6	None
GSH $5x10^{-7}$ + GABA10^{-6}	6.29 ± 3.88	6 ± 5	3 ± 3.66	2 ± 4	6.57 ± 7.96	4 ± 5.5	3.29 ± 4.40	1 ± 3.5	7	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	6.29 ± 3.88	6 ± 5	3 ± 3.66	2 ± 4	6.43 ± 3.58	7 ± 4.5	8.14 ± 7.08	8 ± 7	7	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	6.29 ± 3.88	6 ± 5	3 ± 3.66	2 ± 4	4.14 ± 4.79	1 ± 5.5	5.86 ± 5.91	5 ± 4.5	7	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	6.29 ± 3.88	6 ± 5	3 ± 3.66	2 ± 4	3.14 ± 3.31	2 ± 4.0	5.14 ± 5.25	3 ± 4	7	None

Table 3. The effect of various treatments on the number of large, uncorrelated hypostomal pulses in the a) bath-applied method and b) pipette-applied method. Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range ($m \pm$ i.q.r). Significance was calculated with FANOVAs. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 3
a)

LUHPs								
	C2		T1		T2			
Treatment	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	**n**	**Significant Differences**
GSH $5x10^{-6}$	1.57 ± 1.59	1 ± 3	0.57 ± 0.73	0 ± 1	1.57 ± 1.99	0 ± 3	7	None
GSH $5x10^{-7}$	1.25 ± 1.20	1 ± 2.25	0.5 ± 0.71	0 ± 1	1.25 ± 1.79	0 ± 2.25	8	None
GSH $5x10^{-8}$	2.13 ± 1.83	1.5 ± 3.25	0.63 ± 0.70	0.5 ± 1	0.38 ± 0.48	0 ± 1	8	**T2<C2^, p≤0.0693**
GSH $5x10^{-7}$ + GABA 10^{-6}	2.25 ± 2.49	1.5 ± 4	3 ± 2.83	2.5 ± 3.25	0.88 ± 1.05	0.5 ± 1.25	8	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	1.63 ± 3.60	0 ± 0.5	0.75 ± 0.97	0.5 ± 1	1.13 ± 1.36	0.5 ± 2	8	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	2.09 ± 2.11	1 ± 4	1.55 ± 1.74	1 ± 2.5	1.64 ± 2.62	1 ± 2	11	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	0.63 ± 0.48	1 ± 1	0.5 ± 0.87	0 ± 0.5	0.25 ± 0.43	0 ± 0.25	8	None

Table 3
b)

LUHPS										
	C1		C2		T1		T2			
Treatment	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	n	Significant Differences
GSH $5x10^{-6}$	0.86 ± 0.64	1 ± 0.5	1 ± 2.07	0 ± 0.5	0.57 ± 0.73	0 ± 1	1.14 ± 2.80	0 ± 0	7	None
GSH $5x10^{-7}$	0.86 ± 0.64	1 ± 0.5	1 ± 2.07	0 ± 0.5	4.43 ± 4.03	4 ± 7	3.86 ± 4.12	3 ± 3.5	7	None
GSH $5x10^{-8}$	0.86 ± 0.64	1 ± 0.5	1 ± 2.07	0 ± 0.5	2.17 ± 2.19	1.5 ± 3.25	0.83 ± 0.69	1 ± 0.75	6	None
GSH $5x10^{-7}$ + GABA $1x10^{-6}$	0.86 ± 0.64	1 ± 0.5	1 ± 2.07	0 ± 0.5	3.29 ± 4.86	1 ± 1.5	5.29 ± 7.36	2 ± 4.5	7	**T2>C2*, p≤0.0440**
GSH $5x10^{-7}$ + Phaclofen $1x10^{-8}$	0.86 ± 0.64	1 ± 0.5	1 ± 2.07	0 ± 0.5	5.57 ± 6.28	5 ± 6.5	4.71 ± 4.40	4 ± 4.5	7	**T2>C2^, p≤0.0734**
GSH $5x10^{-7}$ + Baclofen 10^{-8}	0.86 ± 0.64	1 ± 0.5	1 ± 2.07	0 ± 0.5	1.57 ± 1.59	1 ± 1.5	2.57 ± 2.82	2 ± 4	7	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	0.86 ± 0.64	1 ± 0.5	1 ± 2.07	0 ± 0.5	4 ± 3.82	5 ± 6.5	5.57 ± 6.39	3 ± 8	7	None

Table 4: The effect of various treatments on the number of extra-large, uncorrelated hypostomal pulses in the a) bath-applied method and b) pipette-applied method. Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with FANOVAs. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 4
a)

XLUHPs								
Treatment	**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r		
GSH $5x10^{-6}$	5.43 ± 7.29	1 ± 8	2 ± 2.56	1 ± 1.5	3.43 ± 3.89	1 ± 7	7	None
GSH $5x10^{-7}$	3.75 ± 2.49	4 ± 3.5	3.63 ± 3.57	3 ± 2.75	1.75 ± 3.56	0 ± 1.25	8	**T2<C2*, p≤0.0428 T2<T1^, p≤0.0612**
GSH $5x10^{-8}$	10.13 ± 7.27	7.5 ± 7.25	5.25 ± 7.14	1 ± 8.75	5.38 ± 6.82	0.5 ± 12	8	None
GSH $5x10^{-7}$ + GABA 10^{-6}	6.88 ± 4.54	7.5 ± 5.25	6.63 ± 5.17	6.5 ± 9.75	2.75 ± 5.09	1 ± 2	8	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	12.25 ± 7.84	13 ± 10.75	10.5 ± 8.08	10.5 ± 14	10.38 ± 6.56	10.5 ± 10.25	8	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	5.82 ± 6.46	3 ± 12.5	4.55 ± 6.01	0 ± 8.5	3.55 ± 4.92	2 ± 4.5	11	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	7 ± 6.87	5 ± 9	3 ± 3.74	1.5 ± 4	6 ± 5.63	5.5 ± 12	8	None

Table 4
b)

XLUHPS										
Treatment	**C1**		**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r		
GSH $5x10^{-6}$	11.14 ± 3.83	12 ± 7	12.14 ± 6.98	11 ± 11	0.43 ± 0.73	0 ± 0.5	0.29 ± 0.45	0 ± 0.5	7	**T1<C1*, p≤0.0323 T2<C1*, p≤0.0323 T1<C2*, p≤0.0171 T2<C2*, p≤0.0169**
GSH $5x10^{-7}$	11.14 ± 3.83	12 ± 7	12.14 ± 6.98	11 ± 11	12.86 ± 13.31	7 ± 16.5	12.86 ± 10.55	13 ± 20	7	None
GSH $5x10^{-8}$	11.14 ± 3.83	12 ± 7	12.14 ± 6.98	11 ± 11	8.67 ± 11.23	4 ± 5.75	7 ± 10.13	3 ± 5.75	6	None
GSH $5x10^{-7}$ + GABA 10^{-6}	11.14 ± 3.83	12 ± 7	12.14 ± 6.98	11 ± 11	22.71 ± 9.91	23 ± 7.5	15 ± 6.63	16 ± 7	7	**T1>C1*, p≤0.0450**
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	11.14 ± 3.83	12 ± 7	12.14 ± 6.98	11 ± 11	19.43 ± 10.18	20 ± 11	7.71 ± 7.59	2 ± 13.5	7	**T2<T1*, p≤0.00821**
GSH $5x10^{-7}$ + Baclofen 10^{-8}	11.14 ± 3.83	12 ± 7	12.14 ± 6.98	11 ± 11	3.86 ± 6.17	0 ± 5	1.71 ± 3.81	0 ± 0.5	7	**T2<C1*, p≤0.0413 T2<C2*, p≤0.0109 T1<C2*, p≤0.0414**
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	11.14 ± 3.83	12 ± 7	12.14 ± 6.98	11 ± 11	8.43 ± 7.89	10 ± 14.5	5 ± 6.82	2 ± 7.5	7	None

Table 5. The effect of various treatments on the number of pacemaker bursting pulses in the a) bath-applied method and b) pipette-applied method. Data is reported as means and standard error ($\mu \pm$ s.d) and as medians and interquartile range ($m \pm$ i.q.r). Significance was calculated with FANOVAs. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 5
a)

PBPs								
Treatment	**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r		
GSH $5x10^{-6}$	1.14 ± 0.83	1 ± 0	0 ± 0	0 ± 0	0.29 ± 0.70	0 ± 0	7	**T1>C2*, p≤0.0152 T2>C2^, p≤0.0537**
GSH $5x10^{-7}$	0.63 ± 0.48	1 ± 1	0.13 ± 0.33	0 ± 0	0.63 ± 0.48	1 ± 1	8	None
GSH $5x10^{-8}$	1.63 ± 0.99	1.5 ± 1.25	0.75 ± 0.83	0.5 ± 1.25	0.63 ± 0.70	0.5 ± 1	8	**T2>C2*, p≤0.0327**
GSH $5x10^{-7}$ + GABA 10^{-6}	0.63 ± 0.48	1 ± 1	0.63 ± 0.48	1 ± 1	0.25 ± 0.43	0 ± 0.25	8	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	1.25 ± 0.66	1 ± 1	0.88 ± 0.60	1 ± 0.25	1 ± 0.71	1 ± 0.5	8	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	1.09 ± 0.83	1 ± 1.5	1 ± 1.22	1 ± 1.5	0.64 ± 0.92	0 ± 1	11	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen10^{-8}	0.63 ± 0.70	0.5 ± 1	0.25 ± 0.43	0 ± 0.25	0.5 ± 0.5	0.5 ± 1	8	None

Table 5
b)

PBPS										
Treatment	**C1**		**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r		
GSH $5x10^{-6}$	1.43 ± 0.49	1 ± 1	1.43 ± 0.73	2 ± 1	1.14 ± 1.12	1 ± 2	1.14 ± 0.99	2 ± 2	7	None
GSH $5x10^{-7}$	1.43 ± 0.49	1 ± 1	1.43 ± 0.73	2 ± 1	2.14 ± 0.64	2 ± 0.5	1.29 ± 1.03	1 ± 1.5	7	None
GSH $5x10^{-8}$	1.43 ± 0.49	1 ± 1	1.43 ± 0.73	2 ± 1	2 ± 1.41	2 ± 2	1.17 ± 0.69	1 ± 0.75	6	None
GSH $5x10^{-7}$ + GABA 10-6	1.43 ± 0.49	1 ± 1	1.43 ± 0.73	2 ± 1	2.43 ± 0.90	2 ± 1	1.57 ± 0.73	1 ± 1	7	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	1.43 ± 0.49	1 ± 1	1.43 ± 0.73	2 ± 1	2.14 ± 1.25	2 ± 1.5	0.86 ± 0.83	1 ± 1.5	7	**T2<T1^, p≤0.0855**
GSH $5x10^{-7}$ + Baclofen 10^{-8}	1.43 ± 0.49	1 ± 1	1.43 ± 0.73	2 ± 1	1.29 ± 1.48	0 ± 3	1.57 ± 1.18	2 ± 2	7	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	1.43 ± 0.49	1 ± 1	1.43 ± 0.73	2 ± 1	2 ± 0.53	2 ± 0	1.43 ± 0.49	1 ± 1	7	None

Table 6. The effect of various treatments on the number of pulses per pacemaker bursting pulse in the a) bath-applied method and b) pipette-applied method. Data is reported as means and standard error ($\mu \pm$ s.e) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with FANOVAs. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 6

a)

P/PBPs								
Treatment	**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r		
GSH $5x10^{-6}$	9.29 ± 1.93	9 ± 4	0 ± 0	0 ± 0	1.36 ± 1.26	0 ± 0	7	**T2>C2^, p≤0.0538 T1<C2*, p≤0.0151**
GSH $5x10^{-7}$	4.25 ± 1.31	4.5 ± 7.25	1.5 ± 1.40	0 ± 0	6.63 ± 2.40	6 ± 9	8	None
GSH $5x10^{-8}$	9.28 ± 1.41	9.8 ± 3.55	5.56 ± 2.13	3.5 ± 10.13	5.13 ± 1.92	3 ± 11.25	8	None
GSH $5x10^{-7}$ + GABA 10^{-6}	7.75 ± 2.31	9.5 ± 11.75	9.88 ± 3.11	11 ± 15.25	3.63 ± 2.36	0 ± 2.5	8	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	10.13 ± 2.11	9.5 ± 8.25	9.56 ± 2.06	2 ± 5.25	9.63 ± 2.30	11.25 ± 7.13	8	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	8.55 ± 1.89	9.5 ± 11	4.93 ± 1.46	7 ± 8.25	4.82 ± 1.75	0 ± 11	11	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	3.75 ± 1.33	3.5 ± 7.25	2.13 ± 1.37	0 ± 1.5	6 ± 2.19	4.5 ± 12	8	None

Table 6
b)

P/PBPS										
Treatment	**C1**		**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r		
GSH $5x10^{-6}$	9.29 ± 1.52	8.5 ± 5.75	7.29 ± 1.38	8 ± 4.25	3.14 ± 1.20	3.5 ± 4.75	7.55 ± 1.53	8.5 ± 5.09	7	None
GSH $5x10^{-7}$	9.29 ± 1.52	8.5 ± 5.75	7.29 ± 1.38	8 ± 4.25	12.76 ± 1.57	11 ± 4.92	12.02 ± 3.22	13 ± 11.92	7	None
GSH $5x10^{-8}$	9.29 ± 1.52	8.5 ± 5.75	7.29 ± 1.38	8 ± 4.25	6.21 ± 1.43	6.17 ± 1.15	6.83 ± 1.61	7 ± 4.63	6	**T1<C1*, p≤0.0157**
GSH $5x10^{-7}$ + GABA 10^{-6}	9.29 ± 1.52	8.5 ± 5.75	7.29 ± 1.38	8 ± 4.25	11.91 ± 1.64	11 ± 6	16.14 ± 1.80	16 ± 3.75	7	**T2>C2^, p≤0.0569**
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	9.29 ± 1.52	8.5 ± 5.75	7.29 ± 1.38	8 ± 4.25	12.56 ± 4.50	7.5 ± 9.05	9.07 ± 3.38	9.5 ± 16	7	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	9.29 ± 1.52	8.5 ± 5.75	7.29 ± 1.38	8 ± 4.25	2.76 ± 1.22	0 ± 5.83	6.40 ± 1.68	7.33 ± 6.75	7	**T1<C1*, p≤0.00827 T2<C1*, p≤0.0439**
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	9.29 ± 1.52	8.5 ± 5.75	7.29 ± 1.38	8 ± 4.25	14.12 ± 3.45	9.5 ± 9.59	12 ± 1.52	10.5 ± 6.75	7	None

Table 7. The effect of various treatments on the RP system in the a) bath-applied method and b) pipette-applied method. Data is reported as means and standard error ($\mu \pm$ s.e) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with FANOVAs. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 7
a)

RPs								
Treatment	**C2**		**T1**		**T2**		**n**	**Significant Differences**
	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r		
GSH $5x10^{-6}$	2.14 ± 1.08	0 ± 4	5.71 ± 2.26	4 ± 7.5	6.29 ± 2.01	7 ± 6	7	None
GSH $5x10^{-7}$	0.75 ± 0.46	0 ± 1	1.13 ± 0.57	0.5 ± 1.25	0.63 ± 0.35	0 ± 1	8	None
GSH $5x10^{-8}$	6.25 ± 2.09	5 ± 11.5	1.63 ± 0.86	1 ± 0.25	1 ± 0.40	2 ± 0	8	**T2<C2^, p≤0.0751**
GSH $5x10^{-7}$ + GABA 10^{-6}	2.63 ± 0.68	2.5 ± 3.25	2.5 ± 0.83	1.5 ± 3.75	2.13 ± 0.48	2 ± 2.25	8	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	7.63 ± 2.79	5.5 ± 6.5	5.25 ± 2.32	2 ± 5.25	3.38 ± 1.30	2 ± 3.75	8	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	8.73 ± 3.54	3 ± 8	4.27 ± 1.63	2 ± 6.5	3.82 ± 1.52	3 ± 4.5	11	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	8.75 ± 1.48	7.5 ± 6.75	5.88 ± 1.92	4.5 ± 4.5	3.13 ± 1.29	2 ± 3.25	8	**T2<C2*, p≤0.00969**

Table 7
b)

RPS										
	C1		C2		T1		T2		n	Significant Differences
Treatment	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r	μ ± s.e	m ± i.q.r		
GSH $5x10^{-6}$	3.86 ± 1.30	3 ± 5	2.43 ± 1.24	2 ± 2.5	7 ± 2.71	6 ± 6	7 ± 2.36	6 ± 4	7	None
GSH $5x10^{-7}$	3.86 ± 1.30	3 ± 5	2.43 ± 1.24	2 ± 2.5	10.43 ± 5.22	3 ± 13.5	11.57 ± 5.54	4 ± 16	7	None
GSH $5x10^{-8}$	3.86 ± 1.30	3 ± 5	2.43 ± 1.24	2 ± 2.5	2 ± 0.53	1 ± 2.25	1.83 ± 0.46	1 ± 1.5	6	None
GSH $5x10^{-7}$ + GABA 10^{-6}	3.86 ± 1.30	3 ± 5	2.43 ± 1.24	2 ± 2.5	7.43 ± 1.63	9 ± 8	5 ± 1.29	3 ± 3.5	7	**T1>C2*, p≤0.0324**
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	3.86 ± 1.30	3 ± 5	2.43 ± 1.24	2 ± 2.5	3.86 ± 1.06	3 ± 3.5	2.71 ± 1.52	1 ± 3	7	None
GSH $5x10^{-7}$ + Baclofen 10^{-8}	3.86 ± 1.30	3 ± 5	2.43 ± 1.24	2 ± 2.5	4.29 ± 0.45	3 ± 4	3.29 ± 1.17	2 ± 4.5	7	None
GSH $5x10^{-7}$ + Baclofen 10^{-8} + Phaclofen 10^{-8}	3.86 ± 1.30	3 ± 5	2.43 ± 1.24	2 ± 2.5	3.29 ± 1.34	3 ± 5.5	3.29 ± 1.04	2 ± 5	7	None

