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MODULATION OF LOCAL REFLEXES DURING CENTRALLY COMMANDED MOVEMENTS

by

UZMA H. TAHIR

Under the Direction of Donald H. Edwards

ABSTRACT

During centrally orchestrated movements, the nervous system must distinguish between appropriate and inappropriate reflexes. I studied local postural flexion reflexes of the crayfish that are evoked by unexpected touch. An isolated abdomen was used which permitted recording and stimulating of tailfan afferents, nerve cord interneurons, and postural motor neurons. Stimulation of the afferents evoked a postural flexion response of the medium tonic and large phasic motor neurons of the superficial flexor nerve; a flexion motor program was then excited by stimulating descending interneurons. Afferent stimulation evoked a smaller motor response during the motor program than before or after. These results indicate that the postural reflex responses to sensory stimulation are inhibited at a site presynaptic to the motor neurons during the flexion motor program. Application of Picrotoxin (blocked inhibition) to the primary afferent-to-mechanosensory interneuron synapse did not prevent the modulation of the postural flexion reflex during the flexion motor program.

INDEX WORDS: Motor control, Neuromodulation, Crayfish, Abdomen, Posture, Picrotoxin

# MODULATION OF LOCAL REFLEXES DURING CENTRALLY COMMANDED MOVEMENTS

by

UZMA H. TAHIR

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

in the College of Arts and Sciences

Georgia State University

2013

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2013

MODULATION OF LOCAL REFLEXES DURING CENTRALLY COMMANDED MOVEMENTS

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May 2013

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# 1 INTRODUCTION

Behavioral context is essential to the generation of reflexes; to produce smooth voluntary movements inappropriate reflexes must be inhibited or even reversed. For example, a contact-evoked startle response would be appropriate when the contact is unexpected but inappropriate when the contact is expected. Similarly, in the crayfish unexpected touch or water movement can evoke a rapid flexion escape reflex to propel the animal away from the direction of attack. The sensory to interneuron synapses of this pathway are prone to synaptic depression (Krasne 1969). Primary afferent depolarization (PAD) has been shown to protect these synapses from responding to the animal's own movements and from habituating to repeated activation. During centrally commanded movement, PAD reduces the amount of neurotransmitter released onto the mechanosensory interneurons (MSIs) by the primary sensory afferents (Kennedy et. al. 1974). A number of primary afferent depolarization interneurons (PADI) have been identified as part of this escape pathway. They synapse onto the primary sensory afferents where they release the inhibitory neurotransmitter *gamma*-Aminobutyric acid (GABA) to cause PAD (Kirk and Wine 1984).

The same issues of inappropriate reafference might arise during postural movements of the abdomen that occur during a tail flip escape. A slow postural local flexion reflex can be elicited in the isolated abdomen of the crayfish by stimulating the sensory afferent neurons innervating the tailfan of the animal (Figure 3). These afferent neurons synapse onto MSIs at the sixth ganglion neuropil which then project rostrally in the ventral nerve cord and synapse onto a number of neurons throughout the animal. These MSIs excite the neurons of the third superficial flexor nerve (3s) of ganglia 1-5; a purely motor nerve that innervates the superficial flexor muscles of the abdomen (Kennedy and Takeda 1965; Figures 1C and 1D). The superficial flexor muscles form a thin sheet on the ventral surface of the abdomen and are used to flex the abdomen and maintain its posture (Kennedy and Takeda 1965; Figures 1C and

1D). Nerve 3s contains the axons of six motor neurons that are numbered one through six based on the amplitude and shape of their extracellularly recorded action potentials (see Figure 5 insets). All neurons except number five are excitatory; five is an inhibitor of the superficial flexor muscles (Kennedy and Takeda 1965). Neurons one, two, three, and four are tonically active whereas neuron five and six are phasic and usually only fire in response to stimulation (Kennedy and Takeda 1965). Activation of the excitatory motor neurons of nerve 3s causes a contraction of the superficial flexor muscles producing tension which then flexes the abdomen of the animal (Figure 1).

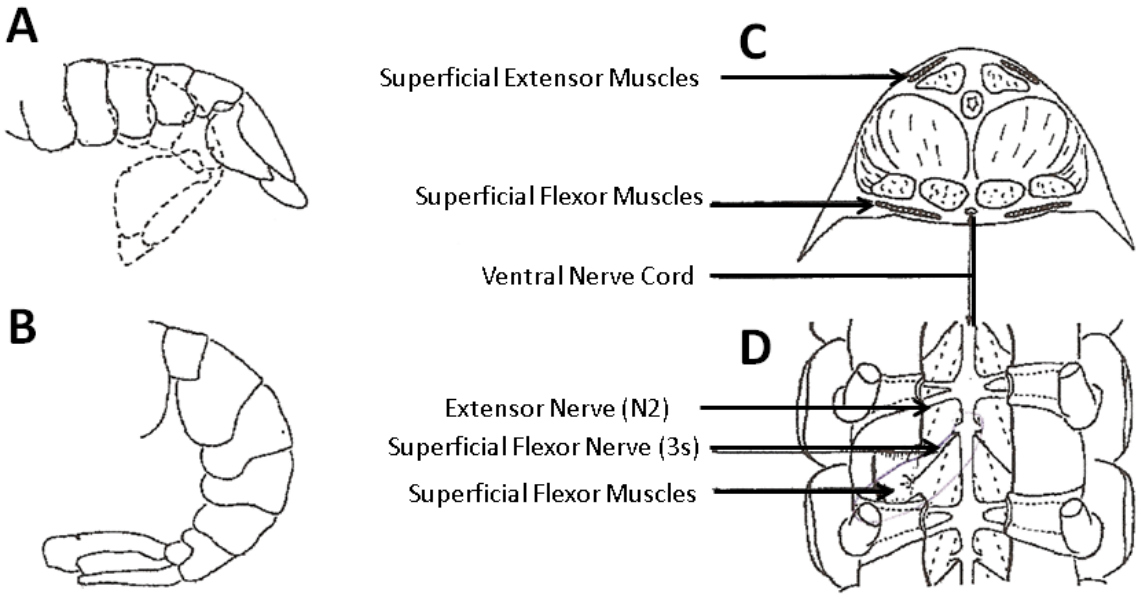


**Figure 1: Postural system of the crayfish abdomen**
**(A) & (B): The extended abdomen is flexed through contractions of the superficial flexor muscles. (C): Transverse section of the abdomen showing the locations of the superficial extensor and flexor muscles as well as the ventral nerve cord. (D) Ventral view of the ventral nerve cord, superficial flexor muscles, superficial flexor nerve (3s), and extensor nerve (N2). Ventral soft cuticle and sternites are absent from drawing to allow visualization of the nervous system and muscles. Adapted from Wine JJ *et. al.* 1974.**

The main purpose of this study is to understand how an animal might modulate local reflexes during a centrally commanded movement. Lightly touching the sensory hairs on the tailfan of the crayfish will elicit a postural flexion reflex response in the behaving animal. However, when the animal is

walking backwards or actively flexing its abdomen, the same tailfan sensory hairs will come into contact with the substrate. In order to prevent inappropriate reflexes and habituation to its own movements it is expected that the local flexion reflexes in response to sensory hair stimulation would be modulated during the centrally commanded flexion movements.

A number of command neurons have been identified in the crayfish ventral nerve cord that can be stimulated in order to produce stereotyped behavioral responses such as abdominal flexion (Wiersma 1958). By stimulating a centrally commanded motor program during ongoing afferent stimulation one can study how a local postural flexion reflex might change during the centrally commanded flexion motor program.

Primary afferent depolarization (PAD) has been shown to modulate local reflexes during centrally commanded movements (Bryan and Krasne 1977a and 1977b). PAD is a depolarizing inhibition that reduces the amount of neurotransmitter released by the primary afferents and has been shown to be mediated by GABA in the crayfish (Cattaert et al. 1992). A number of PAD interneurons (PADIs) have been identified that release GABA onto the presynaptic terminals of the primary afferents and cause PAD during a tail flip escape response in the crayfish (Kirk and Wine 1984). PAD has also been shown to be blocked with the application of picrotoxin (PTX), a blocker of chloride channels associated with GABA receptors (El Manira and Clarac 1991; Cattaert et al. 1992). To see if PAD and GABA played a role in the modulation of the local postural flexion reflex during the centrally commanded flexion motor program I locally applied PTX to the sixth ganglion (see Methods). If PADs associated with GABA at the sixth ganglion primary afferents was responsible for the modulation of the reflex, then the blocking of PAD with PTX would prevent the modulation of the local flexion reflex during the centrally commanded flexion motor program.

Modulation of reflexes has been well studied in the past in the context of the rapid escape system of the abdomen, tailfan movements, and walking but not in the postural system. In the present

study, I generalize the modulation of local reflexes to the slower postural flexion system of the crayfish abdomen. I will also discuss the possible role of GABA in this modulation at the afferent to interneuron synapse of the terminal abdominal ganglion.

Crayfish have been used extensively in the past to study the control of locomotion because of their easy availability, ability to withstand surgery, and large identifiable neurons. The understanding of control of movement in the crayfish can reveal mechanisms that may be evolutionarily conserved and allow the generalization of these mechanisms to humans and assist in the treatment of motor control pathologies.