Table 8: The comparison of SUHP responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was the same as treatment period 2 (T2). Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with Welch two-sample t-tests. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 8

SUHPs					
Treatment	**TB**		**TP**		**Significant Differences**
	μ + s.d	m + i.q.r	μ + s.d	m + i.q.r	
GSH $5x10^{-6}$	249.36 ± 78.78	259 ± 96.75	166.5± 135.27	151.5 ± 222.75	**TB>TP^ p≤0.0702**
GSH $5x10^{-7}$	83.57 ± 54.63	77 ± 80	149.79 ± 69.91	141.5 ± 130	**TB<TP* p≤0.0126**
GSH $5x10^{-8}$	156.58 ± 97.28	112.5 ± 165.5	100.92 ± 96.88	63 ± 85.75	None
GSH $5x10^{-7}$ + GABA 10^{-6}	194.86 ± 136.87	155 ± 179.75	197.07 ± 67.40	184.5 ± 88.75	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	109.57± 50.93	89.5 ± 46.25	181.14 ± 72.13	194.5 ± 74.5	**TB<TP* p≤0.00758**
GSH $5x10^{-7}$ +Baclofen 10^{-8}	165.29 ± 111.01	199 ± 186.5	55.93 ± 21.69	53 ± 27.25	**TB<TP* p≤0.00364**
GSH $5x10^{-7}$ +Phaclofen 10^{-8} + Baclofen 10^{-8}	128 ±75.60	130 ± 81.25	81.64 ± 72.84	50 ± 47.75	None

Table 9: The comparison of MUHP responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was the same as treatment period 2 (T2). Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with Welch two-sample t-tests. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 9

MUHPS					
Treatment	**TB**		**TP**		**Significant Differences**
	μ + s.d	m + i.q.r	μ + s.d	m + i.q.r	
GSH $5x10^{-6}$	7.79 ± 7.00	6 ± 10.75	5.57 ± 5.82	3 ±7	None
GSH $5x10^{-}$	4.79 ± 4.79	4 ± 5.5	7.43 ± 6.02	6.5 ± 10.5	None
GSH $5x10^{-7}$ + GABA 10^{-6}	4.86 ± 6.56	1.5 ± 4.75	4.93 ± 6.64	2 ± 5	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	1.5 ± 1.92	1 ± 2	7.29 ± 5.67	7.5 ± 6.5	**TB<TP*, p≤0.00309**
GSH $5x10^{-7}$ +Baclofen 10^{-8}	7.5 ± 12.70	3 ± 4	5 ± 5.45	3 ± 6.25	None
GSH $5x10^{-7}$ +Phaclofen 10^{-8} + Baclofen 10^{-8}	1.36 ± 2.74	0 ± 1	4.14 ± 4.50	2.5 ± 4.5	**TB<TP^, p≤0.0702**

Table 10: The comparison of LUHP responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was the same as treatment period 2 (T2). Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with Welch two-sample t-tests. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 10

LUHPS					
Treatment	**TB**		**TP**		**Significant Differences**
	μ + s.d	m + i.q.r	μ + s.d	m + iqr	
GSH $5x10^{-6}$	1.07 ± 1.58	0 ± 1.75	0.86 ± 2.07	0 ± 0.75	None
GSH $5x10^{-7}$	0.86 ± 1.46	0 ± 1	4.14 ± 4.09	3.5 ± 6.25	**TB<TP*, p≤0.0146**
GSH $5x10^{-8}$	0.58 ± 0.64	0.5 ± 1	1.5 ± 1.76	1 ± 2	None
GSH $5x10^{-7}$ + GABA 10^{-6}	2.21 ± 2.43	1.5 ± 2.75	4.29 ± 6.32	1.5 ± 1.75	None
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	0.71 ± 0.96	0 ± 1	5.14 ± 5.44	4.5 ± 5.5	**TB<TP*, p≤0.0119**
GSH $5x10^{-7}$ +Baclofen 10^{-8}	1.71 ± 2.37	1 ± 2	2.07 ± 2.34	1.5 ± 2.75	None
GSH $5x10^{-7}$ +Phaclofen 10^{-8} + Baclofen 10^{-8}	0.29 ± 0.59	0 ± 0	4.79 ± 5.32	4 ± 7.25	**TB<TP*, p≤0.00943**

Table 11: The comparison of XLUHP responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was the same as treatment period 2 (T2). Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with Welch two-sample t-tests. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 11

XLUHPS					
Treatment	**TB**		**TP**		**Significant Differences**
	μ ± s.d	m ± i.q.r	μ ± s.d	m ± i.q.r	
GSH $5x10^{-6}$	2.71 ± 3.37	1 ± 4.25	0.36 ± 0.61	0 ± 0.75	**TB>TP*, p≤0.0265**
GSH $5x10^{-8}$	6 ± 7.51	0.5 ± 12	7.83 ± 10.73	3.5 ± 6	None
GSH $5x10^{-7}$ + GABA $1x10^{-6}$	5.29 ± 5.61	2 ± 8.75	18.86 ± 9.27	19 ± 7.75	**TB<TP*, p≤1.82e-4**
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	10.57 ± 7.46	10.5 ± 10.75	13.57 ± 10.72	15 ± 17.5	None
GSH $5x10^{-7}$ +Baclofen 10^{-8}	3.86 ± 5.41	1 ± 5.5	2.79 ± 5.24	0 ± 1	None
GSH $5x10^{-7}$ +Phaclofen 10^{-8} + Baclofen 10^{-8}	5.07 ± 5.11	2.5 ± 10.5	6.71 ± 7.57	2 ± 12.75	None

Table 12: The comparison of PBP responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was the same as treatment period 2 (T2). Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with Welch two-sample t-tests. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 12

PBPS					
Treatment	**TB**		**TP**		**Significant Differences**
	μ + s.d	m + i.q.r	μ + s.d	m + i.q.r	
GSH $5x10^{-6}$	0.14 ± 0.52	0 ± 0	1.14 ± 1.06	1.5 ± 2	**TB<TP*, p≤0.00649**
GSH $5x10^{-7}$	0.43 ± 0.50	0 ± 1	1.71 ± 0.96	2 ± 1	**TB<TP*, p≤3.69e-4**
GSH $5x10^{-8}$	0.83 ± 0.80	1 ± 1.25	1.58 ± 1.19	1 ± 1.25	**TB<TP^, p≤0.0982**
GSH $5x10^{-7}$ + GABA 10^{-6}	0.5 ± 0.5	0.5 ± 1	2 ± 0.93	2 ± 1.75	**TB<TP*, p≤4.99e-5**
GSH $5x10^{-7}$ +Baclofen 10^{-8}	0.86 ± 1.25	0 ± 1	1.43 ± 1.35	1.5 ± 3	None
GSH $5x10^{-7}$ +Phaclofen 10^{-8} + Baclofen 10^{-8}	0.43 ± 0.50	0 ± 1	1.71 ± 0.59	2 ± 1	**TB<TP*, p≤2.60e-6**

Table 13: The comparison of P/PBP responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was the same as treatment period 2 (T2). Data is reported as means and standard error ($\mu \pm$ s.e) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with Welch two-sample t-tests. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 13

P/PBPS					
Treatment	**TB**		**TP**		**Significant Differences**
	μ + s.e	m + i.q.r	μ + s.e	m + i.q.r	
GSH $5x10^{-6}$	0.68 ± 0.65	0 ± 0	5.35 ± 1.14	5 ± 8	**TB<TP*, p≤0.00256**
GSH $5x10^{-7}$	4.64 ± 1.70	0 ± 7.5	12.40 ± 1.80	11.84 ± 7.58	**TB<TP*, p≤0.005567**
GSH $5x10^{-8}$	6.13 ± 1.65	6.5 ± 11.25	6.52 ± 1.16	6.17 ± 3.44	None
GSH $5x10^{-7}$ + GABA 10^{-6}	7.71 ± 2.29	5 ± 14.25	14.02 ± 1.34	14.34 ± 6.0	**TB<TP*, p≤0.0326**
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	8.82 ± 1.66	11 ± 10.13	10.82 ± 2.85	7.63 ± 16.92	None
GSH $5x10^{-7}$ +Baclofen 10^{-8}	3.84 ± 1.31	0 ± 7	4.58 ± 1.15	5.83 ± 7.59	None
GSH $5x10^{-7}$ +Phaclofen 10^{-8} + Baclofen 10^{-8}	4.64 ± 1.51	0 ± 10.5	13.06 ± 1.91	10 ± 7.75	**TB<TP*, p≤0.00272**

Table 14: The comparison of RP responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was the same as treatment period 2 (T2). Data is reported as means and standard error ($\mu \pm$ s.e) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with Welch two-sample t-tests. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 14

RPs					
Treatment	**TB**		**TP**		**Significant Differences**
	μ + s.e	m + i.q.r	μ + s.e	m + i.q.r	
GSH $5x10^{-6}$	6 ± 1.51	4.5 ± 6	7 ± 1.80	6 ± 5.5	None
GSH $5x10^{-7}$	1 ± 0.38	0.5 ± 1	11 ± 3.81	3.5 ± 17.75	**TB<TP*, p≤0.0254**
GSH $5x10^{-8}$	1.67 ± 0.60	1 ± 1.25	1.92 ± 0.38	1 ± 2.25	None
GSH $5x10^{-7}$ + GABA 10^{-6}	2.36 ± 0.55	1.5 ± 3	6.21 ± 1.09	4.5 ± 6.75	**TB<TP*, p≤0.00657**
GSH $5x10^{-7}$ + Phaclofen 10^{-8}	4.79 ± 1.50	2 ± 6.5	3.29 ± 0.94	2 ± 4.5	None
GSH $5x10^{-7}$ +Baclofen 10^{-8}	2.36 ± 0.95	1 ± 2.75	3.79 ± 0.85	2.5 ± 5.75	None
GSH $5x10^{-7}$ +Phaclofen 10^{-8} + Baclofen 10^{-8}	4.93 ± 1.34	4 ± 4.5	3.29 ± 0.85	2.5 ± 6	None

Table 15: The comparison of responses in bath-applied method vs. pipette applied method where treatment period 1 (T1) was different from treatment period 2 (T2). Data is reported as means and standard deviation ($\mu \pm$ s.d) and as medians and interquartile range (m $\pm$ i.q.r). Significance was calculated with Welch two-sample t-tests. Asterisks denote a significant difference. Carets denote a potentially significant difference. a) GSH $5x10^{-7}$M, XLUHPs b) GSH $5x10^{-8}$M, MUHPs c) GSH $5x10^{-7}$M + Phaclofen 10^{-8}M: PBPs.

Table 15

a)

GSH $5x10^{-7}$, XLUHPS			
	$T1_B$	**$T1_P$**	**Significant Differences**
μ ± s.d	3.71 ± 3.81	12.86 ± 13.31	None
m ± i.q.r	3 ± 3.5	7 ± 16.5	
	$T2_B$	**$T2_P$**	
μ ± s.d	2 ± 3.74	12.86 ± 10.55	**$T2_B<T2_P$*, $p\leq0.0469$**
m ± i.q.r	0 ± 1.5	13 ± 20	

b)

GSH $5x10^{-8}$, MUHPS			
	$T1_B$	**$T1_P$**	**Significant Differences**
μ ± s.d	9 ± 7.46	7 ± 7.94	None
m ± i.q.r	8.5 ± 8.5	3 ± 6.75	
	$T2_B$	**$T2_P$**	**Significant Differences**
μ ± s.d	6 ±7	3 ± 2.77	None
m ± i.q.r	2.5 ± 10.50	2 ± 3.5	

c)

GSH $5x10^{-7}$ + Phaclofen 10^{-8}, PBPs			
	$T1_B$	**$T1_P$**	**Significant Differences**
μ ± s.d	0.86 ± 0.64	2.14 ± 1.25	**$T1_P>T1_B$^, $p\leq0.0617$**
m ± i.q.r	1 ± 0.5	2 ± 1.5	
	$T2_B$	**$T2_P$**	
μ ± s.d	1 ± 0.76	0.86 ± 0.83	None
m ± i.q.r	1 ± 1.0	1 ± 1.5	

Table 16: The comparison of the dose response of GSH $5x10^{-8}$, GSH $5x10^{-7}$, GSH $5x10^{-6}$ in the a) bath-applied method and b) pipette-applied method. Data is reported as means and standard deviation ($\mu \pm$ s.d) for SUHPs, MUHPs, LUHPs, XLUHPs, PBPs and as means and standard error ($\mu \pm$ s.e) for P/PBPs and RPs. Asterisks denote a significant difference. Carets denote a potentially significant difference.

Table 16:

a)

GSH: Bath-Applied				
	$5x10^{-8}$	**$5x10^{-7}$**	**$5x10^{-6}$**	**Significant Differences**
SUHPs	165.29 ±94.00	84.07 ± 55.15	249.36 ± 78.78	**$5x10^{-7} < 5x10^{-6}$, p≤0.00195*, $5x10^{-7} < 5x10^{-8}$,p≤0.0604^**
MUHPs	6.86 ± 7.03	4.79 ± 4.78	7.79 ± 7.00	None
LUHPs	0.57 ± 0.62	0.86 ± 1.46	1.07 ± 1.33	None
XLUHPs	6 ± 7.20	2.93 ± 3.86	3.71 ± 5.72	None
PBPs	0.78 ± 0.77	0.43 ± 0.49	0.14 ± 0.52	**$5x10^{-6} < 5x10^{-8}$ p≤0.00444***
P/PBPs	6.11 ± 1.44	4.64 ± 1.59	0.68 ± 0.66	**$5x10^{-6} < 5x10^{-8}$ p≤0.0184***
RPs	1.5 ± 0.50	0.79 ± 0.33	6 ± 1.51	**$5x10^{-6} > 5x10^{-7}$ p≤0.0104***

b)

GSH: Pipette-Applied				
	$5x10^{-8}$	**$5x10^{-7}$**	**$5x10^{-6}$**	**Significant Differences**
SUHPs	165.29 ± 94.00	149.79 ± 69.91	166.50 ± 135.27	None
MUHPs	6.89 ± 7.03	7.43 ± 6.02	5.57 ± 5.82	None
LUHPs	1.5 ± 1.76	4.42 ± 4.23	1 ± 2.20	**$5x10^{-6} < 5x10^{-7}$, p≤0.032***
XLUHPs	7.83 ± 10.73	12.33 ± 12.09	0.42 ± 0.64	**$5x10^{-6} < 5x10^{-7}$, p≤0.0140***
PBPs	1.58 ± 1.19	1.92 ± 0.86	1 ± 1.08	**$5x10^{-6} < 5x10^{-7}$, p≤0.0483***
P/PBPs	6.52 ± 1.16	12.46 ± 1.43	4.53 ± 1.07	**$5x10^{-6} < 5x10^{-7}$, p≤0.0379***
RPs	1.5 ± 0.50	11 ± 3.81	7 ± 1.80	None

Figure 1. Schematic diagram of an ablated hypostome.

Figure 1.

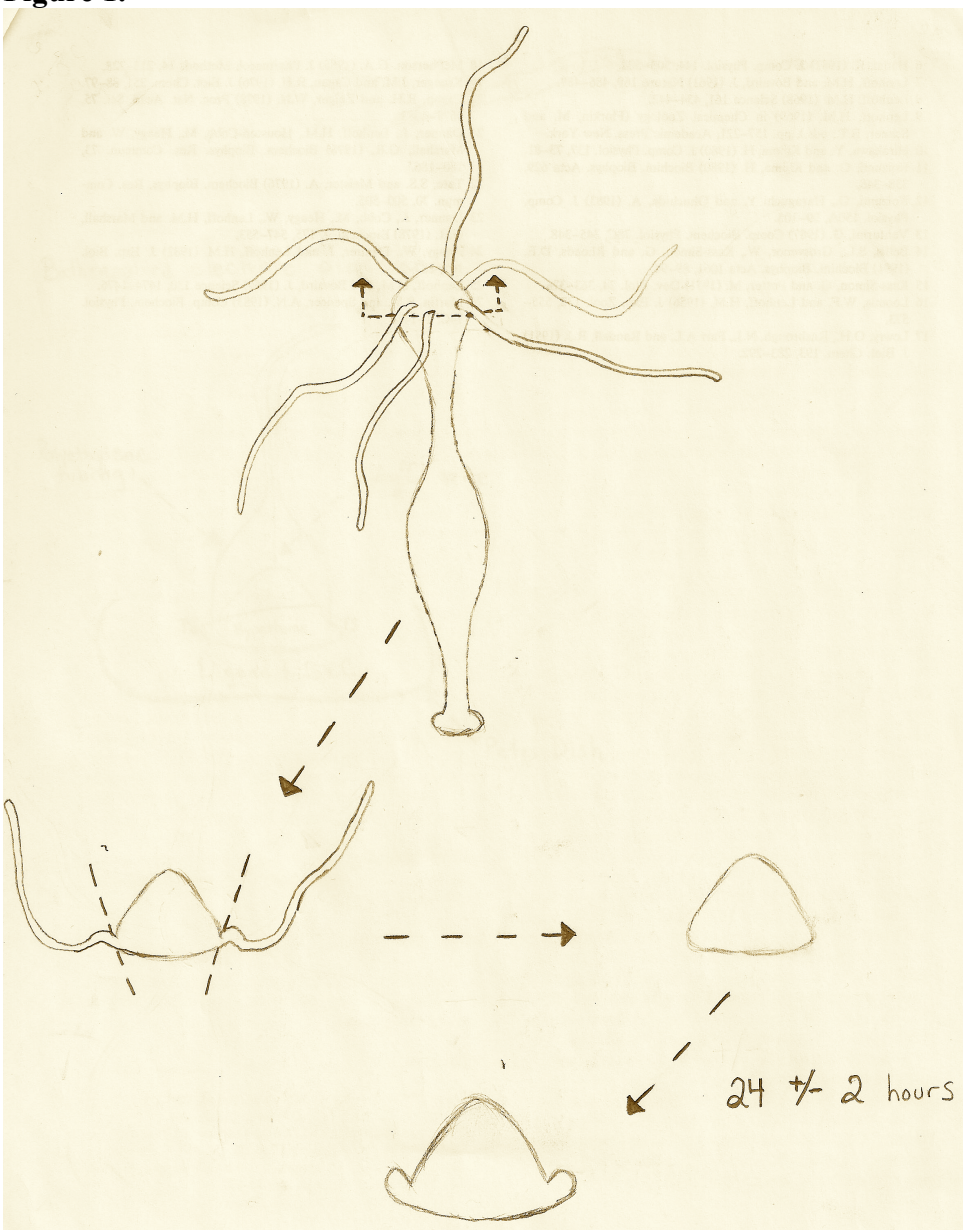


Figure 2. A side view of an ablated hypostome preparation after 24-hr regeneration. Tentacle stubs are labeled TS. The hypostome (mouth) is labeled M.

Figure 2.

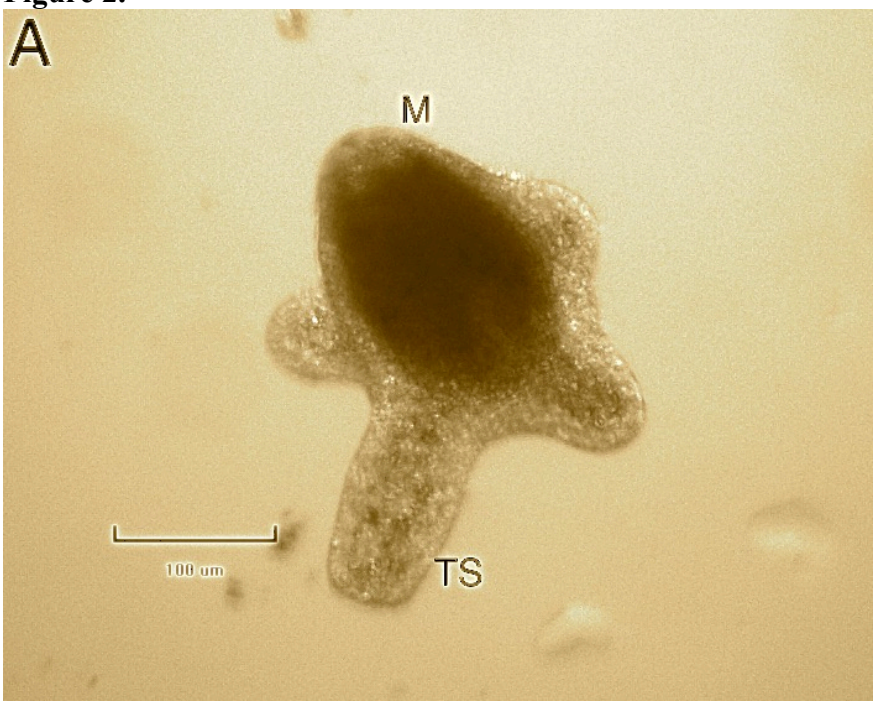


Figure 3. Schematic diagram of bath-applied method electrode placement.

Figure 3.

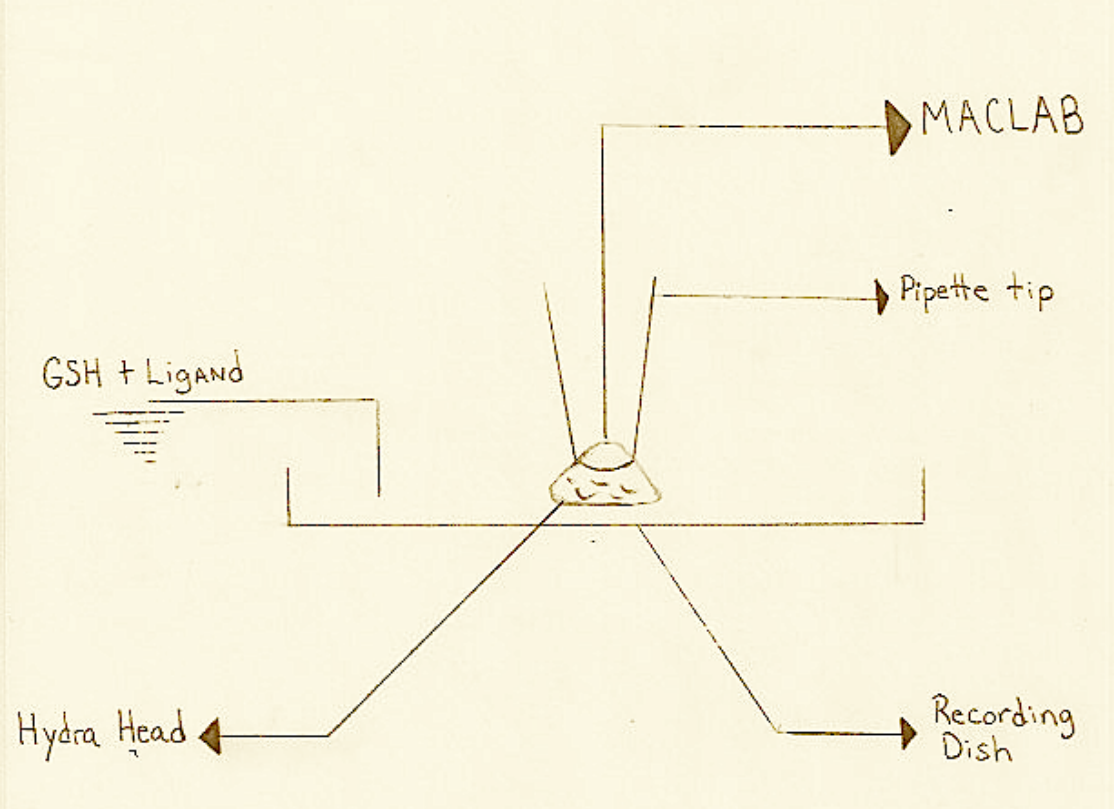
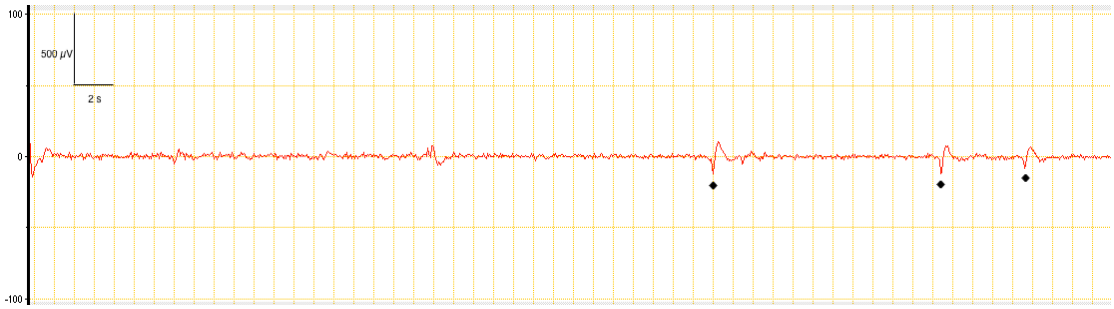


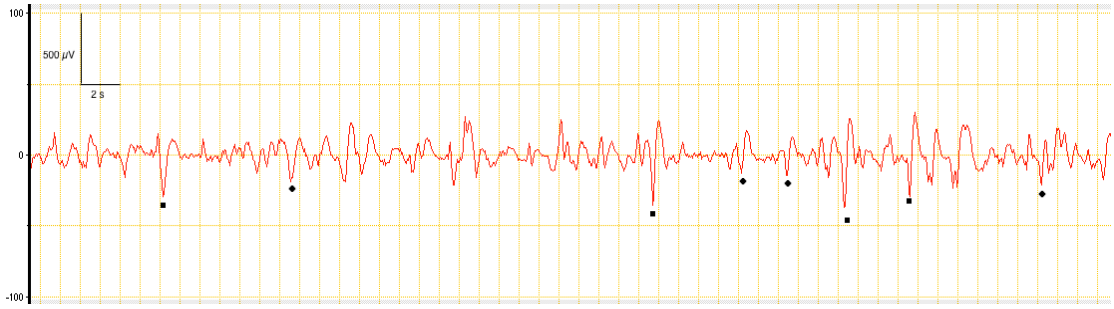
Figure 4. Sample recording from the bath-applied method with GSH $5x10^{-6}$ M. a) control period (C2); b) treatment period (T1); c) treatment period (T2). Samples were taken from comparable times after the addition of the test substance in each treatment period, and control period C2. Diamonds (SUHPs), squares (MUHPs) and stars (LUHPs).

Figure 4.

a.



b.



c.

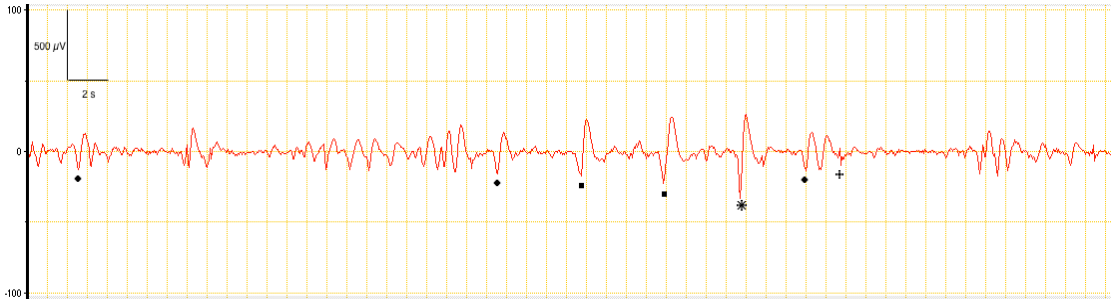


Figure 5. Schematic diagram of pipette-applied electrode placement.

Figure 5.

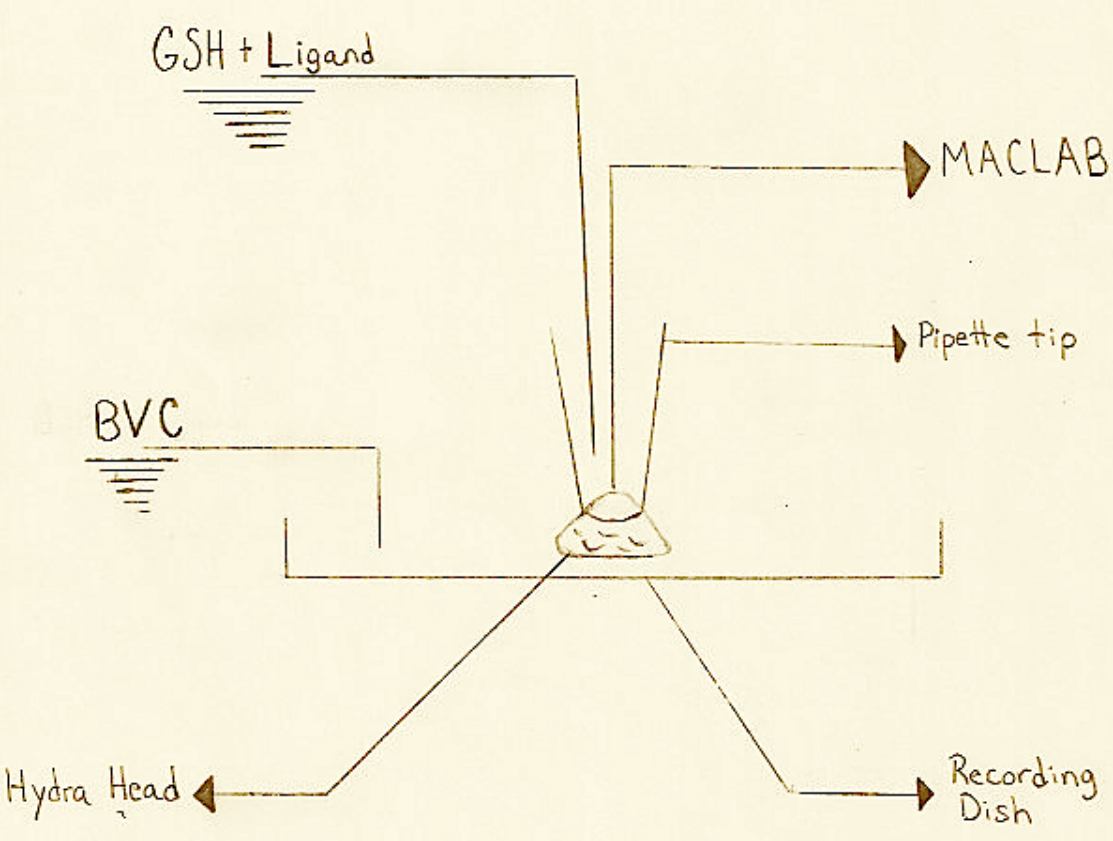
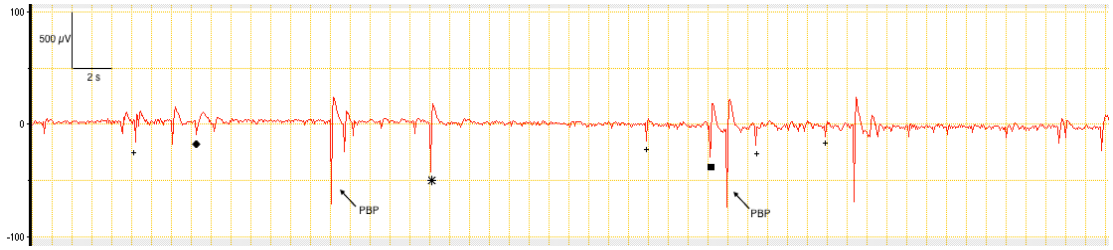

GSH + Ligand
MACLAB
Pipette tip
BVC
Hydra Head
Recording Dish

Figure 6. Sample recording from the pipette-applied method with GSH $5x10^{-6}$M. a) treatment period (T1); b) treatment period (T2). Samples were taken from comparable times after the addition of the test substance in each treatment period. Diamonds (SUHPs), crosses (RPs), squares (MUHPs), stars (LUHPs) and arrows (PBPs).

Figure 6.

a.



b.

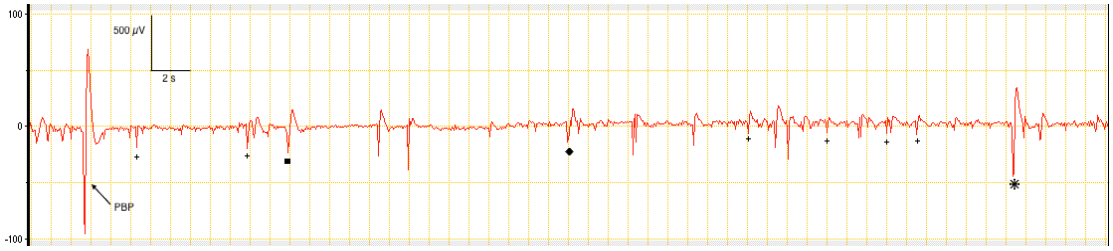


Figure 7. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on SUHPs in the bath-applied method. Control period BVC (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d). * (significant differences).

Figure 7.

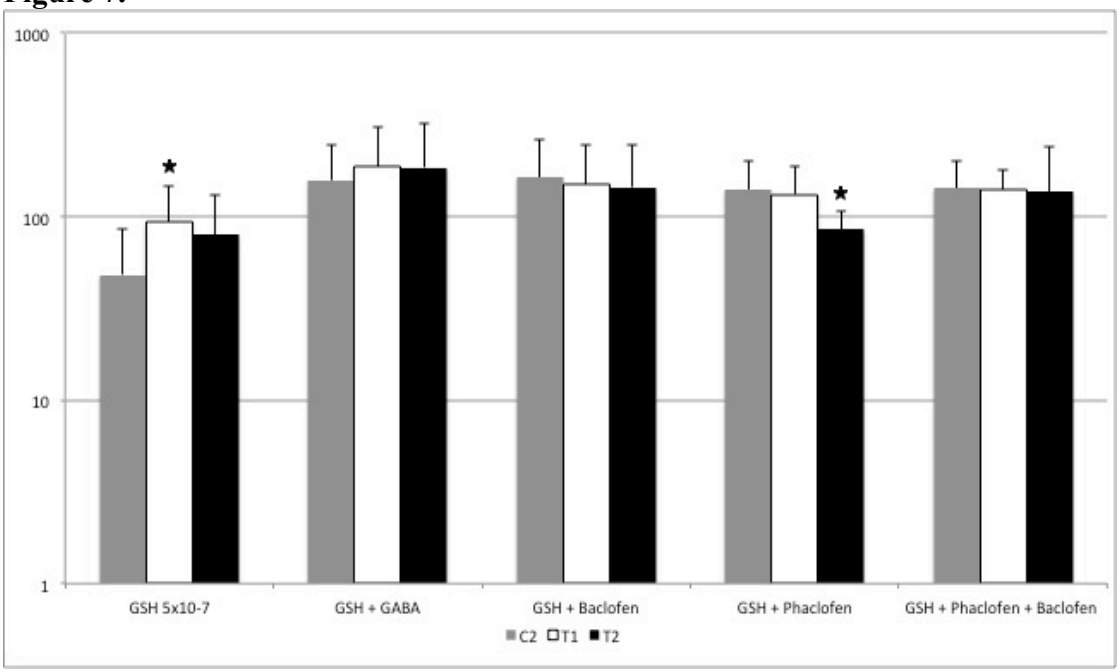

1000
100
10
1
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C2 T1 T2

Figure 8. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on XLUHPs in the bath-applied method. Control period BVC (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d). * (significant differences), ** (potentially significant differences).

Figure 8.