# 2 METHODS

## 2.1 Experimental Animals

Male and female crayfish (*Procambarus clarkii),* measuring 8.5-13cm in body length from rostrum to telson were purchased from a commercial supplier, Atchafalaya Biological Supply (Raceland, LA). All animals were housed communally in freshwater aquaria on a 12:12h light dark cycle.

## 2.2 Dissection

Animals were cold-anesthetized and perfused with 100ml of saline. Crayfish hemolymph and saline was collected and mixed with the remaining saline (about 2L). The abdomen was isolated from the cephalothorax and pinned ventral side up in a Sylgard-lined dish. The abdominal ventral soft cuticle, sternites, and ventral artery were removed to expose the six abdominal ganglia and abdominal motor roots. The fast flexor nerves (deep nerves 3 of ganglia 1-5) were also cut to prevent muscle contractions of the large flexor muscles during the experiments. All preparations were constantly perfused with cold oxygenated saline ((mM) NaCl, 205; KCl, 5.3; CaCl2, 13.5; MgCl2, 2.45 Hepes, 2.39; Glucose, 1.99; pH 7.55).

## 2.3 Extracellular recordings and stimulation

To stimulate the sensory afferents innervating the tailfan, an extracellular stimulating hook electrode was placed onto the second nerve of the terminal (sixth) abdominal ganglion (A6N2) (Figure 2). This nerve contains the axons of the mechanosensory afferents innervating the right uropod of the abdominal tailfan (Figure 3). Nerve A6N2 was stimulated (0.3ms duration, 3-6V) once every 10s to elicit a brief (<500ms) flexion reflex in the postural motor neurons. To record flexion postural motor nerve responses, an extracellular hook recording electrode was placed on the superficial branch of nerve three of the fourth abdominal ganglion (A43s) (Figure 2). In order to record the responses of the nerve innervating the antagonists of the superficial flexor motor neurons, the superficial extensors, a recording suc-

tion electrode was placed in the second nerve of the fourth abdominal ganglion (A4N2). A4N2 is a mixed sensory and motor nerve and to eliminate any sensory afferent spikes, the nerve was cut peripherally. A second extracellular hook stimulating electrode was placed on bundles separated from the ventrolateral portion of the nerve cord between ganglia two and three (A2A3) in order to stimulate descending command fibers causing abdominal flexion (Wiersma 1958). As the afferent stimulation continued, command fibers were stimulated (13.3 Hz, 0.3ms duration per stimulus, 3-7V) to elicit a centrally commanded flexion motor program. Since A4N2 innervates the superficial extensor motor neurons, I expected a decreasing in the firing of the excitatory superficial extensor motor neurons during the centrally commanded flexion motor program.

Command fiber stimulation allowed me to ask if the reflex response of the superficial flexor motor neurons to afferent stimulation changed during a centrally commanded flexion motor program. The afferent stimulation was continued after the motor program terminated to test whether the effects of the motor program persisted after the motor program ended. A single trial consisted of 15 total stimuli with about 5 stimuli before the motor program, 5 during and 5 after. For each animal, several trials were performed before, during and after application of PTX.

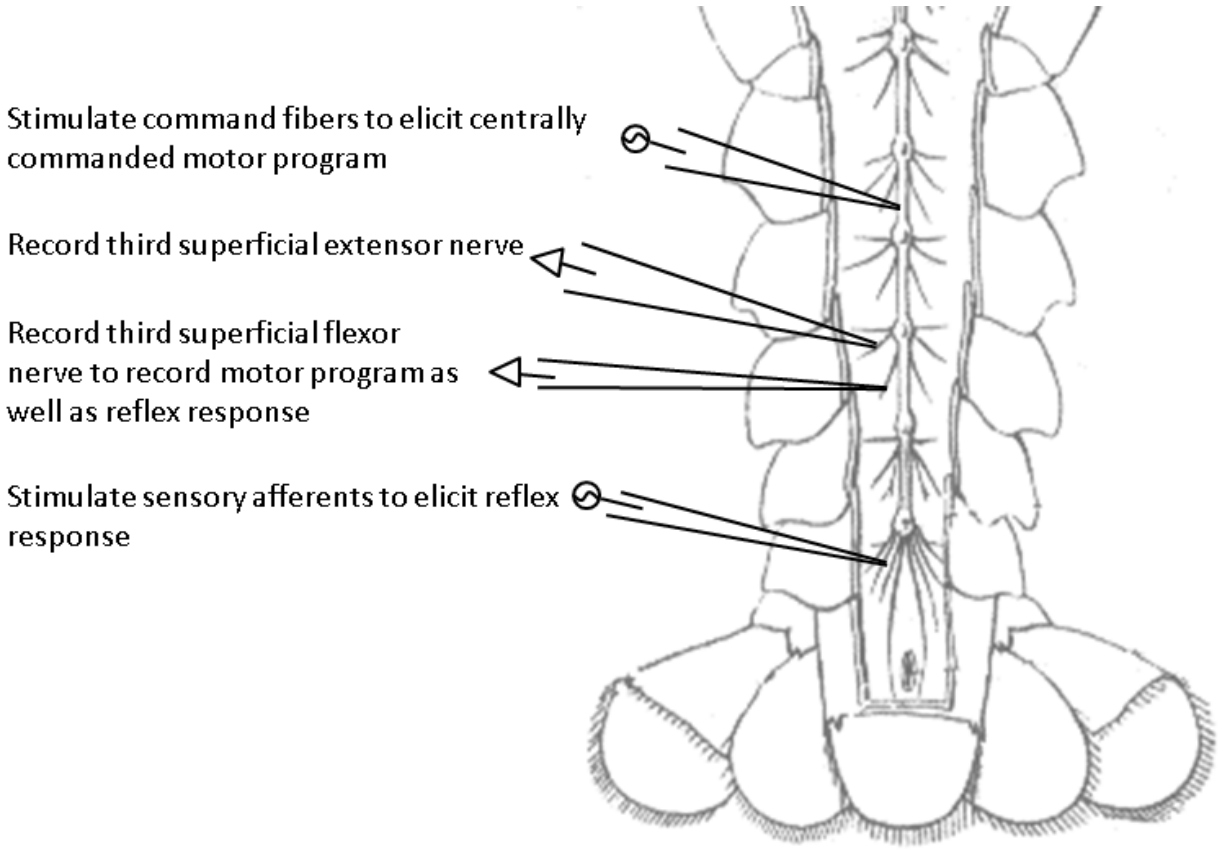


**Figure 2: Isolated abdominal preparation with recording and stimulating electrodes. Abdomen was pinned ventral side up in a sylgard lined dish and constantly perfused with saline. Sensory afferents of the terminal abdominal ganglion (A6N2) were stimulated to elicit a flexion reflex response of the ipsilateral superficial flexor motor neurons. Motor neuron responses were recorded at the third superficial flexor motor nerve (A43s) and the superficial extensor nerve (A4N2). As afferent stimulation continued, descending command fibers of postural flexion were stimulated (A2A3). Afferent stimulation continued after the termination of the motor program. Open triangles are recording electrodes and open circles are stimulating electrodes. Drawing of isolated abdomen is adapted from Huxley 1880.**

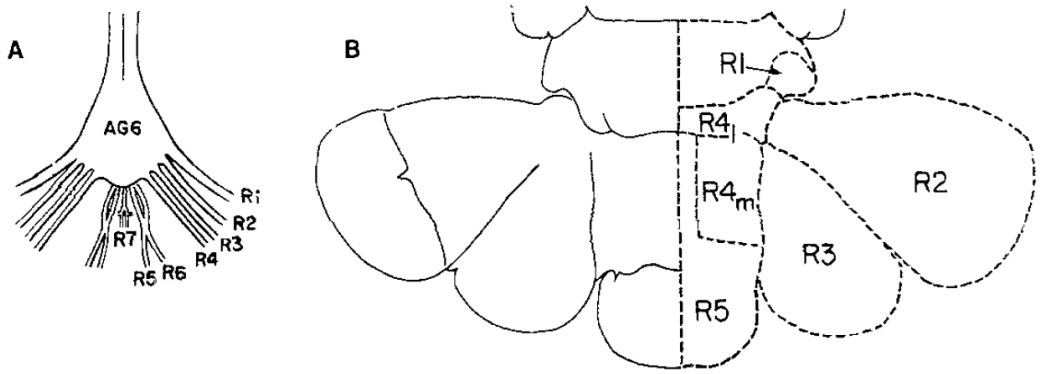


**Figure 3: Innervations of mechanosensory afferents of the terminal abdominal ganglion (A) Ventral view of the sixth abdominal ganglion (AG6) and roots. (B) Tailfan and receptive fields of sensory afferents in roots of A6. R= Root, AG6= sixth abdominal ganglion. Figure is adapted from Calabrese 1976.**

### 2.4 Application of picrotoxin

For trials with picrotoxin (PTX; Sigma), PTX was applied locally to the sixth abdominal ganglion by sliding a piece of Sylgard under the ganglion and building a Vaseline well around the single ganglion. PTX solution (40 µM in crayfish saline) was injected directly into the well. To confirm there were no leaks, fast green (Allied chemical) was added to the PTX solution.