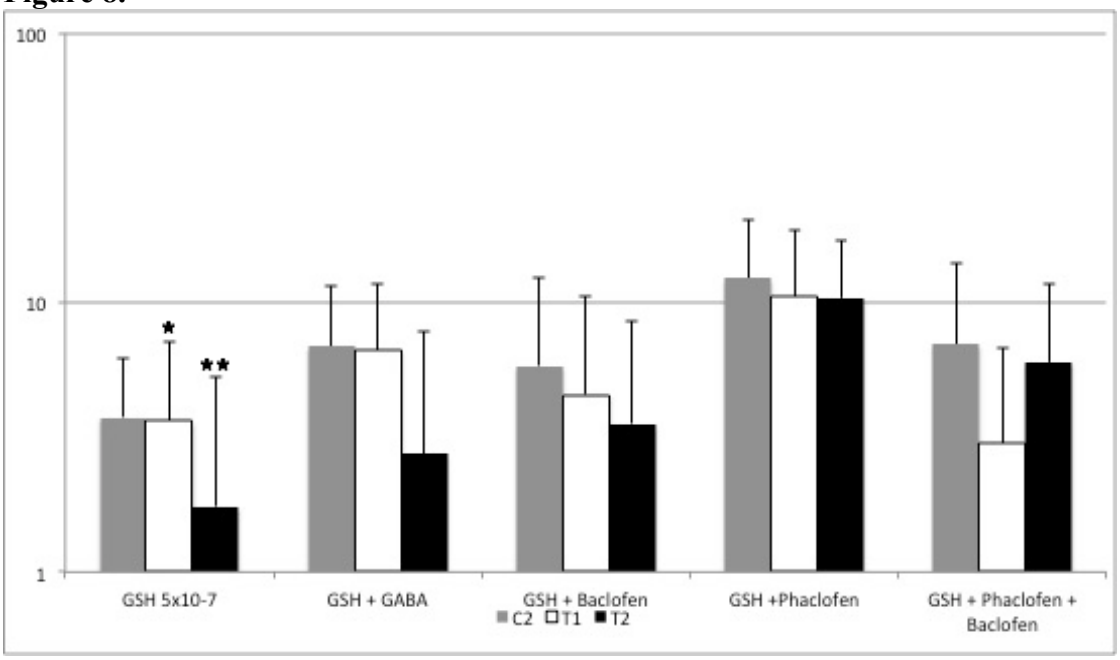

100
10
1
*
**
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH +Phaclofen
GSH + Phaclofen + Baclofen
C2 T1 T2

Figure 9. The bath-applied method effect on the GSH dose response for SUHPs, MUHPs and RPs. Data is reported as mean and standard deviation (SUHPs, MUHPs) ($\mu \pm$ s.d.) and means and standard error (RPs) ($\mu \pm$ s.e). Black diamonds (SUHPs), squares (MUHPs), white diamonds (RPs).

Figure 9.

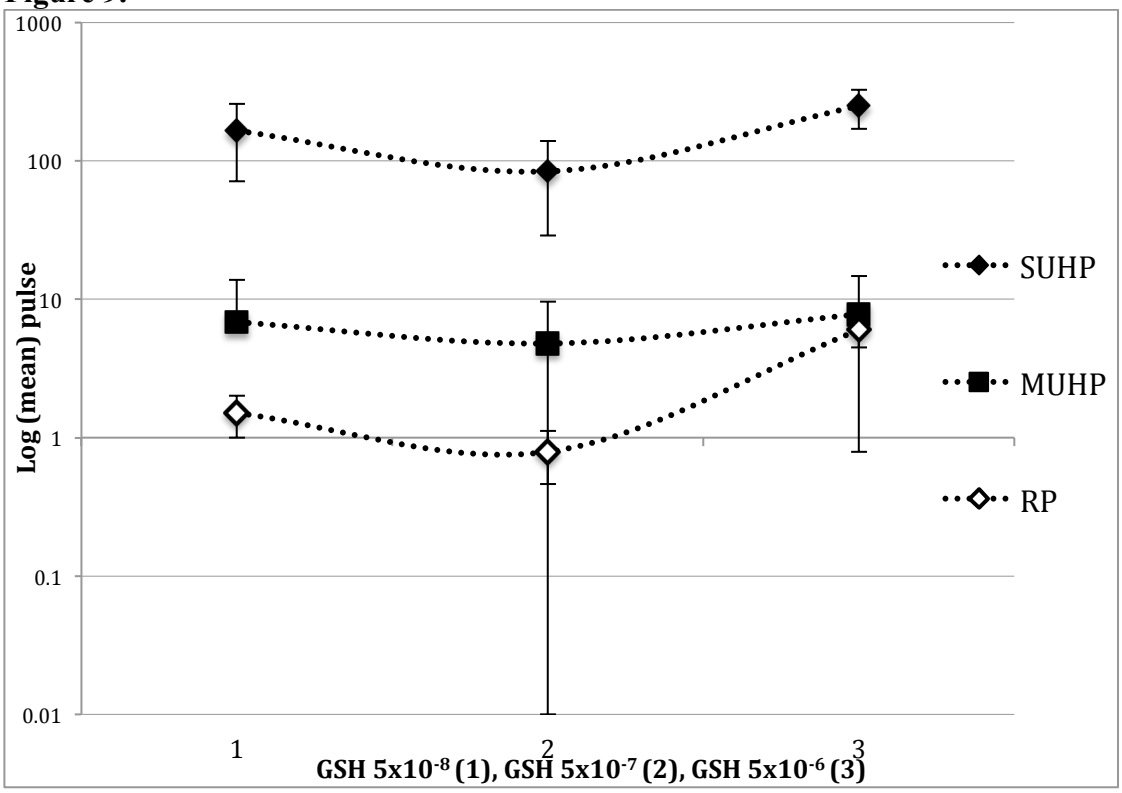

1000
100
10
1
0.1
0.01
Log (mean) pulse
1
2
3
GSH 5x10-8 (1), GSH 5x10-7 (2), GSH 5x10-6 (3)
SUHP
MUHP
RP

Figure 10. The bath-applied method effect on the GSH dose response for LUHPs and XLUHPs. Control is (C2) from bath-applied BVC. Data is reported as mean and standard deviation ($\mu \pm$ s.d.). Stars (LUHPs) and circles (XLUHPs).

Figure 10.

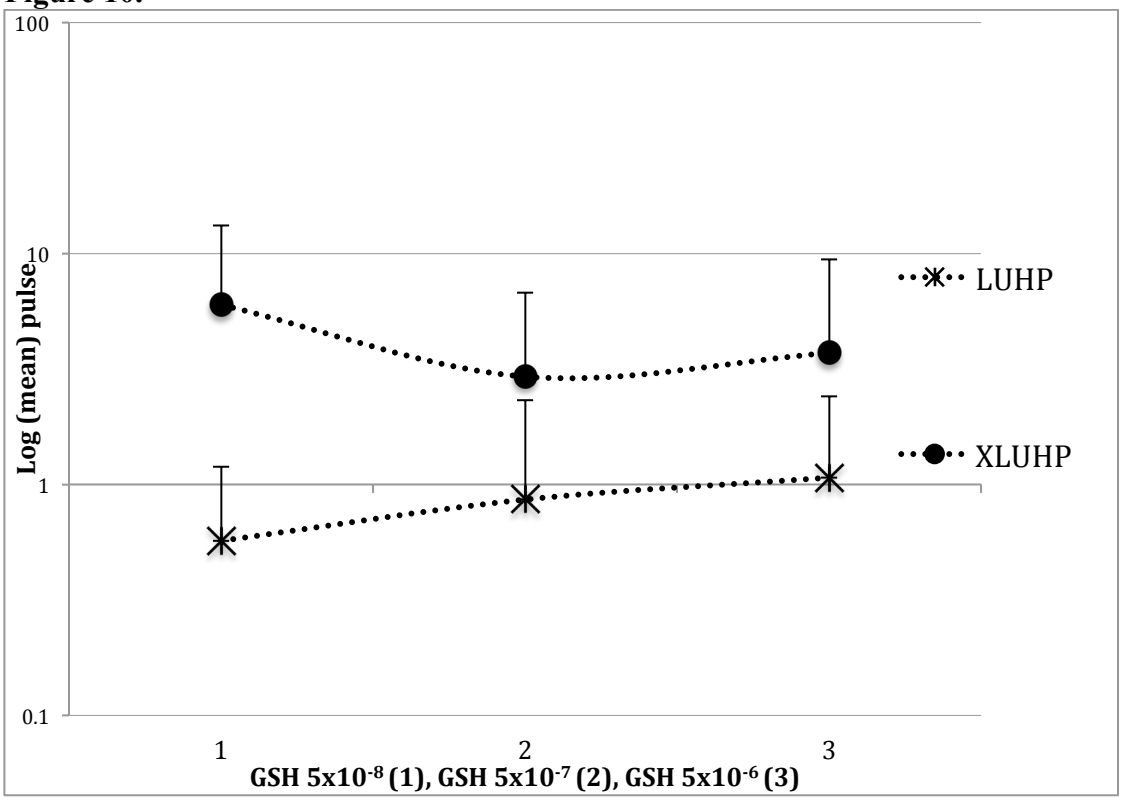

100
10
1
0.1
Log (mean) pulse
LUHP
XLUHP
1
2
3
GSH 5x10-8 (1), GSH 5x10-7 (2), GSH 5x10-6 (3)

Figure 11. The bath-applied method effect on the GSH dose response for PBPs and P/PBPs. Control is (C2) from bath-applied BVC. Data is reported as mean and standard deviation (PBPs) ($\mu \pm$ s.d.) and means and standard error (P/PBPs) ($\mu \pm$ s.e). Black triangles (PBPs) and clear triangles (P/PBPs).

Figure 11.

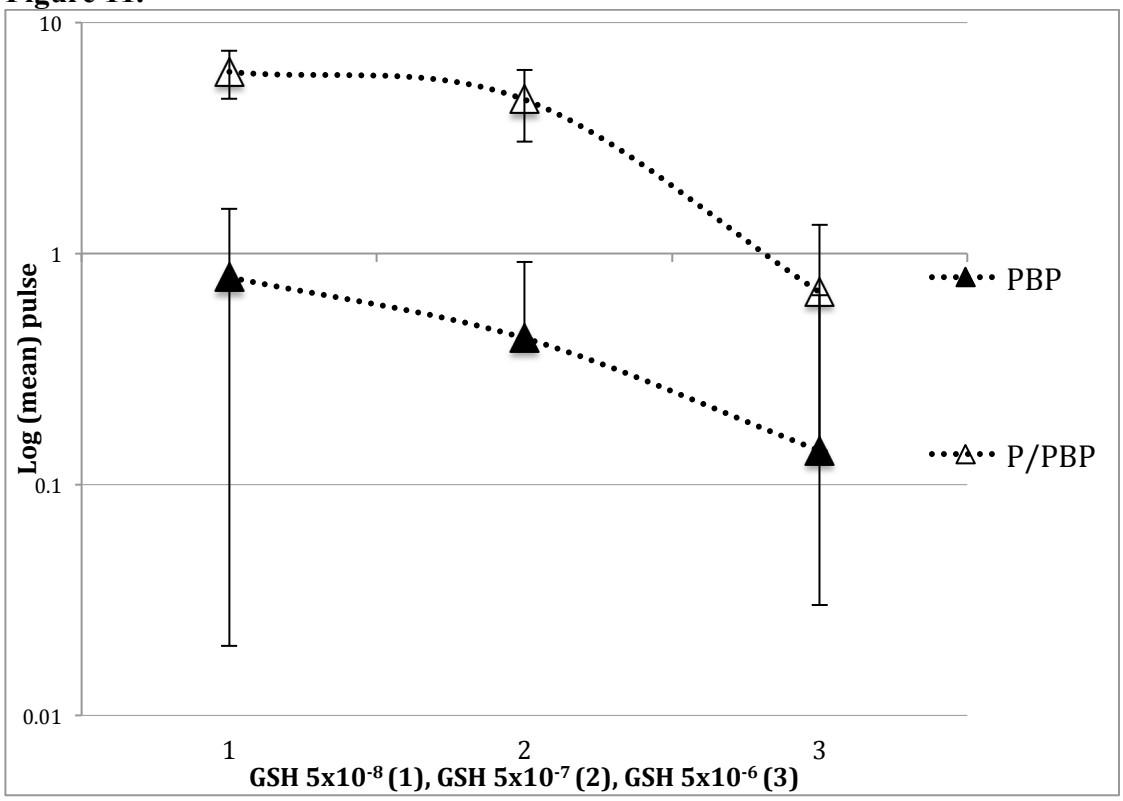

10
1
0.1
0.01
Log (mean) pulse
1
2
3
GSH 5x10-8 (1), GSH 5x10-7 (2), GSH 5x10-6 (3)
PBP
P/PBP

Figure 12. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on MUHPs in the pipette-applied method. Control period BVC (C1) (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d). ** (potentially significant differences).

Figure 12.

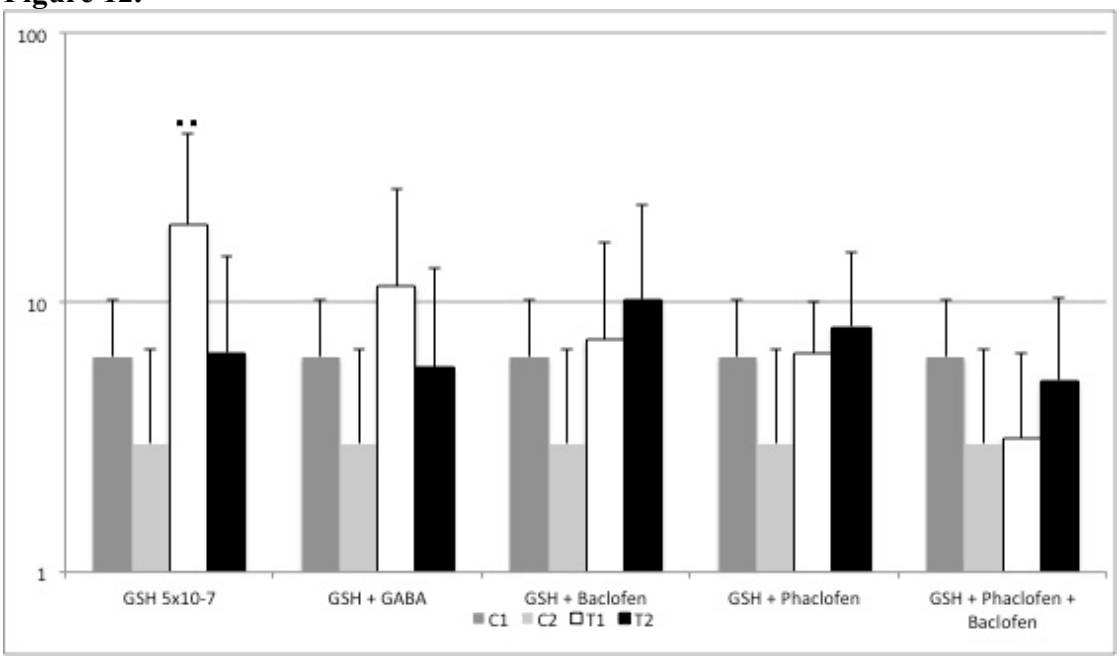

100
10
1
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C1
C2
T1
T2

Figure 13. The pipette-applied method effect on the GSH dose response for LUHPs and XLUHPs. Control is (C1) and (C2) from bath-applied BVC. Data is reported as mean and standard deviation ($\mu \pm$ s.d.). Stars (LUHPs) and circles (XLUHPs).

Figure 13.

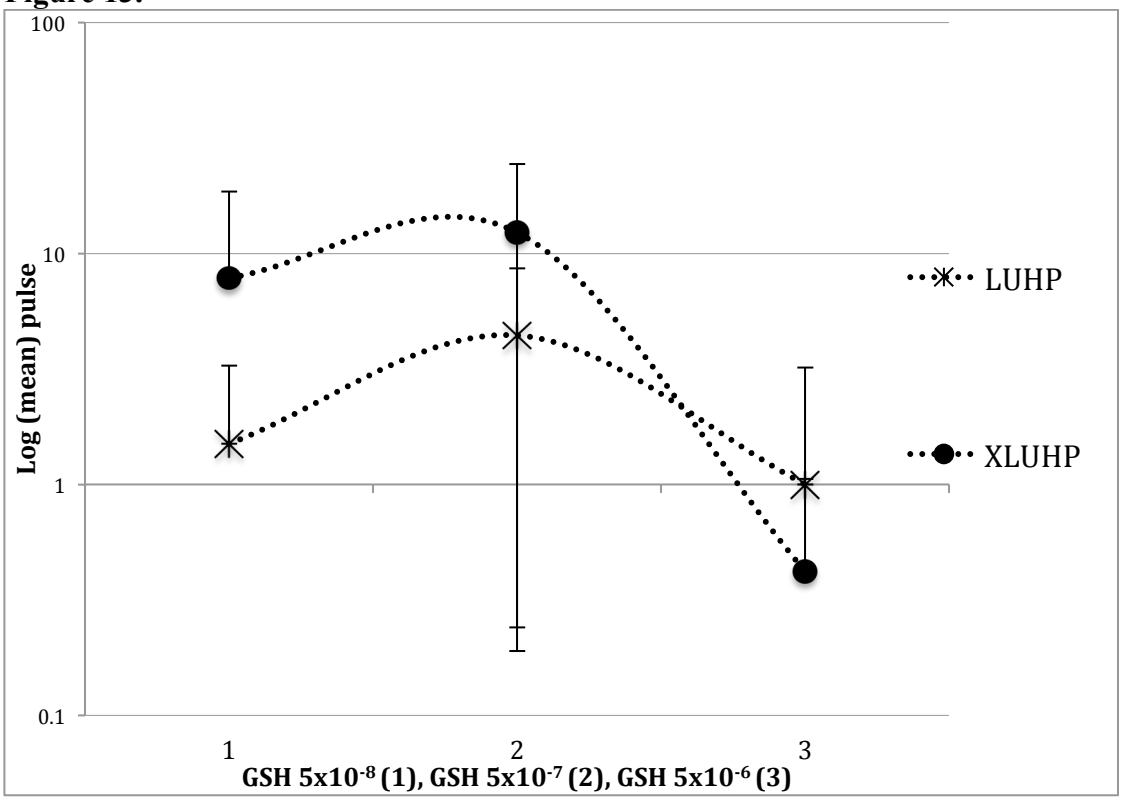

100
10
1
0.1
Log (mean) pulse
LUHP
XLUHP
1
2
3
GSH 5x10-8 (1), GSH 5x10-7 (2), GSH 5x10-6 (3)

Figure 14. The pipette-applied method effect on the GSH dose response for PBPs and P/PBPs. Control is (C1) and (C2) from bath-applied BVC. Data is reported as mean and standard deviation (PBPs) ($\mu \pm$ s.d.) and means and standard error (P/PBPs) ($\mu \pm$ s.e). Black triangles (PBPs) and clear triangles (P/PBPs).