### 2.5 Data analysis

To examine how each of the six motor neurons of the superficial flexor nerve responded to the afferent stimulation before, during and after the motor program, the spikes of the motor neurons were sorted using DataView (http://www.st-andrews.ac.uk/~wjh/dataview/). Peristimulus histograms were created using MatLab (www.mathworks.com) with stimulation onset at T=0. Blue histograms represent data from before the centrally commanded motor program, red histograms are during and green histograms are after. Bar graphs of average firing frequency of each of the motor neurons were also created in MatLab. Responses of the motor neurons to afferent stimulation and average firing frequencies were averaged within a single animal across several trials before, during and after the motor program. These

were then pooled across multiple animals. Error bars are standard error of the mean (SEM) unless otherwise noted.

# 3 RESULTS

## 3.1 Trace of the local flexion reflex responses to afferent stimulation and the centrally commanded flexion motor program

### *3.1.1 Sample of a single trial*

Afferent stimulation was delivered once every 10s causing a reflex response each time in the postural motor nerve (Figure 4: A6N2 stimulation, second trace). As the afferent stimulation continued, command fibers of postural flexion were excited causing a flexion motor program (Figure 4- A2A3 stimulation- top trace). The flexion motor program was defined as an increase in the firing of the largest phasically active superficial flexor motor neuron, cell 6, during the entire command fiber stimulation, at a minimum of 20Hz (large cell in A43s- Figure 4: Extracellular recording: superficial flexor motor nerve (A43s), third trace). This increase in superficial flexor motor neuron activity during the command fiber stimulation was often coupled with a decrease in firing of the motor neurons of the antagonistic nerve, the extensor motor nerve of the fourth ganglion (A4N2) ( Figure 4: extracellular recording: extensor motor nerve, bottom trace).

After the motor program terminated, the afferent stimulation continued. Responses of the motor neurons of the superficial flexor nerve of the fourth ganglion (A43s) can be seen in the third trace (Figure 4- Extracellular Recording: superficial flexor motor nerve). Baseline activity levels and reflex responses to afferent stimulation can be seen before and after the motor program. The overall superficial flexor nerve activity levels were highest and superficial extensor activity lowest during the centrally commanded motor program.

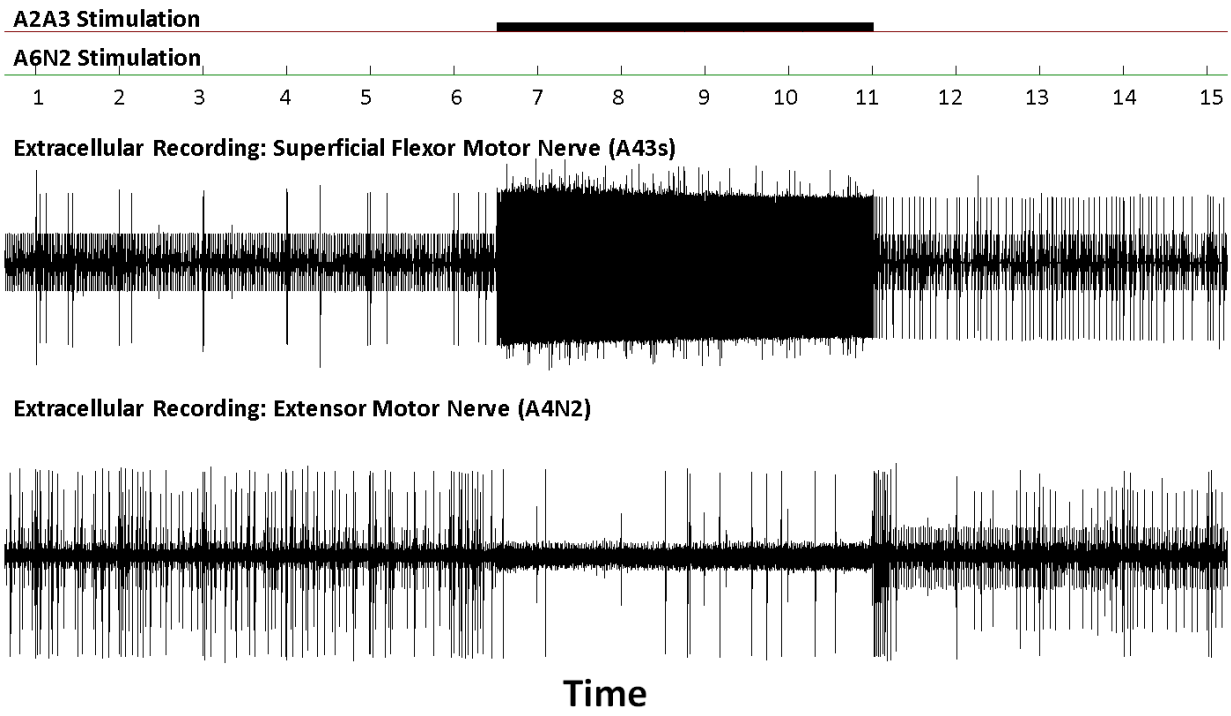


**Figure 4: Sample recording of the local flexion reflex responses to afferent stimulation and the centrally commanded flexion motor program**
**Afferent stimulation (A6N2) was delivered once every 10s numbered 1-15 in the second trace (A2A3 stimulation). Command fiber stimulation (A2A3) elicited an abdominal flexion motor program (black bar: top trace, A2A3 stimulation). Extracellular recording of superficial flexor motor nerve (A43s) is shown in the third trace and extracellular recording of extensor motor nerve (A4N2) is shown in the bottom trace. Note: increase in activity levels of the superficial flexor nerve and decrease in activity of superficial extensor motor nerve activity during command fiber stimulation.**

### *3.1.2 Sample postural flexion reflex response to afferent stimulation before the centrally commanded flexion motor program*

Cell 6 is a phasically active cell and usually only fired in response to the afferent stimulation (Kennedy and Takeda 1965) and in the example in figure 5, cell 6 fired two spikes after afferent stimulation (Figure 5: afferent stimulation is indicated by the arrow in the second trace). Cell 5 is an inhibitory motor neuron, smaller than cell 6, and is also phasically active; in the example in figure 5, cell 5 fired a single spike after the afferent stimulation. Cells 3 and 4 are medium sized tonically active cells and usually increased their firing as part of the reflex response to afferent stimulation. Cells 1 and 2 are the small tonically active cells (Kennedy and Takeda 1965).

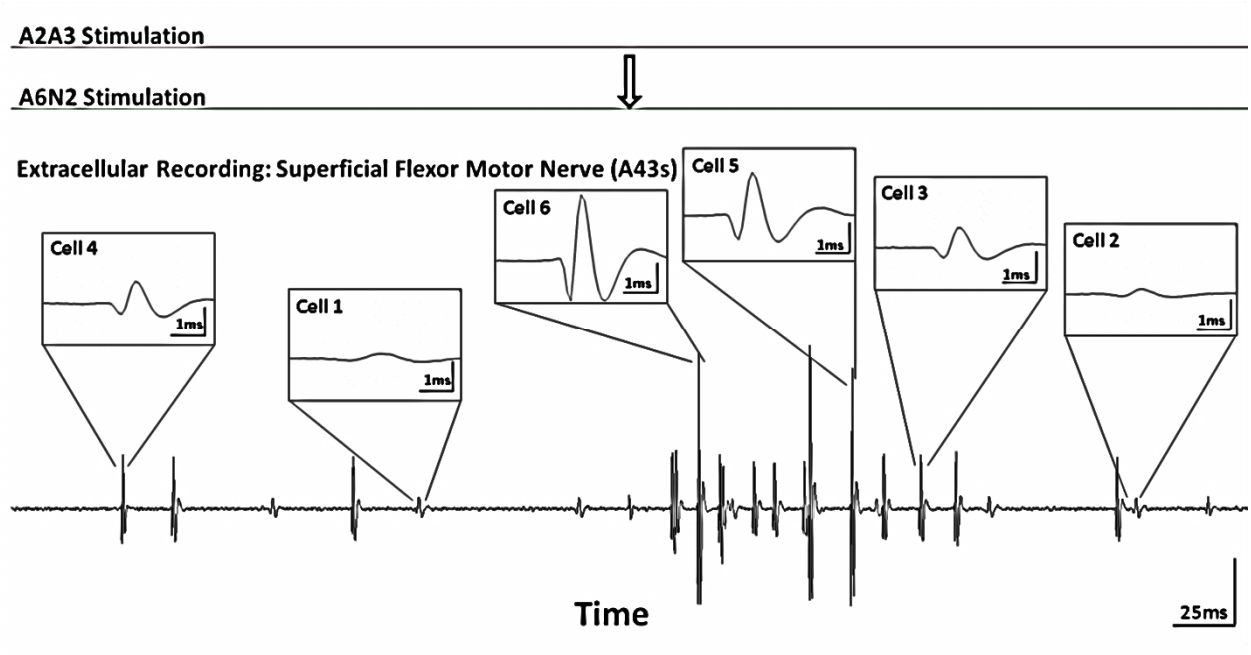


**Figure 5: Recording of superficial flexor motor neurons of the superficial flexor nerve of the fourth abdominal ganglion (A43s) in response to afferent stimulation of the sixth abdominal ganglion**
**The arrow in the middle trace indicates the time of a single ipsilateral afferent shock. Insets demonstrate waveforms of the different motor neurons (time and voltage scale is the same across motor neurons).**

### *3.1.3 Sample postural flexion reflex response to afferent stimulation during the centrally commanded flexion motor program*

During the centrally commanded motor program the overall nerve activity levels were much higher (Figure 6: Extracellular recording: superficial flexor motor nerve (A43s)). Cell 6 fired regularly during the motor program at a minimum of 20Hz. Cells 1-4 are also be identifiable during the motor program. Cell 5 is did not fire in this particular example.

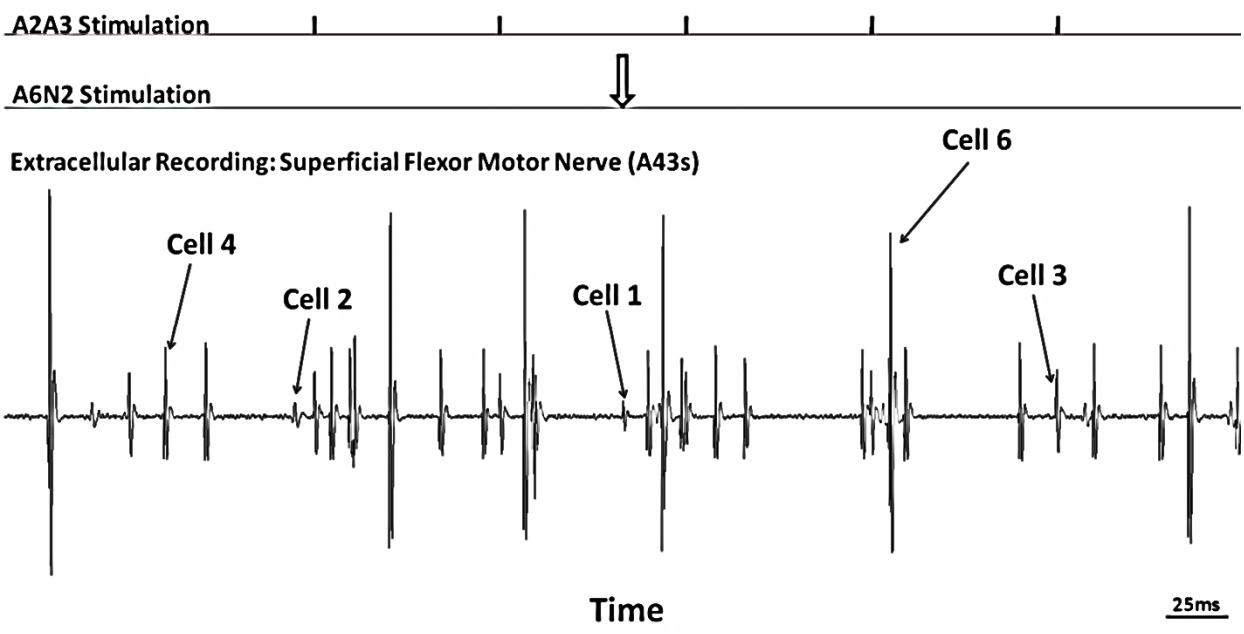


**Figure 6: Responses of superficial flexor motor neurons of the superficial flexor nerve of the fourth abdominal ganglion (A43s) to afferent stimulation of the sixth abdominal ganglion during the centrally commanded flexion motor program**
**A2A3 stimulation (top trace) indicates command fiber stimulation (13.3 Hz) of bundles separate from connectives between ganglia 2 and 3 (A2A3) causing centrally commanded abdominal flexion. The arrow (second trace) indicates a single afferent stimulus at the second nerve of the sixth abdominal ganglion (A6N2). Note: overall nerve activity levels were much higher during the motor program when compared to before the motor program (Figure 5).**

### *3.1.4 Sample postural flexion reflex response to afferent stimulation after the centrally commanded flexion motor program*

After the termination of the motor program cell 6 returned to only firing in response to afferent stimulation and in this example fired 3 spikes after the afferent stimulation (Figure 7). Cells 1-4 also returned to their baseline activity levels after the motor program.

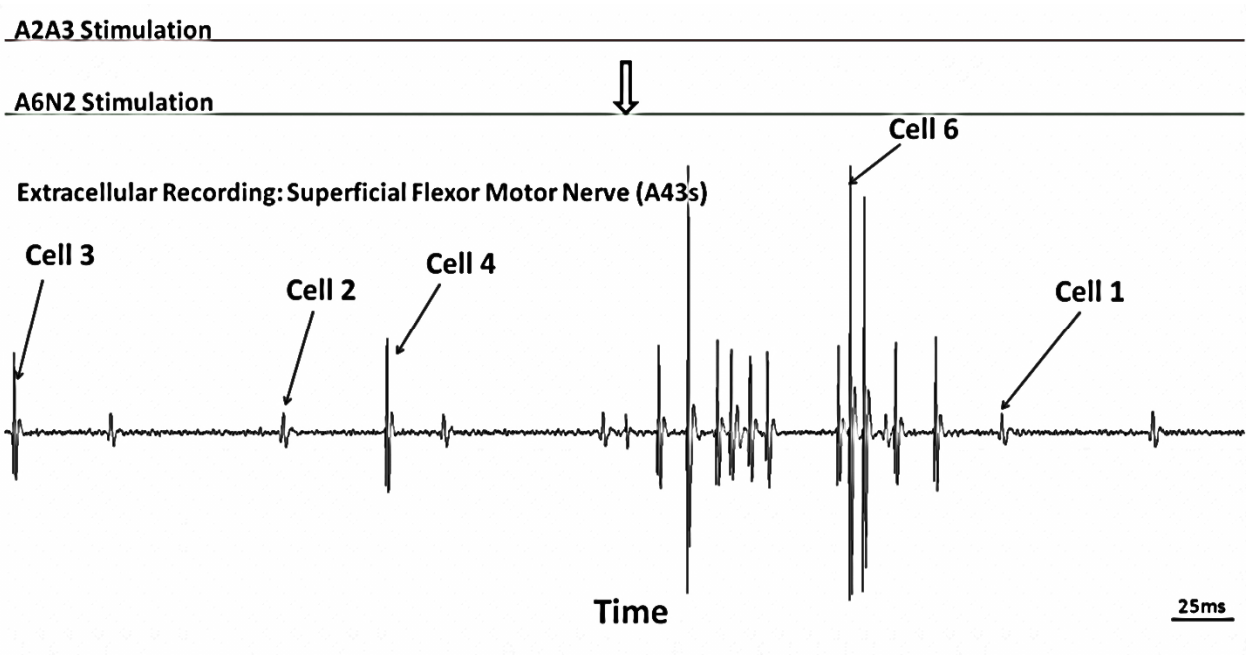


**Figure 7: Responses of superficial flexor motor neurons of the superficial flexor nerve of the fourth abdominal ganglion (A43s) to afferent stimulation of the sixth abdominal ganglion, after the centrally commanded flexion motor program.**
**The arrow (middle trace) indicates a single afferent stimulus (A6N2). Note: overall nerve activity levels were much lower after the centrally commanded motor program when compared to during the motor program (Figure 6) and similar to before the motor program (Figure 5).**

## 3.2 Modulation of the local flexion reflex responses to afferent stimulation during the centrally commanded flexion motor program and the effects of local application ofpicrotoxin at the sixth abdominal ganglion

### *3.2.1 Cell 3*

#### *3.2.1.1 Reflex response of Cell 3 to afferent stimulation is inhibited during the centrally commanded motor program*

Before the onset of the motor program the average firing frequency of cell 3 was 9.90 ± 3.70 Hz (here and throughout the text: average instantaneous firing frequency over entire 500ms before afferent stimulation ± SEM) (Figure 11: blue bar, before PTX). Cell 3 was excited in response to afferent stimulation, and this excitation lasted about 300ms (Figure 8A). During the centrally commanded flexion motor program the baseline activity of cell 3 increased to 28.06 ± 7.76 Hz (Figure 11: red bar, before PTX) and the cell did not respond to the afferent stimulation (8B). After the termination of the motor program, the baseline firing frequency before afferent stimulation of cell 3 was lower than before or during the centrally commanded motor program, 5.55 ± 1.78 Hz (Figure 11: green bar, before PTX). Also, after the motor program cell 3 was once again excited by the afferent stimulation lasting about 300ms (Figure 8C).

Cell 3 was excited by the afferent stimulation before and after the motor program. During the motor program, when overall activity levels of the neuron were higher, the cell did not respond to afferent stimulation, suggesting the reflex response of cell 3 to afferent stimulation was inhibited during the centrally commanded flexion motor program. Due to the increase in baseline firing frequency of cell 3, there is a possibility that there might be maximum firing frequency of cell 3 and that during the motor program cell 3 was at this maximum. Because of this increase, cell 3 may have become incapable of re-

sponding to afferent stimulation and the lack of response of cell 3 during the motor program was not due to motor program modulation.