Figure 14.

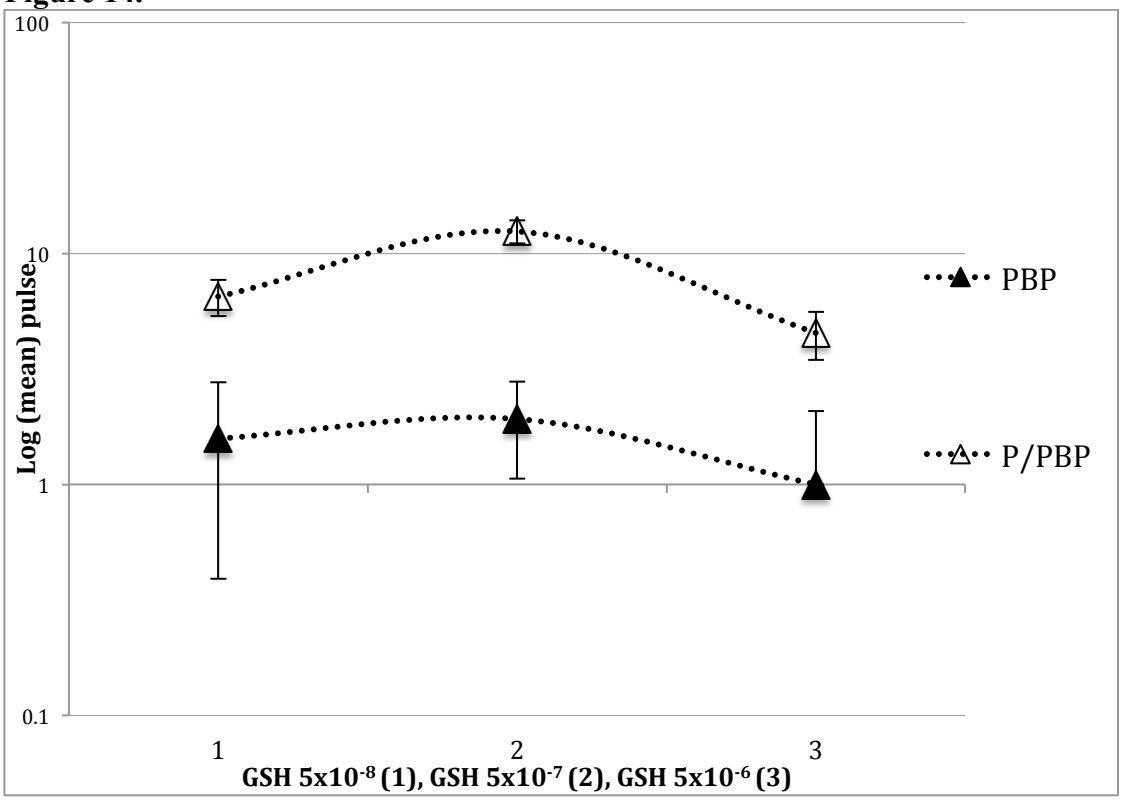

100
10
1
0.1
Log (mean) pulse
PBP
P/PBP
1
2
3
GSH 5x10-8 (1), GSH 5x10-7 (2), GSH 5x10-6 (3)

Figure 15. The pipette-applied method effect on the GSH dose response for SUHPS, MUHPs and RPs. Control is (C1) and (C2) from bath-applied BVC. Data is reported as mean and standard deviation (SUHPs, MUHPs) ($\mu \pm$ s.d.) and means and standard error (RPs) ($\mu \pm$ s.e). Black diamonds (SUHPs), squares (MUHPs) and white diamonds (RPs).

Figure 15.

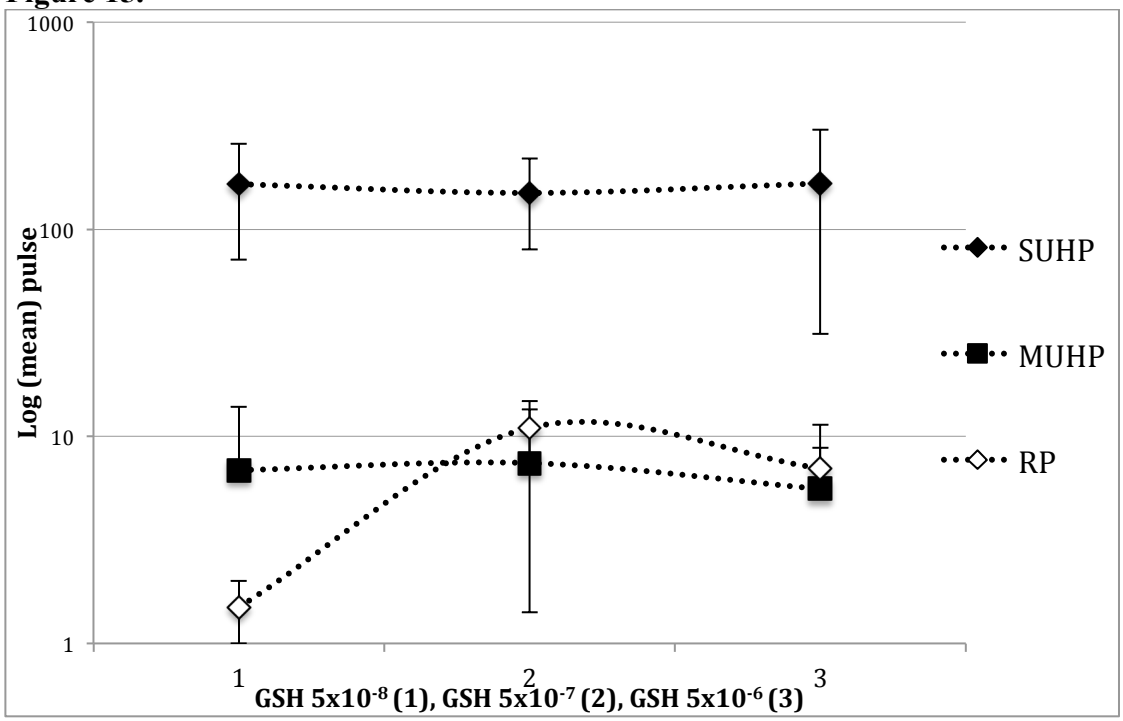

1000
100
10
1
Log (mean) pulse
1
2
3
GSH 5x10-8 (1), GSH 5x10-7 (2), GSH 5x10-6 (3)
SUHP
MUHP
RP

Figure 16. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on MUHPs in the bath-applied method. Control period BVC (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d). * (significant differences), ** (potentially significant differences).

Figure 16.

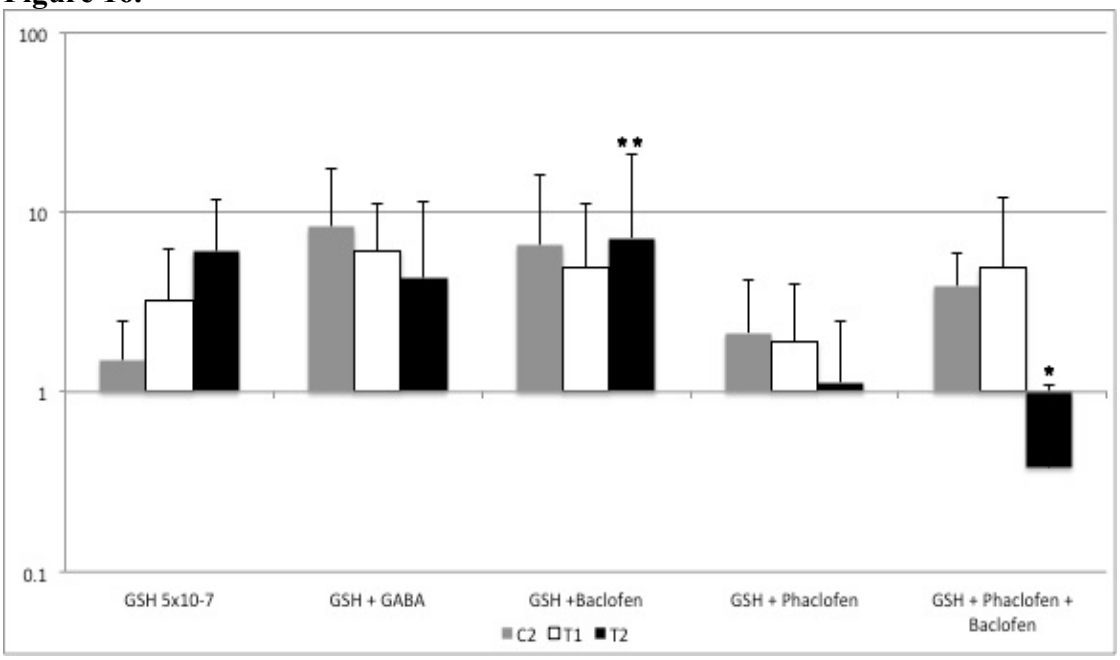

100
10
1
0.1
**
*
GSH 5x10-7
GSH + GABA
GSH +Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C2
T1
T2

Figure 17. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on LUHPs in the bath-applied method. Control period BVC (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d).

Figure 17.

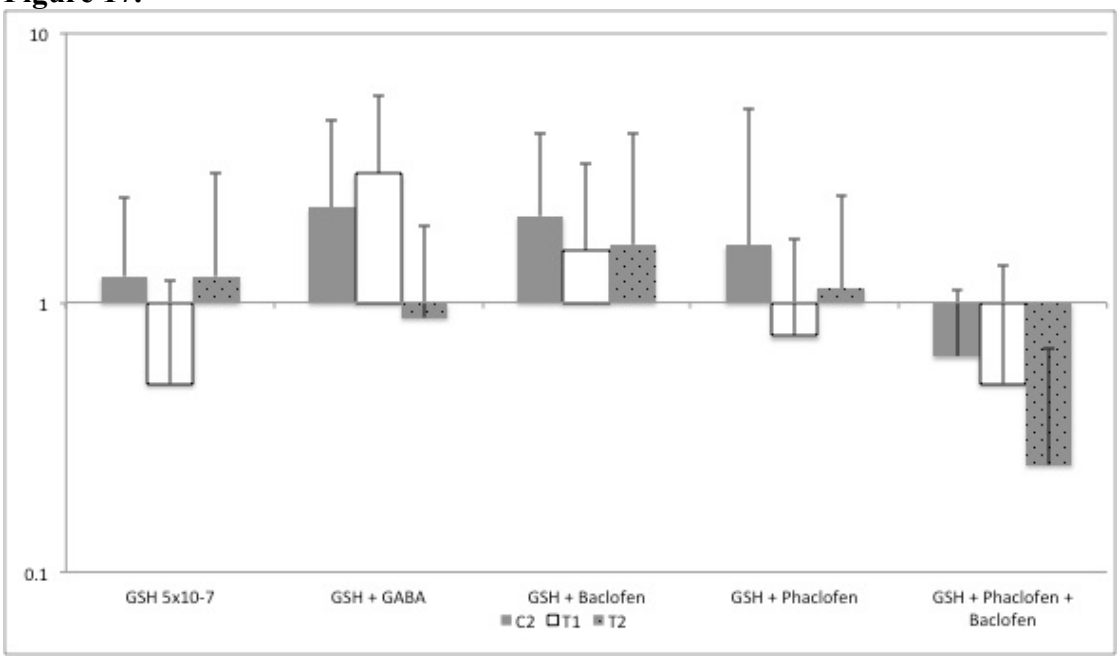

10
1
0.1
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C2
T1
T2

Figure 18. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on PBPs in the bath-applied method. Control period BVC (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d).

Figure 18.

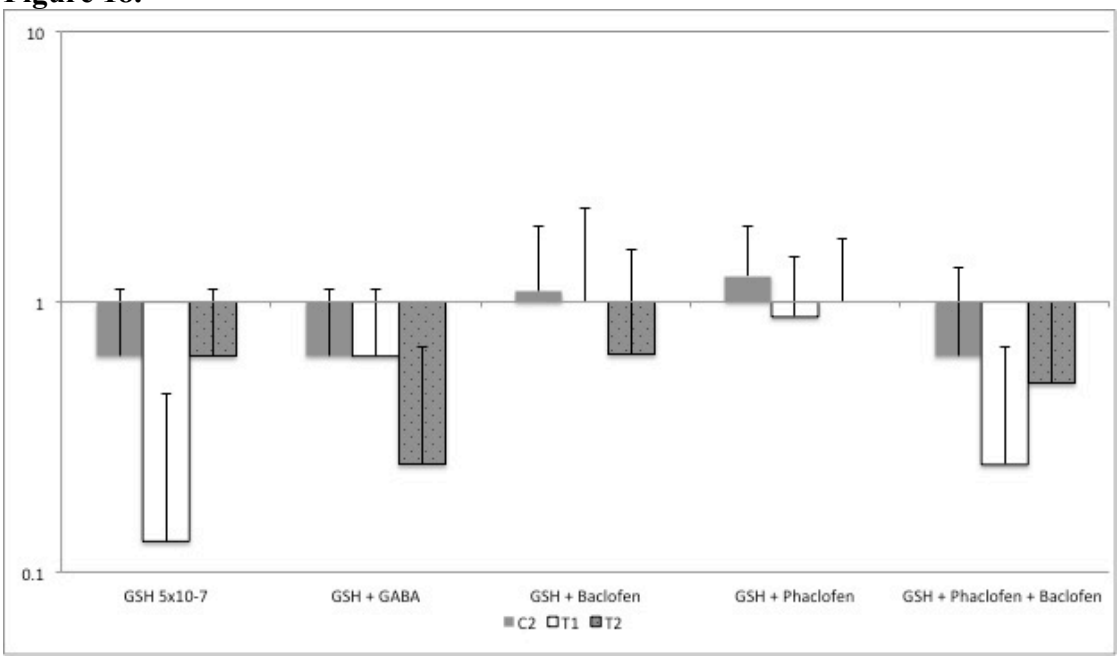

10
1
0.1
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C2
T1
T2

Figure 19. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on P/PBPs in the bath-applied method. Control period BVC (C2). Data is reported as means and standard error ($\mu \pm$ s.e).

Figure 19.

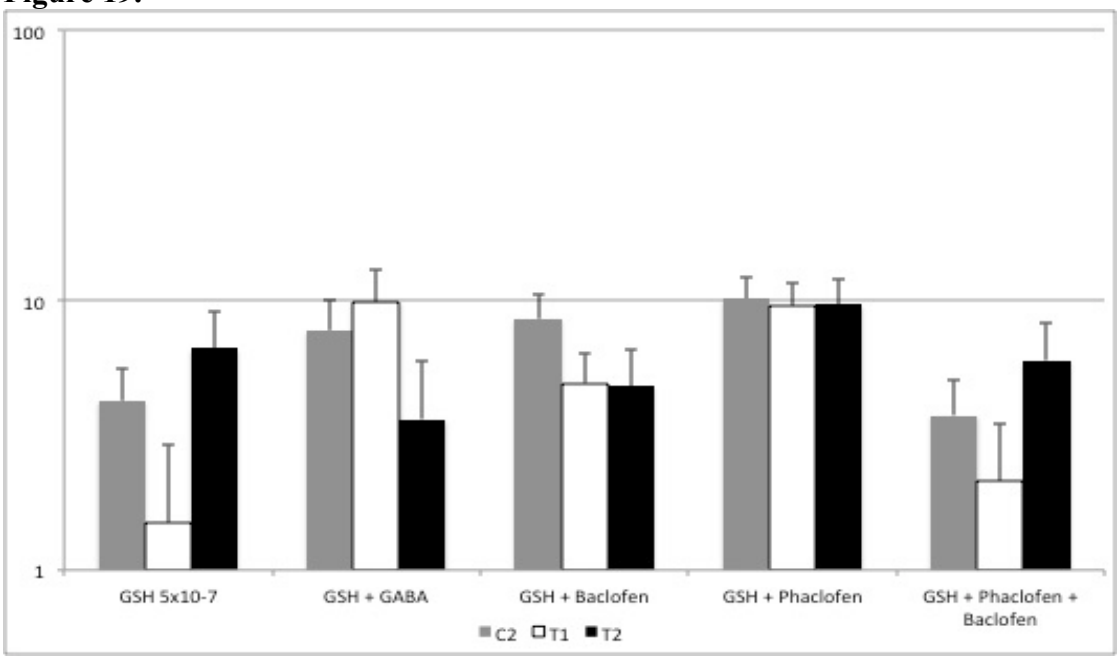

100
10
1
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C2
T1
T2

Figure 20. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on RPs in the bath-applied method. Control period BVC (C2). Data is reported as means and standard error ($\mu \pm$ s.e). * (significant differences).

Figure 20.