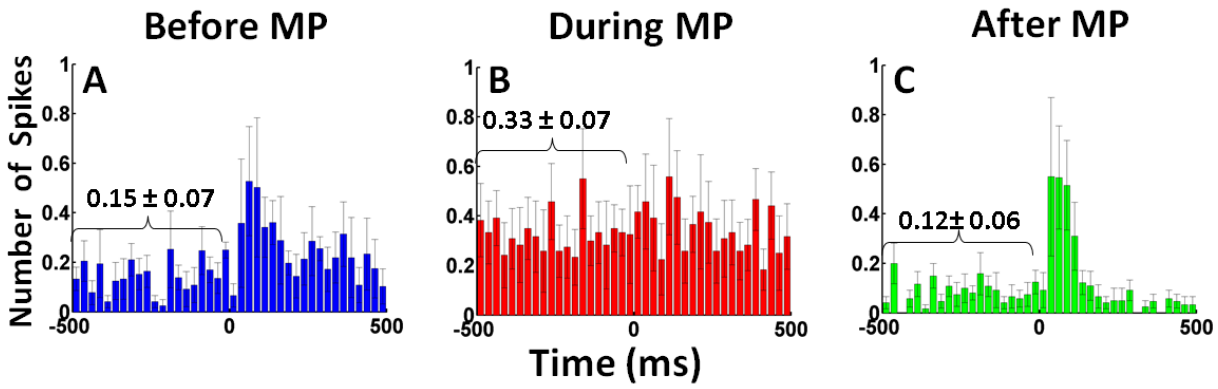


**Figure 8: Reflex response of cell 3 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2)**
**(A): Blue histogram is response to afferent stimulation before centrally commanded motor program. (B): Red histogram is response to afferent stimulation during the motor program. (C): Green histogram is response to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across 4 animals ± SEM. T=0=afferent stimulation at A6N2.**

*3.2.1.2 Hypothesized circuitry of the inhibition of the local postural flexion reflex response of cell 3 to afferent stimulation during the centrally commanded motor program*

As part of the reflex circuitry, input to the sixth ganglion afferents excites mechanosensory interneurons (MSIs) of the abdominal nerve cord (Figure 9). These MSIs then project rostrally and excite cell 3 of the superficial flexor nerve of the fourth ganglion that is responsible for postural flexion through contraction of the superficial flexor muscles. When a centrally commanded motor program for flexion is excited though command fibers that excite the same motor neuron, this causes a centrally commanded flexion of the abdomen (Figure 9). However, the nerve cord command fibers for postural flexion may not only excite the motor neurons of the superficial flexor nerve, they may also send descending projections

that could inhibit incoming sensory information and therefore, prevent the response to afferent stimulation; as the data suggest for cell 3. The modulation of the response to afferent stimulation of the postural motor neuron could occur through synaptic recruitment of modulatory interneurons; as has been shown in the past for the fast flexor motor neurons during the escape system of the crayfish. Modulation could be directed pre- or post-synaptically at either of the two synapses of the reflex circuitry: afferent to MSI or MSI to motor neuron. Reducing the efficacy of either of these synapses would reduce the excitation of the postural motor neuron as part of the local flexion reflex to afferent stimulation and would account for the lack of response to afferent stimulation during the motor program of cell 3.

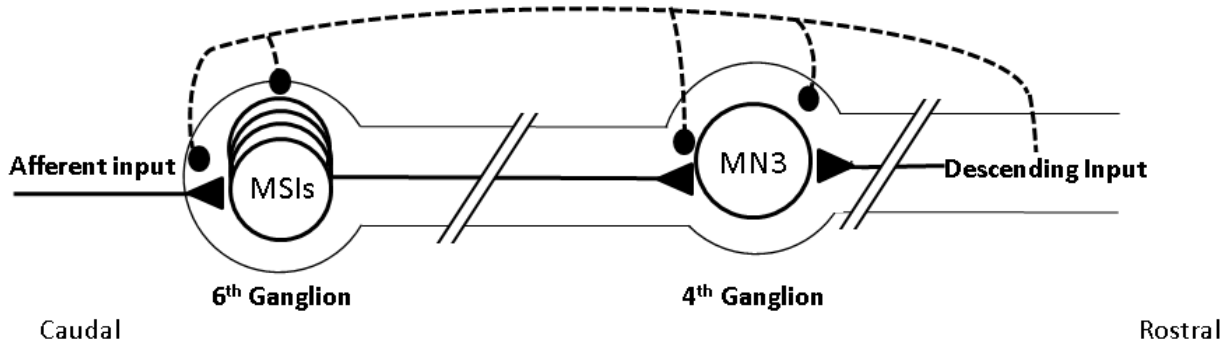


**Figure 9: Hypothesized circuitry of modulation of the local postural flexion reflex response of cell 3 to afferent stimulation during the centrally commanded motor program**
**Filled circles are inhibitory synapses and filled triangles are excitatory synapses. MSIs: Mechanosensory Interneurons (population). MN3: Motor Neuron 3. Dotted lines are hypothesized descending modulation during the centrally commanded motor program.**

*3.2.1.3 Inhibition of reflex response to afferent stimulation of cell 3 during the centrally commanded motor program is not prevented with the application of picrotoxin to the sixth abdominal ganglion*

To test whether GABA-mediated primary afferent depolarization (PAD) was the mechanism of the inhibition of the response to afferent stimulation during the centrally commanded motor program, picrotoxin (PTX) was applied to the sixth ganglion synapse (See Introduction and Methods). With PTX application, cell 3 was excited in response to the afferent stimulation before and after the centrally commanded motor program, and did not respond to the afferent stimulation during the motor program

(Figure 10D-F). After the removal of PTX, the average firing frequency of cell 3 was lower than before or with PTX (Figure 11: compare blue bars before PTX (9.90 ± 3.70 Hz) *vs.* with PTX (6.59 ± 2.95 Hz) *vs.* after PTX (0.74 ± 0.25 Hz)). These data suggest that local application of PTX to the sixth ganglion did not prevent the inhibition of the reflex response of cell 3 to afferent stimulation during the centrally commanded flexion motor program.

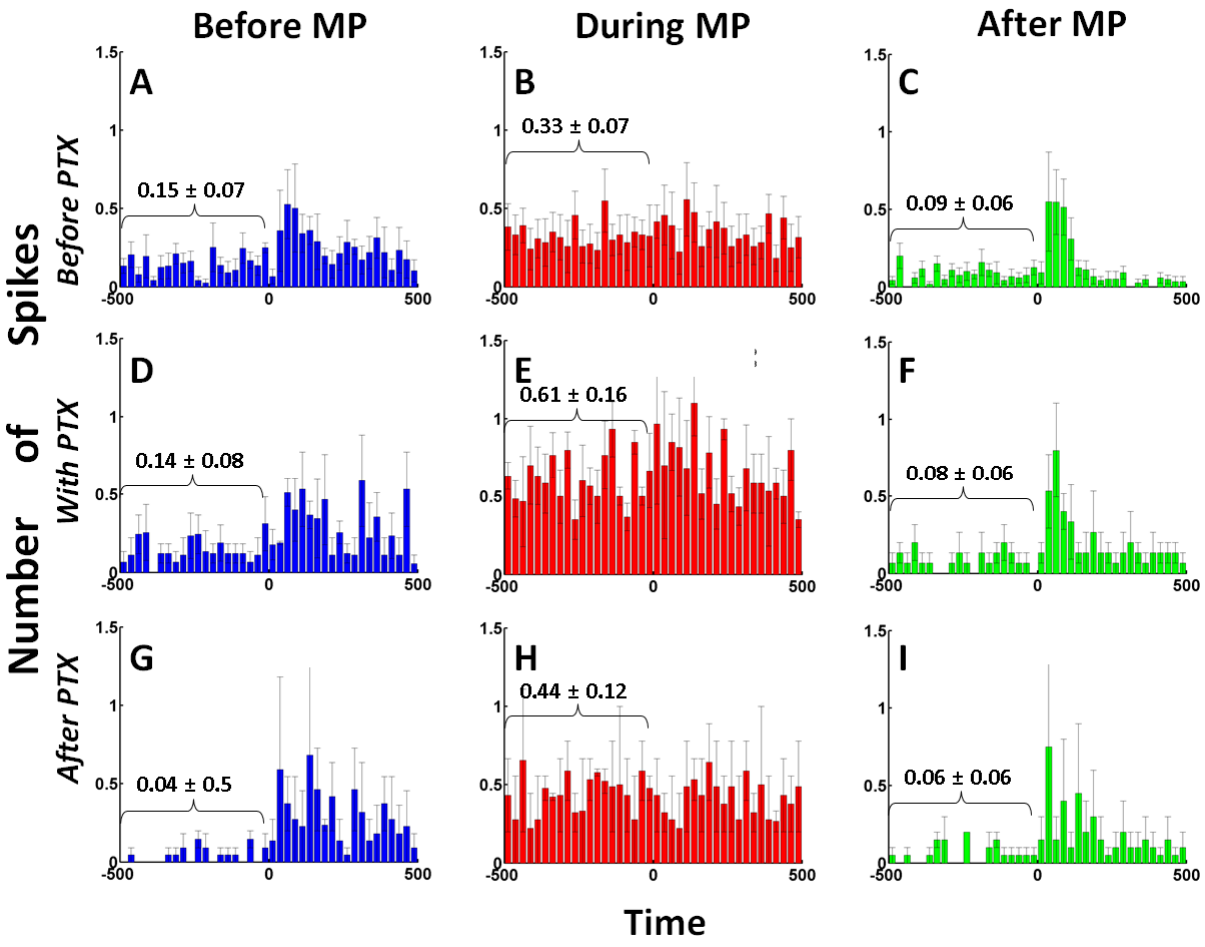


**Figure 10: Reflex response of cell 3 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2) before, with and after the local application of picrotoxin.**
**(A), (D), and (G): Blue histograms are responses to afferent stimulation before centrally commanded motor program. (B), (E), and (H): Red histograms are response during the motor program. (C), (F), and (I): Green histograms are responses to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across multiple animals ± SEM. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. T=0=afferent stimulation at A6N2.**