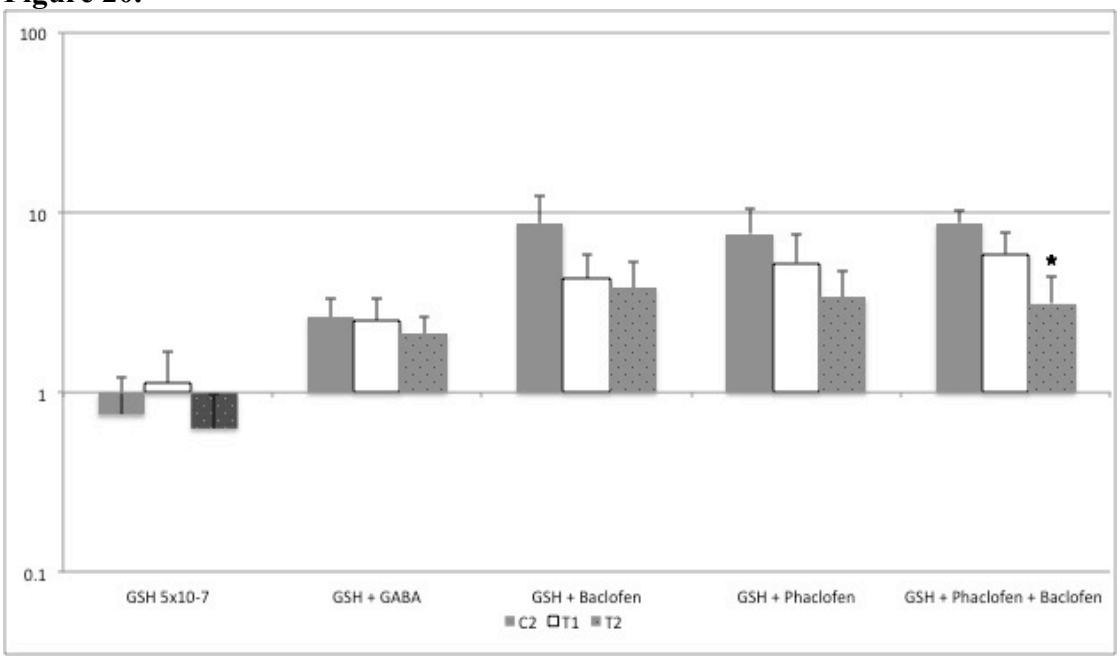

100
10
1
0.1
*
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C2 T1 T2

Figure 21. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on LUHPs in the pipette-applied method. Control period BVC (C1) (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d). * (significant differences), ** (potentially significant differences).

Figure 21.

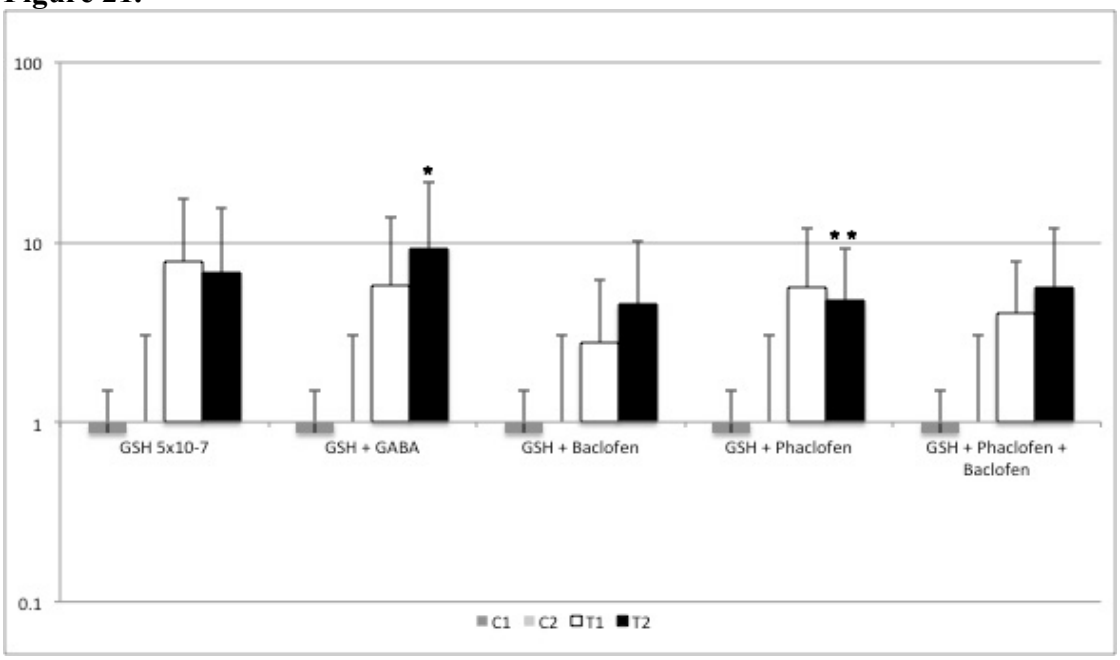

100
10
1
0.1
*
* *
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C1
C2
T1
T2

Figure 22. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on P/PBPs in the pipette-applied method. Control period BVC (C1) (C2). Data is reported as means and standard error ($\mu \pm$ s.e). * (significant differences), ** (potentially significant differences).

Figure 22.

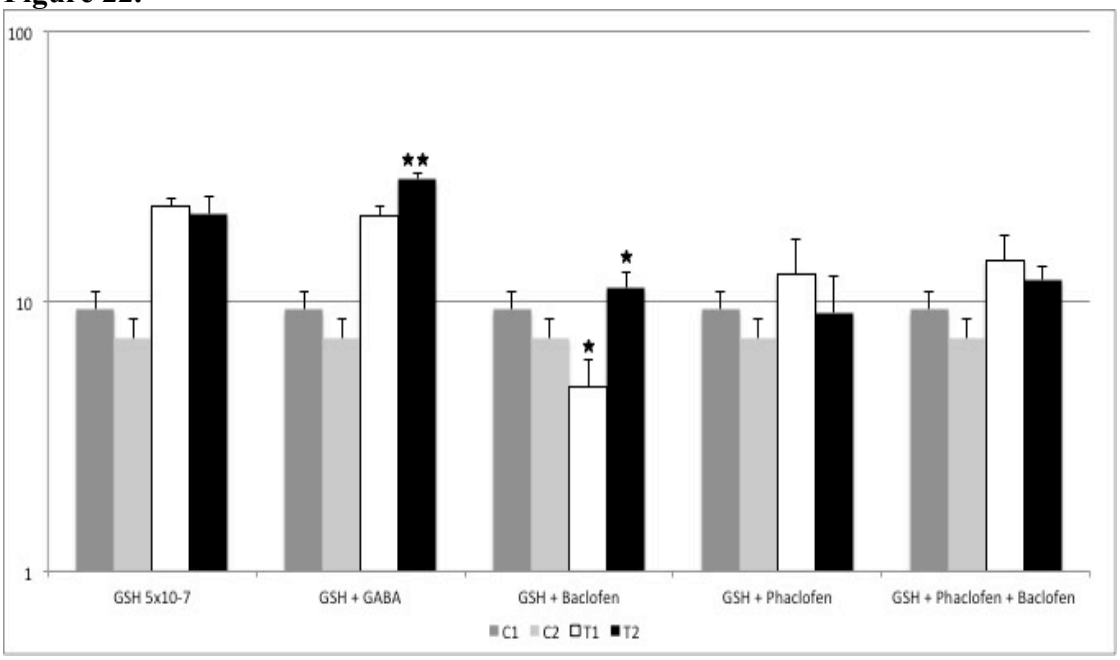

100
10
1
**
*
*
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C1 C2 T1 T2

Figure 23. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on XLUHPs in the pipette-applied method. Control period BVC (C1) (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d). * (significant differences).

Figure 23.

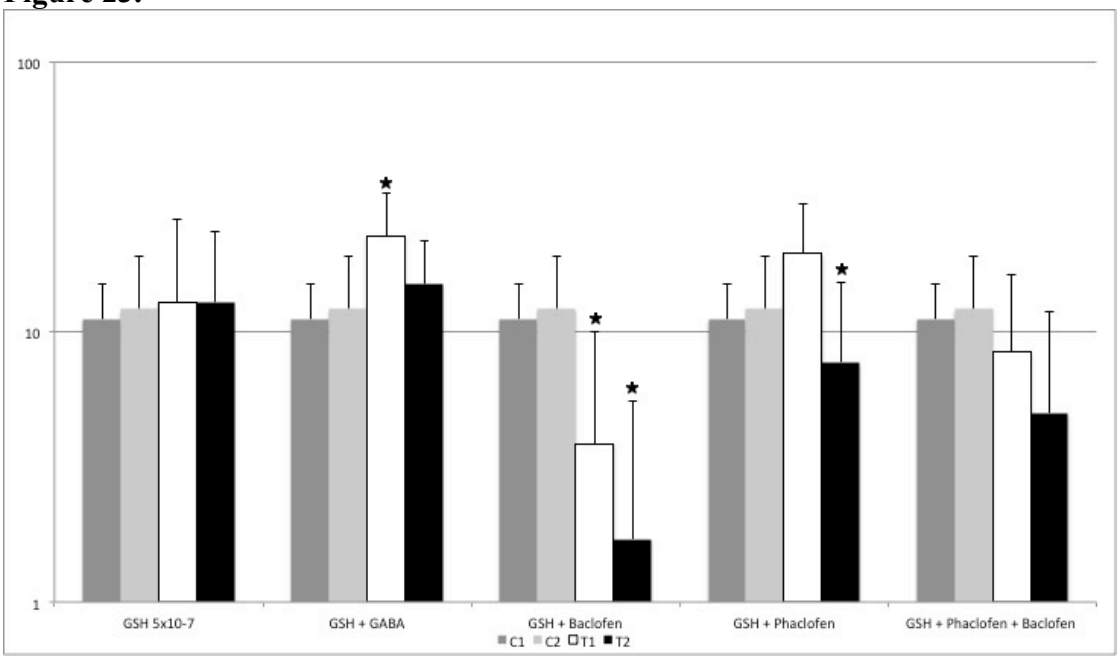

100
10
1
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C1 C2 T1 T2

Figure 24. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on RPs in the pipette-applied method. Control period BVC (C1) (C2). Data is reported as means and standard error ($\mu \pm$ s.e). * (significant differences).

Figure 24.

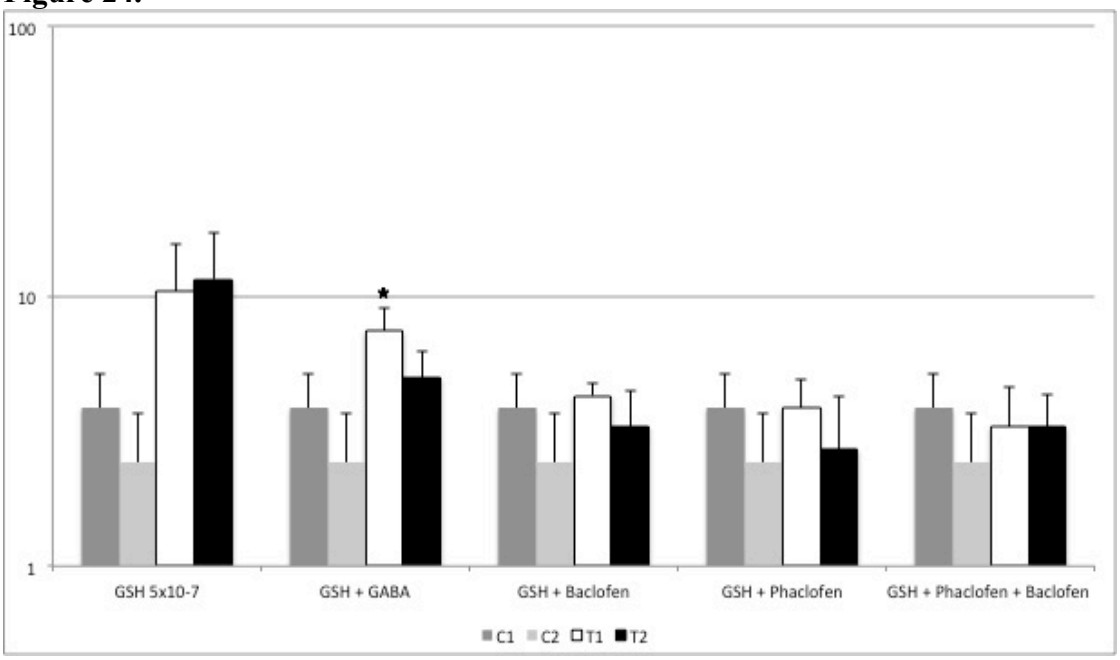
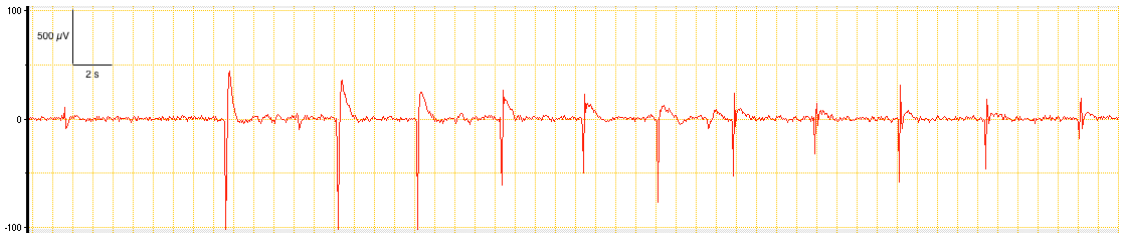

100
10
1
*
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C1
C2
T1
T2

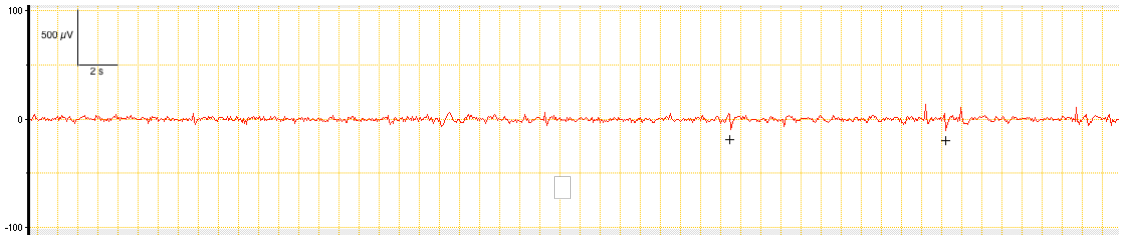
Figure 25. Sample recording with GSH $5x10^{-7}$ and GABA 10^{-6} in a) control (C2) bath-applied b) GSH $5x10^{-7}$ + GABA 10^{-6} (T2) bath-applied method c) control (C2) bath-applied method d) GSH $5x10^{-7}$ + GABA 10^{-6} pipette-applied method. Diamonds (SUHPs), squares (MUHPs), stars (LUHPs) and arrows (PBPs).

Figure 25.

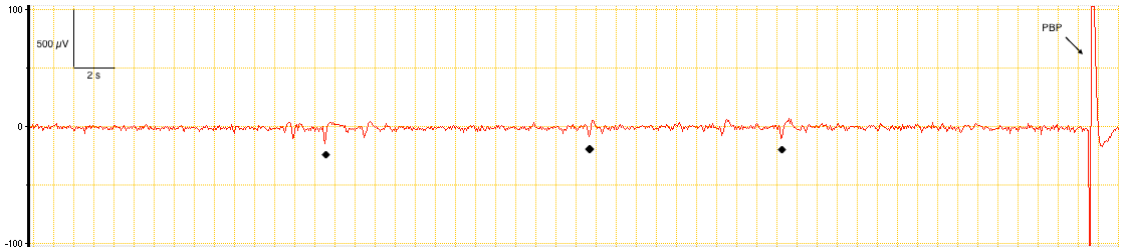
a.



b.



c.



d.

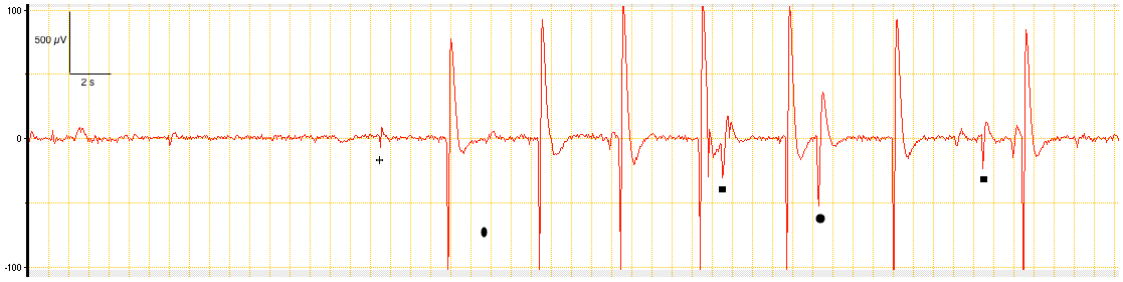


Figure 26. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on SUHPs in the pipette-applied method. Control period BVC (C1) (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d). * (significant differences), ** (potentially significant differences).

Figure 26.

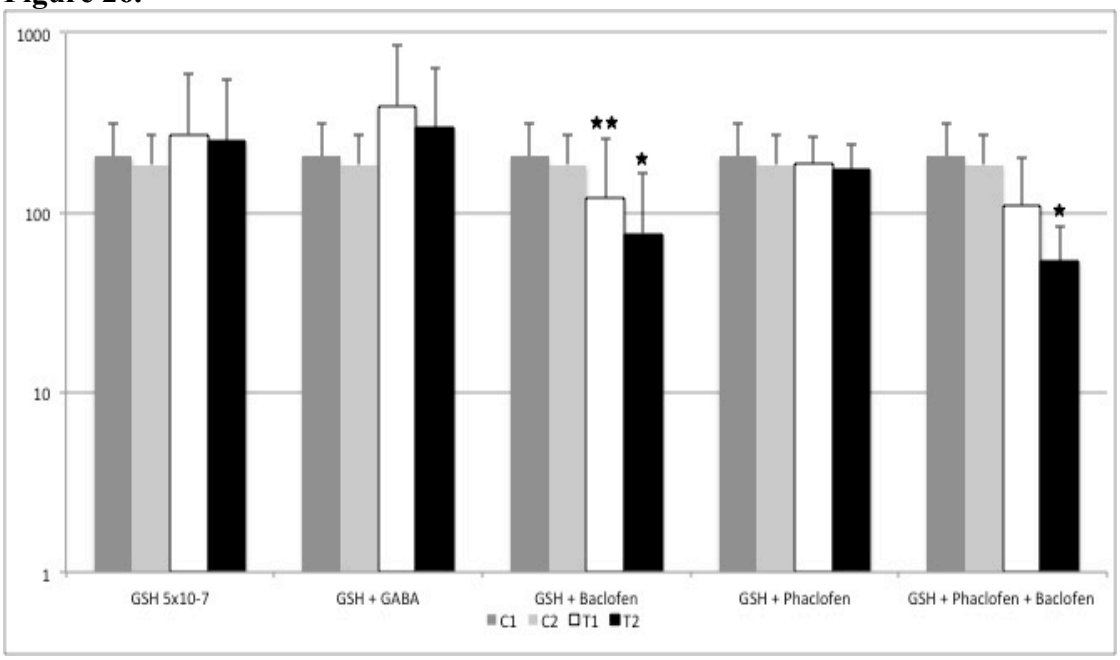
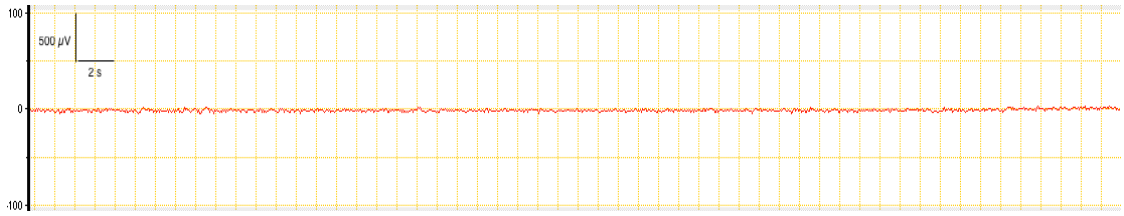

1000
100
10
1
**
*
*
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
C1 C2 T1 T2

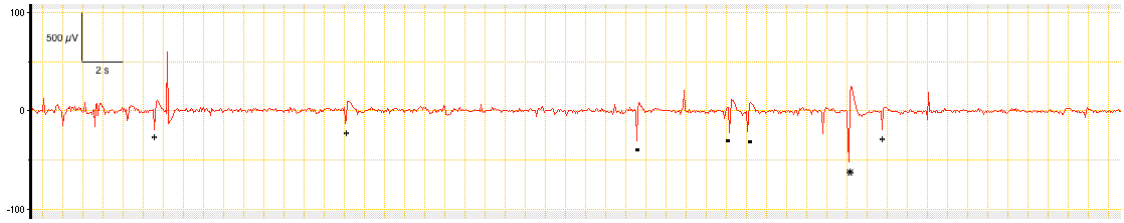
Figure 27: Sample recording from the bath-applied and pipette-applied experiment. a) control period BVC (C2); b) treatment period (T1) with GSH $5x10^{-7}$M pipette-applied c) treatment period (T1) with GSH $5x10^{-7}$M + Baclofen $1x10^{-8}$M bath-applied d) treatment period (T1) with GSH $5x10^{-7}$M + Baclofen $1x10^{-8}$M pipette-applied . Samples were taken from equivalent times after the addition of the test substance in each period, and control period (C2). Diamonds (SUHPs), squares (MUHPs), stars (LUHPs), crosses (RPs) and arrows (PBPs).

Figure 27.

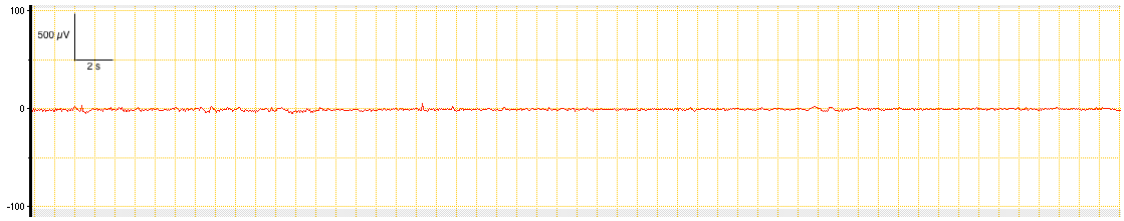
a.



b.



c.



d.

Figure 28. Log (mean) effects of GSH $5x10^{-7}$, GSH $5x10^{-7}$ + GABA 10^{-6}, GSH $5x10^{-7}$ + Phaclofen 10^{-8}, GSH $5x10^{-7}$ + Baclofen 10^{-8}, GSH $5x10^{-7}$ + Phaclofen 10^{-8} + Baclofen 10^{-8} on PBPs in the pipette-applied method. Control period BVC (C1) (C2). Data is reported as means and standard deviations ($\mu \pm$ s.d). ** (potentially significant differences).

Figure 28.

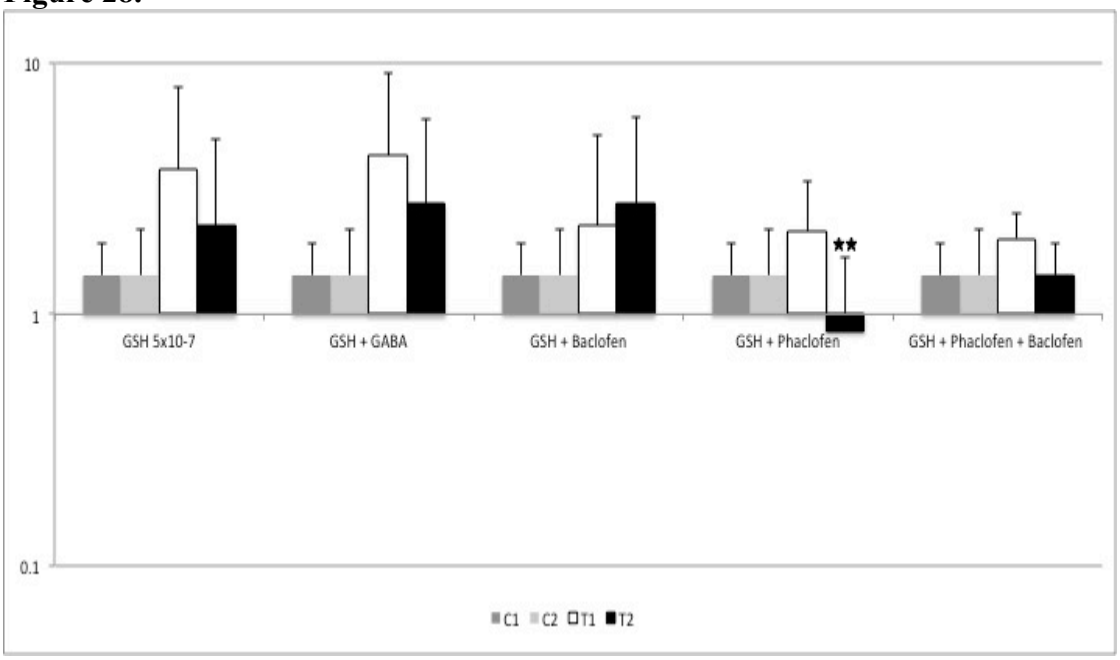
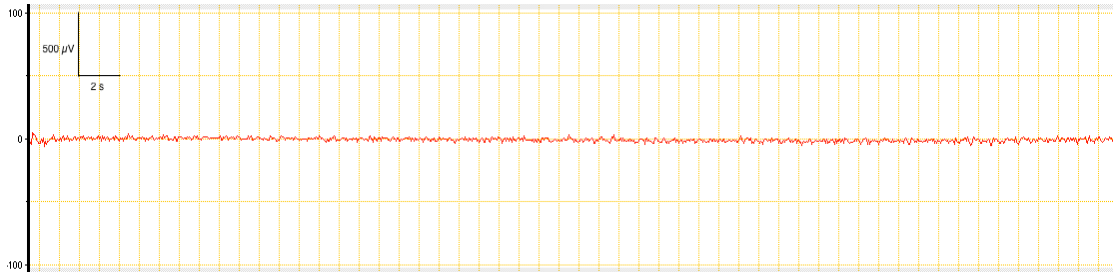

10
1
0.1
GSH 5x10-7
GSH + GABA
GSH + Baclofen
GSH + Phaclofen
GSH + Phaclofen + Baclofen
**
C1 C2 T1 T2

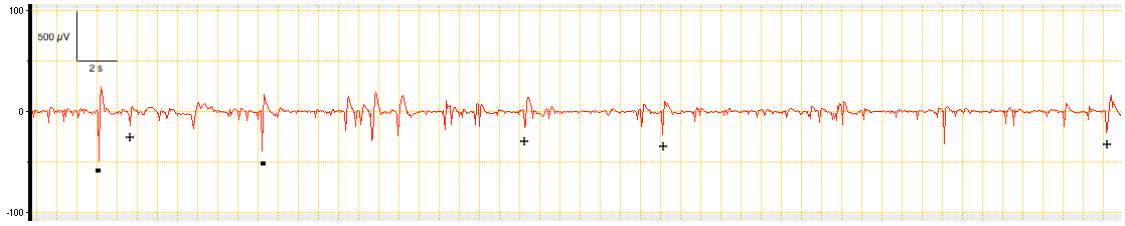
Figure 29. Sample recording from the bath-applied and pipette-applied method. a) control period BVC (C2) b) treatment period (T2) with GSH $5x10^{-7}$M pipette-applied c) treatment period (T2) with GSH $5x10^{-7}$M + Phaclofen $1x10^{-8}$M bath-applied d) treatment period (T2) with GSH $5x10^{-7}$M + Phaclofen $1x10^{-8}$M pipette-applied. Samples were taken from equivalent times after the addition of the test substance in each period, and control period. Diamonds (SUHPs), squares (MUHPs), stars (LUHPs), arrows (PBPs) and crosses (RPs).

Figure 29.

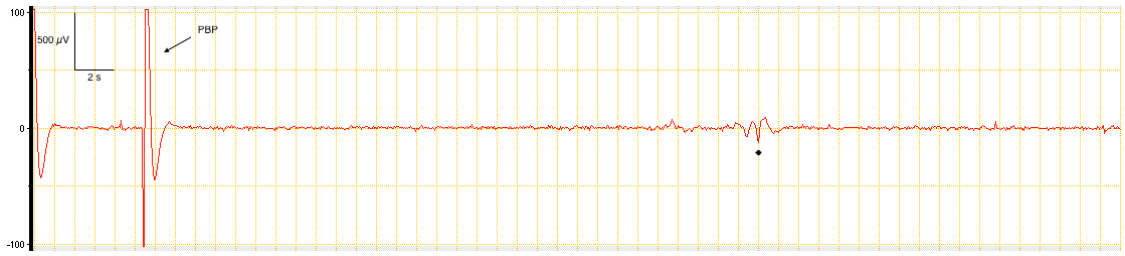
a.



b.



c.



d.

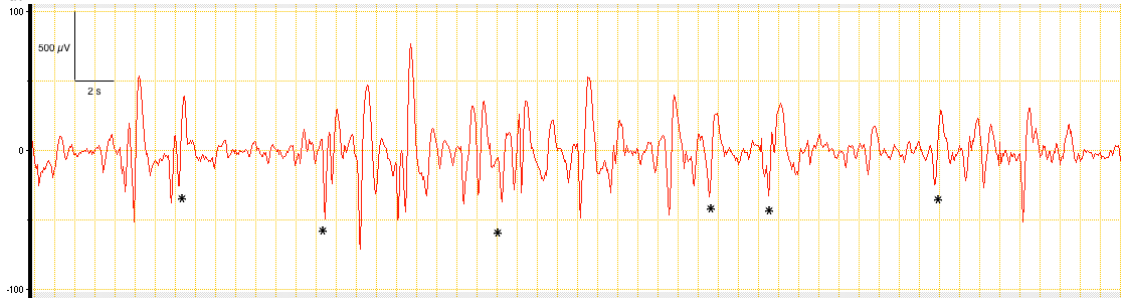
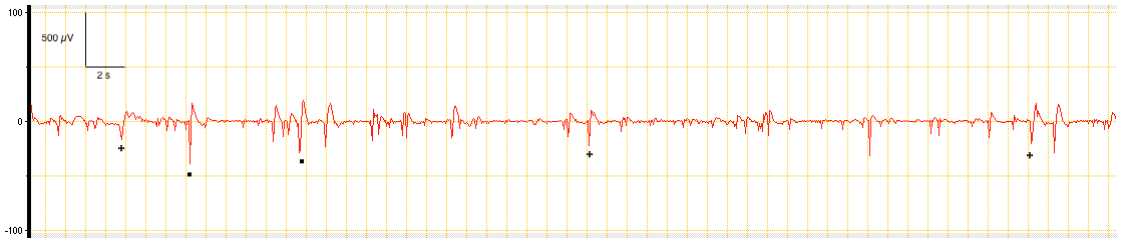


Figure 30: Sample recording from the bath-applied method and the pipette-applied method. a) control period BVC (C2) b) treatment period (T2) with GSH $5x10^{-7}$ M pipette-applied c) treatment period (T2) with GSH $5x10^{-7}$ M + Baclofen $1x10^{-8}$M + Phaclofen $1x10^{-8}$M bath-applied d) treatment period (T2) with GSH $5x10^{-7}$ M + Baclofen $1x10^{-8}$M + Phaclofen pipette-applied. Samples were taken from equivalent times after the addition of the test substance in each period, and control period. Diamonds (SUHPs), squares (MUHPs) and crosses (RPs).

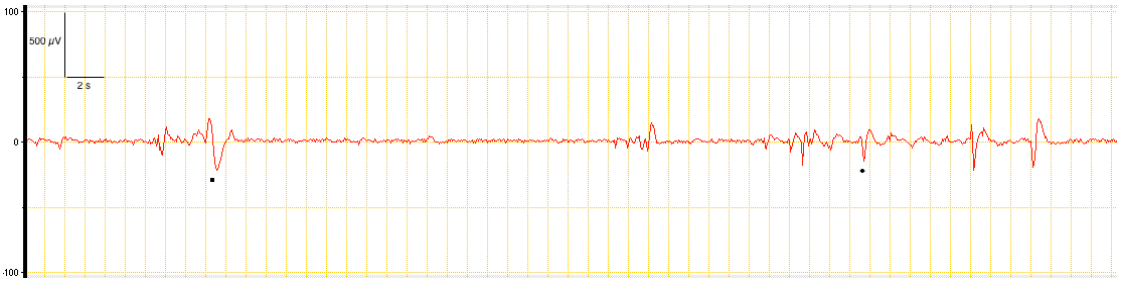
Figure 30:

a.

b.



c.



d.

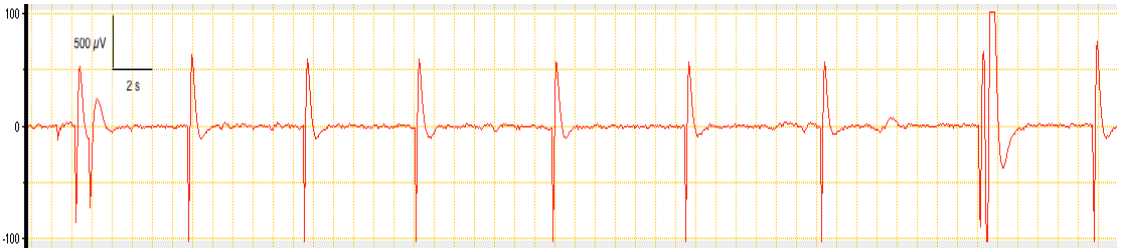
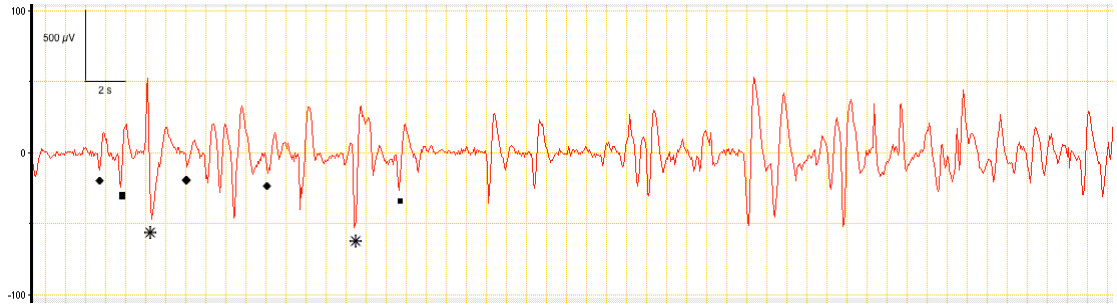


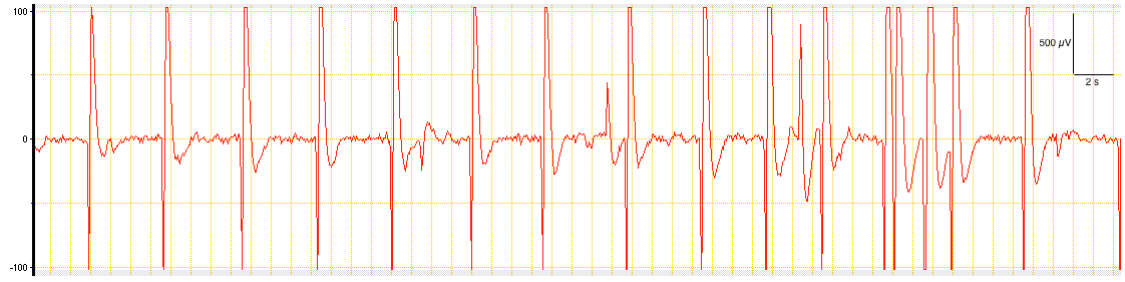
Figure 31: Sample recording from the pipette-applied experiment. a) control period BVC (C2) b) treatment period (T2) with GSH $5x10^{-7}$. c) treatment period (T2) with GSH $5x10^{-7}$ + GABA 10^{-6} c) treatment period (T2) with GSH $5x10^{-7}$ + Baclofen 10^{-8} d) treatment period (T2) with GSH $5x10^{-7}$M + Phaclofen $1x10^{-8}$M e) treatment period (T2) with GSH $5x10^{-7}$M + Baclofen $1x10^{-8}$M + Phaclofen $1x10^{-8}$M. Samples were taken from equivalent times after the addition of the test substance in each period, and control period (C2). Diamonds (SUHPs), squares (MUHPs) and stars (LUHPs).

Figure 31.

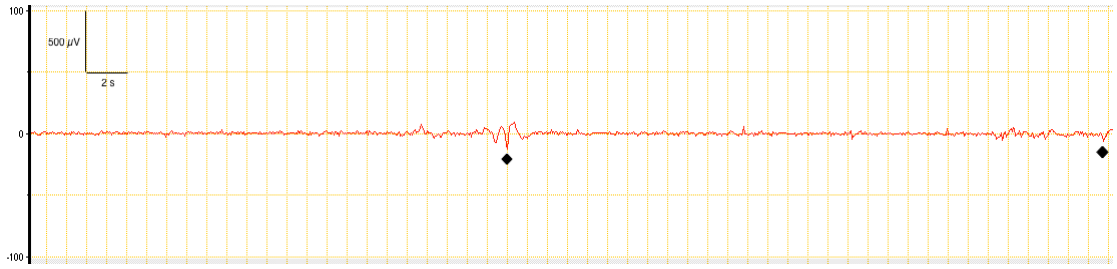
a.