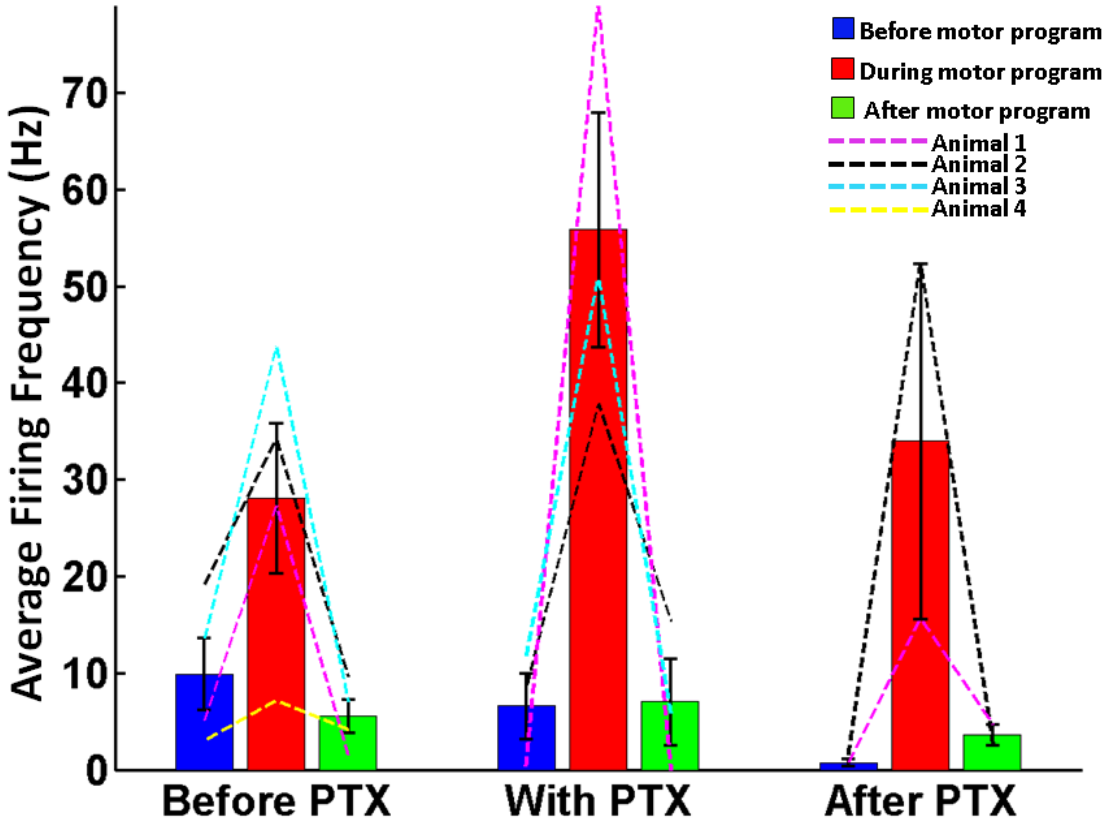


**Figure 11: Average firing frequencies of cell 3 of superficial flexor nerve 3 of the fourth abdominal ganglion (A43s)**
**Average firing frequency is average over entire 500ms before afferent stimulation. Blue bars are averages across animals before the centrally commanded motor program, red are averages across animals during the motor program and green are averages across animals after the motor program. Dotted lines are averages within a single animal. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. Error bars are ± SEM.**

*3.2.1.4 Updated hypothesized circuitry of modulation of the local postural flexion reflex response of cell 3 to afferent stimulation during the centrally commanded motor program*

Before the application of PTX, it was shown that the reflex response to afferent stimulation of cell 3 was inhibited during the centrally commanded motor program. It was hypothesized that this modulation could occur at pre- or post-synaptically at either of two synapses of the reflex circuitry: afferent to MSI or MSI to motor neuron (Figure 12A). To test whether GABA-mediated primary afferent depolarization (PAD) was the mechanism of this inhibition, picrotoxin was applied to the sixth ganglion synapse.

Application of PTX locally to the sixth ganglion did not to prevent the inhibition of the reflex response to afferent stimulation during the centrally commanded flexion motor program. The modulation may be directed at the second synapse in the circuit: the MSIs to motor neuron synapse which occurs in the fourth ganglion. In order to test this possibility, future experiments would need to include the application of PTX to the fourth ganglion synapse. If GABA-mediated inhibition is directed at the fourth ganglion, then application of PTX would prevent the modulation of the descending modulation during the centrally commanded motor program.

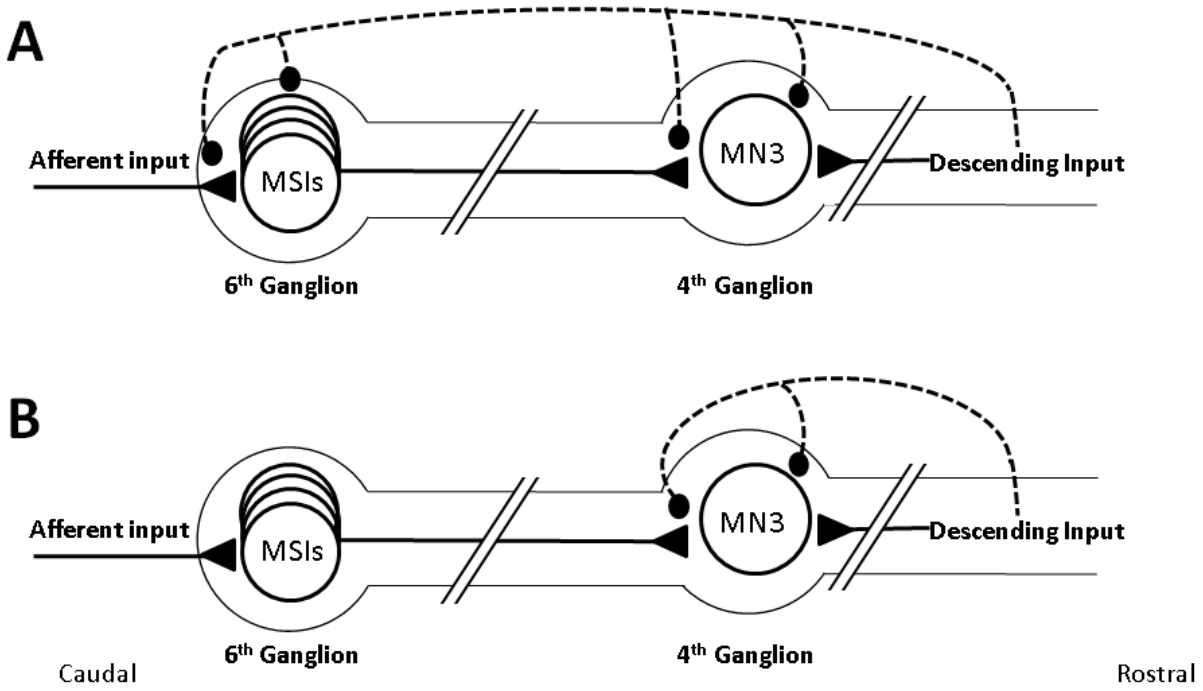


**Figure 12: Updated hypothesized circuitry of modulation of the local postural flexion reflex response of cell 3 to afferent stimulation during the centrally commanded motor program**
**(A) Old hypothesis possible mechanism of descending inhibition during the centrally commanded motor program inhibiting the response to afferent stimulation directed at both the sixth and fourth ganglion. (B) New hypothesis of descending inhibition during the centrally commanded motor program directed at the fourth ganglion synapse only.**
**Filled circles are inhibitory synapses and filled triangles are excitatory synapses. MSIs: Mechanosensory Interneurons (population). MN3: Motor Neuron 3. Dotted lines are hypothesized descending inhibition during the centrally commanded motor program.**

As stated previously the present experiments do not rule out the possibility of a ceiling effect during the centrally commanded motor program. In order to test this possibility, future experiments

would have to test the maximal firing rate of cell 3 and ensure that recruitment of the motor neuron during the centrally commanded motor program is below this maximal rate.

### *3.2.2 Cell 4*

#### *3.2.2.1 Reflex response to afferent stimulation of cell 4 is not inhibited during the centrally commanded motor program*

Before the motor program, cell 4 was excited by the afferent stimulation and this excitation lasted about 200ms (Figure 13A). During the centrally commanded motor program the average firing frequency of cell 4 was much higher than before the motor program, 9.45 ± 4.24 Hz before the motor program *vs.* 25.92 ± 5.51Hz during the motor program (Figure 16). After the termination of the motor program the average firing frequency of cell 4 returned to a similar firing frequency as before the motor program, 9.45 ± 4.24 Hz before the motor program *vs.* 7.99 ± 3.70Hz after the motor program (Figure 16). During and after the motor program, cell 4 was slightly excited by the afferent stimulation lasting 150ms (Figure 13B and 13C). Cell 4 was excited in response to afferent stimulation before, during and after the motor program. These data suggests that, unlike cell 3, the reflex response of cell 4 to afferent stimulation was not inhibited during the centrally commanded motor program when the overall activity of cell 4 was higher. A reduction was seen in the amount of excitation in response to afferent stimulation during the centrally commanded motor program (excitation in response to afferent stimulation in Figure 13A vs. 13B). Similar to cell 3, it could be that there was a maximum firing rate as to how much this neuron could fire, and during the motor program, the cell was still able to respond to the afferent stimulation but was incapable of firing as much as it had before the motor program.