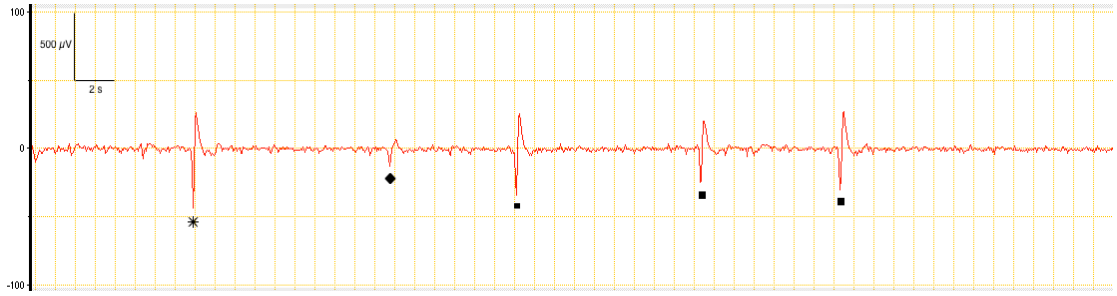
b.



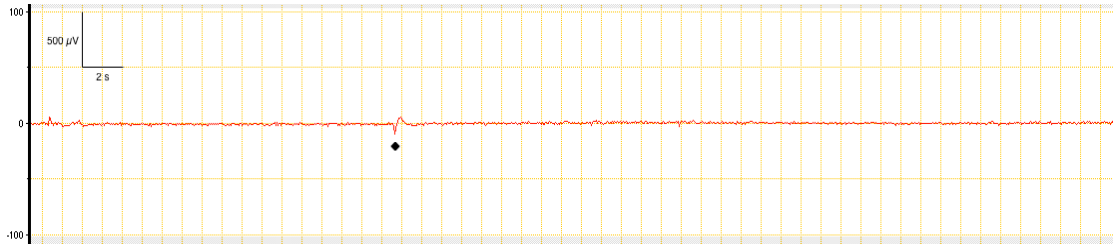
c.



d.



e.



APPENDIX: Raw Data

GSH $5x10^{-6}$: Bath-Applied Method

GSH $5x10^{-6}$M C2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	74	8	3	0	1	13	0
2	118	0	3	10	1	10	6
3	134	6	0	1	1	8	0
4	30	0	0	0	0	0	0
5	17	0	0	0	1	7	0
6	319	0	4	21	3	9	2
7	168	1	1	6	1	18	7
Sum	860	15	11	38	8	65	15

GSH $5x10^{-6}$M T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	313	21	0	1	0	0	0
2	298	9	0	1	0	0	0
3	343	13	1	0	0	0	5
4	353	15	1	0	0	0	2
5	235	21	2	2	0	0	17
6	306	1	0	2	0	0	12
7	211	2	0	8	0	0	4
Sum	2059	82	4	14	0	0	40

GSH $5x10^{-6}$M T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	117	9	2	0	0	0	2
2	225	4	0	0	0	0	0
3	148	0	0	0	0	0	2
4	102	5	5	9	2	9.5	8
5	243	1	4	5	0	0	7
6	322	7	0	1	0	0	17
7	275	1	0	9	0	0	8
Sum	1432	27	11	24	2	9.5	44

GSH $5x10^{-6}$: Pipette-Applied Method

GSH $5x10^{-6}$M T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	351	6	2	0	0	0	23
2	226	2	0	0	0	0	2
3	251	2	0	0	2	3.5	6
4	159	1	1	1	0	0	7
5	41	4	0	0	1	4	9
6	31	11	1	2	3	9	2
7	30	0	0	0	2	5.5	0
Sum	1089	26	4	3	8	22	49

GSH $5x10^{-6}$M T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	424	9	8	1	0	6.33	21
2	256	19	0	0	2	12.5	4
3	331	1	0	1	0	4.5	3
4	144	2	0	0	0	0	8
5	35	5	0	0	2	9	6
6	28	16	0	0	2	12	7
7	24	0	0	0	2	8.5	0
Sum	1242	52	8	2	8	52.83	49

GSH $5x10^{-7}$: Bath-Applied

GSH $5x10^{-7}$M C2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	50	1	0	8	1	7	0
2	44	1	3	4	0	0	1
3	10	3	1	1	1	5	0
4	27	2	2	6	1	10	0
5	2	0	0	0	0	0	0
6	56	1	1	5	1	4	4
7	66	3	3	4	1	8	1
8	131	1	0	2	0	0	0
Sum	386	12	10	30	5	34	6

GSH $5x10^{-7}$M T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	191	1	0	12	1	12	0
2	118	8	0	2	0	0	2
3	43	3	0	0	0	0	1
4	74	0	1	3	0	0	1
5	10	0	0	0	0	0	0
6	83	5	0	5	0	0	5
7	80	2	1	4	0	0	0
8	144	7	2	3	0	0	0
Sum	743	26	4	29	1	12	9

GSH $5x10^{-7}$M T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	159	0	0	11	1	12	1
2	50	18	0	0	1	21	0
3	127	10	0	0	0	0	0
4	24	2	5	1	1	8	1
5	2	6	0	0	1	6	0
6	72	5	3	0	1	6	0
7	137	7	2	2	0	0	3
8	63	0	0	0	0	0	0
Sum	634	48	10	14	5	53	5

GSH $5x10^{-7}$: Pipette-Applied

GSH $5x10^{-7}$M T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	225	11	7	0	1	12	39
2	244	8	11	5	3	10.67	2
3	169	1	0	18	2	9	3
4	48	16	1	20	2	17.5	0
5	70	17	0	0	2	11	0
6	121	9	8	7	3	8.67	23
7	215	16	4	40	2	20.5	6
Sum	1092	78	31	90	15	89.34	73

GSH $5x10^{-7}$M T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	215	0	0	0	0	0	40
2	91	13	4	0	3	11.67	1
3	264	3	13	13	2	13	1
4	82	1	2	23	1	20	1
5	108	2	3	22	2	15.5	4
6	83	2	5	5	0	0	28
7	162	5	0	27	1	24	6
Sum	1005	26	27	90	9	84.17	81

GSH $5x10^{-8}$: Bath-Applied

GSH $5x10^{-8}$M C2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	112	0	0	12	1	12	12
2	184	6	4	7	1	14	13
3	294	6	2	25	3	10.6	1
4	42	0	0	0	0	0	14
5	106	21	5	6	2	12	0
6	272	6	1	17	2	8	9
7	121	7	4	6	3	8.6	0
8	172	2	1	8	1	9	1
Sum	1303	48	17	81	13	74.2	50

GSH $5x10^{-8}$M T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	253	1	0	1	0	0	1
2	98	8	0	0	0	0	8
3	348	9	2	8	2	7	1
4	36	1	0	0	0	0	1
5	91	12	1	0	1	16	0
6	127	23	1	21	2	9.5	1
7	174	2	0	11	1	12	1
8	261	4	1	1	0	0	0
Sum	1388	60	5	42	6	44.5	13

GSH $5x10^{-8}$M T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	276	0	0	0	0	0	2
2	98	1	1	16	1	12	3
3	137	4	1	11	1	11	0
4	57	0	1	0	0	0	0
5	91	13	0	0	2	6	2
6	267	18	0	15	1	12	1
7	67	0	0	1	0	0	0
8	176	1	0	0	0	0	0
Sum	1169	37	3	43	5	41	8

GSH $5x10^{-8}$: Pipette-Applied

GSH $5x10^{-8}$M T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	360	23	6	33	1	13	1
2	38	3	0	2	4	5.25	4
3	62	11	1	5	3	6.33	1
4	80	3	4	9	3	6.67	1
5	26	2	2	3	1	6	1
6	215	0	0	0	0	0	4
Sum	781	42	13	52	12	37.25	12

GSH $5x10^{-8}$M T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	64	8	1	29	2	13.5	1
2	18	5	1	0	1	6	1
3	34	3	1	7	2	9.5	1
4	108	1	2	2	1	8	3
5	39	1	0	4	1	4	1
6	167	0	0	0	0	0	4
Sum	430	18	5	42	7	41	11

GSH + GABA: Bath-Applied

GSH GABA C2							
Trial	SUHP	MUHP	LUHP	XLUHPS	PBP	P/B	RP
1	162	7	3	11	1	18	4
2	227	30	7	8	0	0	1
3	306	1	0	4	0	0	5
4	253	12	4	15	1	14	4
5	78	1	4	7	1	10	5
6	106	2	0	2	0	0	1
7	79	12	0	0	1	11	0
8	50	1	0	8	1	9	1
Sum	1261	66	18	55	5	62	21

GSH GABA T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	169	0	3	13	1	16	6
2	185	17	2	4	0	0	1
3	391	6	3	9	1	10	4
4	390	3	0	1	0	0	6
5	94	9	7	10	1	26	0
6	127	2	1	14	1	15	1
7	49	5	8	1	1	12	0
8	84	7	0	1	0	0	2
Sum	1489	49	24	53	5	79	20

GSH GABA T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	141	1	2	16	1	19	4
2	210	0	0	2	0	0	1
3	492	0	0	0	0	0	2
4	280	22	3	2	0	0	3
5	42	1	1	0	0	0	1
6	79	1	1	2	0	0	4
7	79	1	0	0	1	10	0
8	160	8	0	0	0	0	2
Sum	1483	34	7	22	2	29	17

GSH + GABA: Pipette-Applied

GSH GABA T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	235	6	1	5	2	7	12
2	154	1	1	22	4	7	10
3	256	25	15	16	1	20	13
4	365	4	1	26	2	11	3
5	147	8	3	23	3	9.67	9
6	277	2	2	27	2	15	2
7	141	0	0	40	3	13.67	3
Sum	1575	46	23	159	17	83.34	52

GSH GABA T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	127	1	9	8	2	8.5	12
2	112	0	2	19	1	15	7
3	147	13	22	3	3	13	6
4	191	2	1	24	1	25	3
5	193	6	2	19	2	19.5	3
6	178	1	0	16	1	16	1
7	236	0	1	16	1	16	3
Sum	1184	23	37	105	11	113	35

GSH + Phaclofen: Bath-Applied

GSH Phaclofen C2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/PBP	RP
1	115	0	0	7	0	0	6
2	103	1	2	4	2	7	2
3	46	0	11	11	2	11	1
4	260	5	0	16	1	17	8
5	171	2	0	15	2	8	10
6	162	6	0	0	1	5	27
7	120	2	0	25	1	14	5
8	147	1	0	20	1	19	2
Sum	1124	17	13	98	10	81	61

GSH Phaclofen T1							
Trial	SUHP	MUHP	LUHP		PBP	P/PBP	RP
1	115	0	0	19	1	14	1
2	87	2	0	7	1	9	0
3	50	2	3	16	1	12	3
4	245	7	1	0	0	0	17
5	138	0	0	14	1	15	16
6	110	1	1	2	0	0	2
7	185	2	0	23	2	11.5	1
8	128	1	1	3	1	15	2
Sum	1058	15	6	84	7	76.5	42

GSH Phaclofen T2							
Trial	SUHP	MUHP	LUHP		PBP	P/PBP	RP
1	91	0	0	15	1	12	0
2	73	0	1	8	2	7.5	1
3	52	0	0	0	0	0	2
4	88	1	0	13	1	12	10
5	86	0	2	5	0	0	9
6	81	4	2	5	1	20	3
7	133	2	0	21	2	10.5	2
8	76	2	4	16	1	15	0
Sum	680	9	9	83	8	77	27

GSH + Phaclofen: Pipette-Applied

GSH Phaclofen T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	320	12	5	37	1	37	9
2	260	9	19	25	2	23	0
3	133	2	9	24	4	7.75	2
4	200	7	1	17	3	6.33	2
5	93	6	0	3	0	0	6
6	103	8	5	10	2	7.5	5
7	205	1	0	20	3	6.33	3
Sum	1314	45	39	136	15	87.91	27

GSH Phaclofen T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	204	9	4	15	1	21	12
2	195	8	14	0	1	22	1
3	74	4	7	15	2	11	0
4	294	23	5	2	0	0	0
5	194	11	2	1	0	0	0
6	131	2	1	2	0	0	2
7	130	0	0	19	2	9.5	4
Sum	1222	57	33	54	6	63.5	19

GSH + Baclofen: Bath-Applied

GSH Baclofen C1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	303	4	0	0	0	0	3
2	76	7	6	11	2	9.5	1
3	206	8	1	14	2	11.5	2
4	228	3	4	15	1	16	2
5	45	1	0	0	2	16.5	0
6	26	0	0	0	2	5.5	4
7	299	34	4	3	0	0	3
8	72	1	0	0	0	0	4
9	234	3	0	0	1	17	21
10	155	3	1	16	1	10	16
11	162	8	7	5	1	8	40
Sum	1806	72	23	64	12	94	96

GSH BACLOFEN T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	350	4	1	0	0	0	1
2	207	3	3	17	2	7	0
3	93	4	2	6	1	7	2
4	217	0	0	13	0	0	2
5	59	0	0	0	4	13.75	0
6	28	0	0	0	0	0	0
7	237	22	4	0	0	0	13
8	39	0	0	0	1	9	5
9	127	3	5	3	1	10	0
10	166	2	1	0	0	0	8
11	127	16	1	11	2	7.5	16
Sum	1650	54	17	50	11	54.25	47

GSH BACLOFEN T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	30	3	0	2	0	0	4
2	329	47	9	2	0	0	1
3	286	2	1	0	0	0	3
4	207	3	2	4	1	4	0
5	41	0	0	0	3	11	0
6	39	0	0	0	0	0	0
7	191	17	2	10	1	11	7
8	40	2	0	0	0	0	5
9	141	0	1	5	1	12	0
10	154	2	0	0	0	0	4
11	132	3	3	16	1	15	18
Sum	1590	79	18	39	7	53	42

GSH + Baclofen: Pipette-Applied

GSH Baclofen T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	66	1	1	0	0	0	2
2	91	10	5	9	3	7.67	3
3	38	0	1	0	0	0	8
4	86	13	2	1	3	5.66	4
5	93	1	2	17	3	6	2
6	64	1	0	0	0	0	1
7	39	3	0	0	0	0	10
Sum	477	29	11	27	9	19.33	30

GSH Baclofen T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	48	0	0	0	0	0	2
2	50	5	8	11	2	10.5	1
3	26	3	0	0	3	7.33	8
4	64	19	2	0	3	9	0
5	31	8	3	1	1	12	1
6	31	5	5	0	2	6	3
7	56	1	0	0	0	0	8
Sum	306	41	18	12	11	44.83	23

GSH + Baclofen + Phaclofen: Bath-Applied

GSH PHAC BAC C2							
Trial	SUHP	MUHP	LUHP	XLUHPS	PBP	P/B	RP
1	69	3	1	0	0	0	11
2	142	5	1	1	0	0	5
3	114	1	0	10	1	8	5
4	100	3	1	1	0	0	16
5	241	2	0	19	2	7	4
6	127	7	0	1	0	0	8
7	238	3	1	15	1	8	14
8	116	7	1	9	1	7	7
Sum	1147	31	5	56	5	30	70

GSH PHAC BAC T1							
Trial	SUHP	MUHP	LUHP	XLUHPS	PBP	P/B	RP
1	71	10	0	0	1	6	10
2	165	5	2	3	0	0	6
3	178	0	0	7	0	0	0
4	82	1	0	0	0	0	4
5	142	0	0	11	1	11	5
6	155	1	0	0	0	0	3
7	160	0	0	2	0	0	18
8	165	22	2	1	0	0	1
Sum	1118	39	4	24	2	17	47

GSH PHAC BAC T2							
Trial	SUHP	MUHP	LUHP	XLUHPS	PBP	P/B	RP
1	118	2	1	0	0	0	4
2	85	0	0	9	1	9	4
3	37	0	0	12	1	12	0
4	9	0	0	2	0	0	2
5	84	0	0	13	1	12	1
6	178	0	0	0	0	0	0
7	328	0	1	12	1	15	12
8	251	1	0	0	0	0	2
Sum	1090	3	2	48	4	48	25

GSH + Baclofen + Phaclofen: Pipette-Applied

Phac Bac T1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	246	10	10	12	2	24	0
2	92	2	0	0	1	32	8
3	253	5	5	20	3	7.33	3
4	36	4	0	0	2	6.5	3
5	47	0	0	0	2	8.5	9
6	53	0	5	17	2	11	0
7	42	1	8	10	2	9.5	0
Sum	769	22	28	59	14	98.83	23

GSH Phac Bac T2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	47	17	18	2	1	14	7
2	62	6	1	0	1	7	6
3	116	0	0	18	2	10.5	2
4	37	6	12	0	1	18	0
5	68	2	0	0	2	17	6
6	27	2	3	13	2	8.5	2
7	17	3	5	2	1	9	0
Sum	374	36	39	35	10	84	23

BVC: Pipette-Applied

BVC C1							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/B	RP
1	156	6	1	15	1	15	5
2	249	0	0	12	2	8.5	0
3	455	12	1	15	2	9.5	3
4	128	9	2	5	1	8	0
5	156	6	1	15	1	15	7
6	123	2	0	8	2	4	10
7	173	9	1	8	1	5	2
Sum	1440	44	6	78	10	65	27

BVC C2							
Trial	SUHP	MUHP	LUHP	XLUHP	PBP	P/PBP	RP
1	136	4	0	20	2	10.5	0
2	129	0	0	18	1	10	0
3	262	2	6	11	2	8	3
4	130	11	1	8	1	8	2
5	136	4	0	20	2	10.5	0
6	146	0	0	8	2	4	10
7	355	0	0	0	0	0	2
Sum	1294	21	7	85	10	51	17

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