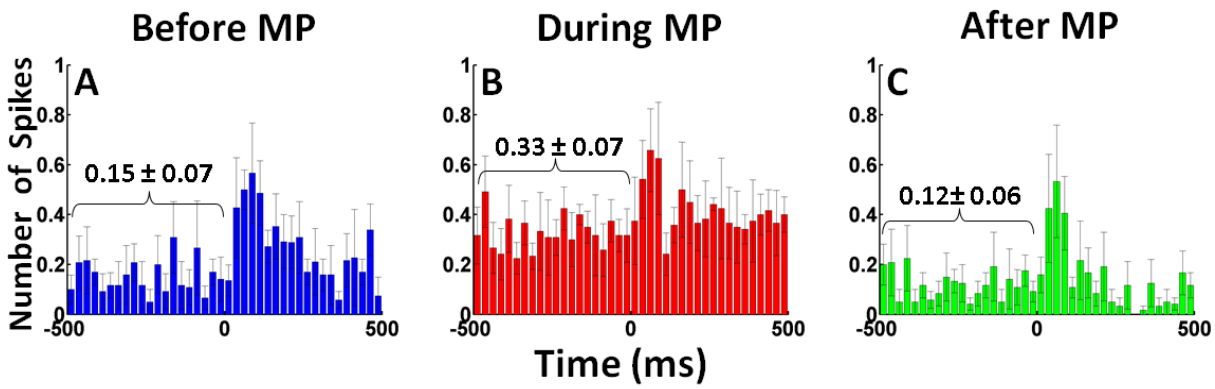


**Figure 13: Reflex response of cell 4 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2)**
**(A): Blue histogram is response to afferent stimulation before centrally commanded motor program. (B): Red histogram is response to afferent stimulation during the motor program. (C): Green histogram is response to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across 4 animals ± SEM. T=0=afferent stimulation at A6N2.**

*3.2.2.2 Hypothesized circuitry of response of cell 4 to afferent stimulation before, during and after the motor program*

Similar to cell 3, afferent input the sixth ganglion excite MSIs which then project rostrally and excite cell 4 (Figure 14). Descending command fibers of postural flexion excite the same motor neuron during the centrally commanded motor program. Unlike cell 3, the reflex response to afferent stimulation was not inhibited during the centrally commanded motor program. The data suggest that cell 4 does not receive descending modulation during the centrally commanded motor program. As stated previously this does not rule out the effect of possible ceiling effect.

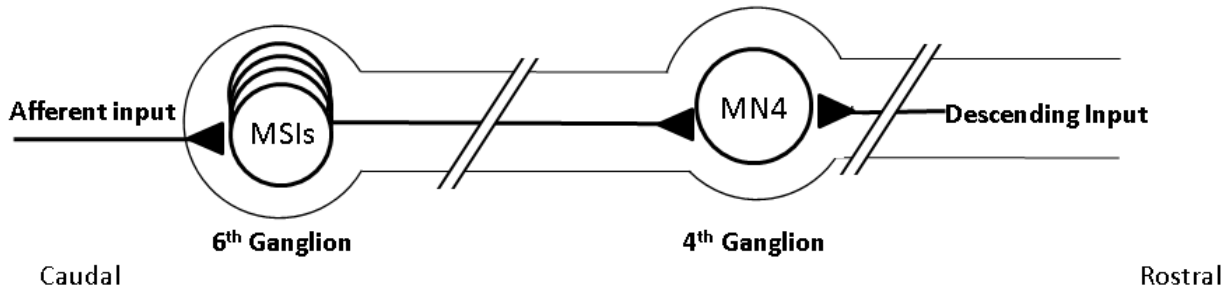


**Figure 14: Hypothesized circuitry of reflex response of cell 4 to afferent stimulation before, during and after the centrally commanded motor program**
**Filled circles are inhibitory synapses and filled triangles are excitatory synapses. MSIs: Mechanosensory Interneurons (population). MN4: Motor Neuron 4.**

*3.2.2.3 Cell 4 is more excited in response to afferent stimulation with the local application of picrotoxin at the sixth abdominal ganglion*

With the application of picrotoxin at the sixth abdominal ganglion, cell 4 was excited in response to afferent stimulation and this excitation lasted longer with the application of PTX than before PTX (Figure 15A *vs*.15G). During the motor program the overall activity levels of cell 4 were higher than before the motor program, 12.02 ± 8.55 Hz before the motor program *vs.* 34.89 ± 11.77 Hz during the motor program (Figure 16). The average firing frequency of the cell was also higher during the motor program with the application of PTX when compared to before PTX, 25.92 ± 5.51 Hz before PTX vs. 34.87 ± 11.77 with PTX (Figure 16). Cell 4 did not respond to the afferent stimulation during the motor program with PTX application (Figure: 15E). After the termination of the motor program, the overall activity level of cell 4 returned to similar levels as before the motor program, 12.02 ± 8.55 Hz before the motor program vs. 9.83 ± 6.49 Hz after the motor program (Figure 16) and was once again excited by the afferent stimulation (Figure 15F). After the removal of PTX, cell 4 was briefly excited by the afferent stimulation before and after the motor program and did not respond to the afferent stimulation during the motor program (Figure 15 G, H, and I).

These data suggest that the reflex response of cell 4 was not initially inhibited during the centrally commanded motor program, however, with the application of PTX the cell no longer responded to

afferent stimulation during the motor program. It should be noted that during the motor program before the application of PTX the firing frequency of cell 4 was lower than during the motor program with PTX application (Figure 16). It could be that before the application of PTX cell 4 responded to afferent stimulation with a slight excitation during the centrally commanded motor program because the baseline firing frequency of the cell was at the level where the cell could still respond. With the application of PTX, however, the baseline firing frequency of the cell was higher during the motor program than it was before the application of PTX, and now the cell is no longer capable of responding to the afferent stimulation.

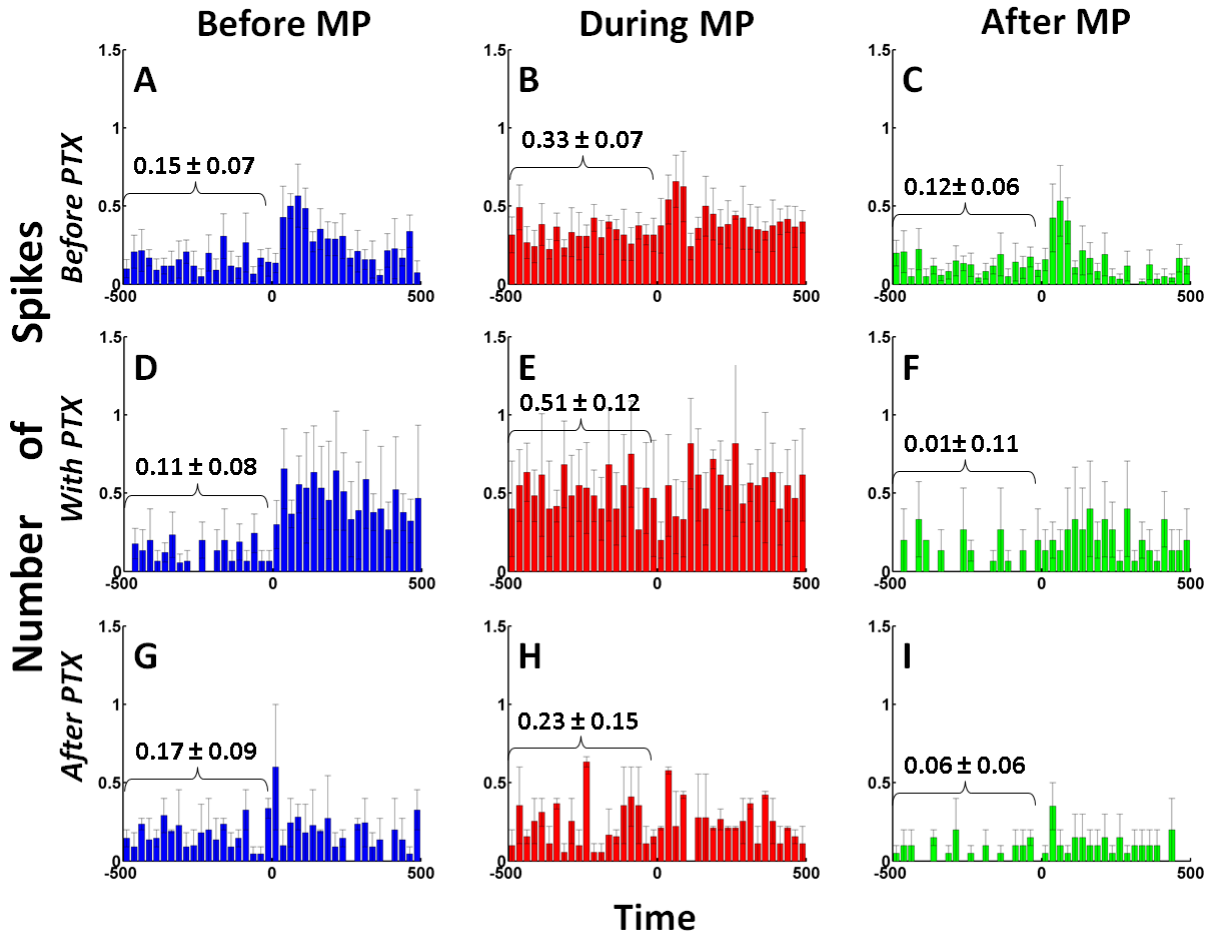


**Figure 15: Reflex response of cell 4 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2) before, with and after the local application of picrotoxin.**

**(A), (D), and (G): Blue histograms are responses to afferent stimulation before centrally commanded motor program. (B), (E), and (H): Red histograms are response during the motor program. (C), (F), and (I): Green histograms are responses to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across multiple animals ± SEM. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. T=0=afferent stimulation at A6N2.**

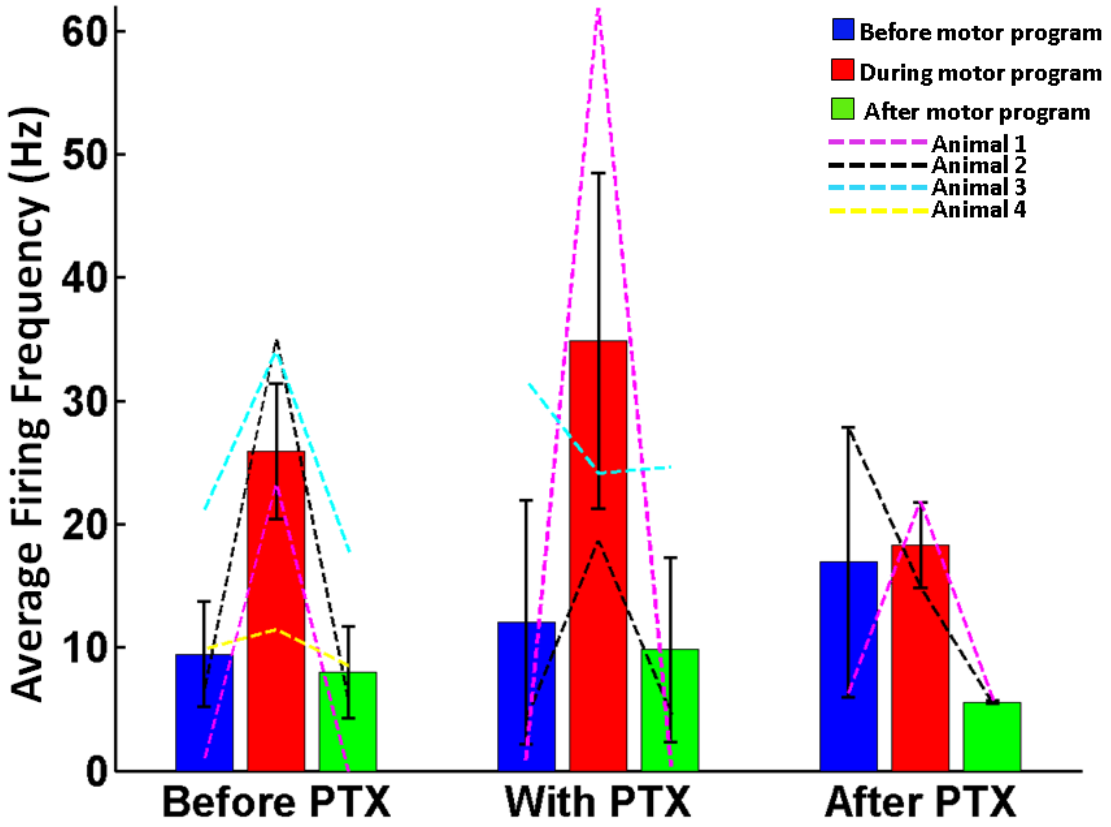


**Figure 16: Average firing frequencies of cell 4 of superficial flexor nerve 3 of the fourth abdominal ganglion (A43s)**
**Average firing frequency is average over entire 500ms before afferent stimulation. Blue bars are averages across animals before the centrally commanded motor program, red are averages across animals during the motor program and green are averages across animals after the motor program. Dotted lines are averages within a single animal. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. Error bars are ± SEM.**

#### *3.2.2.4 Hypothesized circuitry of longer response of cell 4 to afferent stimulation with the application of picrotoxin*

A disinhibitory pathway at the sixth ganglion could explain the longer lasting excitation of cell 4 in response to afferent stimulation with the application of picrotoxin (PTX) (compare response to afferent stimulation in figures 15A and 15D). Under normal, before PTX, conditions the response of cell 4 to afferent stimulation may be mediated by the level of activation of the MSIs which project rostrally from the sixth ganglion and excite cell 4 in response to afferent stimulation (Figure 17A). These MSIs may be tonically inhibited by tonically inhibiting local interneurons (Figure 17). PTX was applied locally to the sixth abdominal ganglion and is a known blocker of inhibitory synapses. If PTX was blocking inhibition that would otherwise shorten the response of the motor neurons to afferent stimulation, then it is expected that with this inhibition onto the MSIs removed, the response to afferent stimulation would be longer, which was observed for cell 4 with the local application of PTX. Removal of this inhibition of the MSIs would permit them to respond to the afferent stimulation more vigorously and excite motor neuron 4 for a longer period of time.

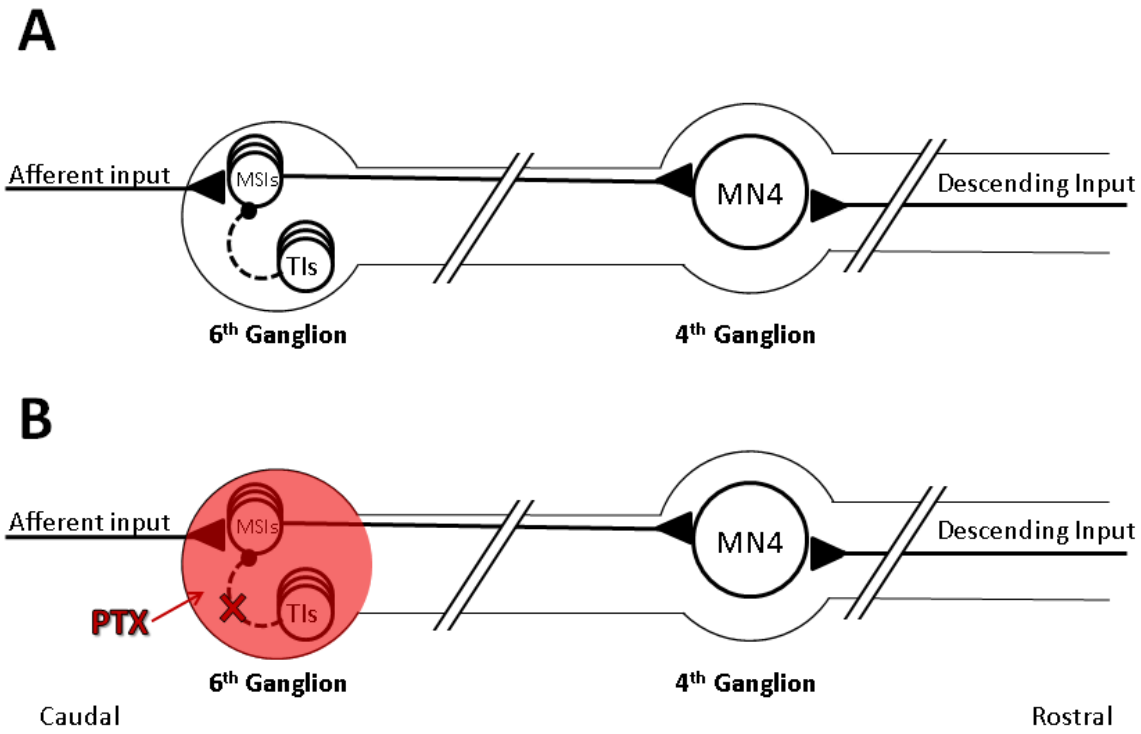


**Figure 17: Hypothesized circuitry of longer response to afferent stimulation of cell 4 with the application of picrotoxin.**
**(A) Before the application of PTX, local tonic inhibition (TI) at the 6th ganglion sets the tonic activity response of the motor neuron 4 to afferent stimulation through inhibition of the MSIs. MSIs (mechanosensory interneurons) mediate the response to mechanosensory input from the afferent. (B) With the application of PTX, (blocks inhibition) at the 6th ganglion local inhibition is removed. Tonic inhibition of the MSIs is prevented causing tonic activity of to increase and a longer response of cell 4 to afferent stimulation. Filled circles are inhibitory synapses and filled triangles are excitatory synapses. MSIs: Mechanosensory Interneurons (population). MN4: Motor Neuron 4. TI: tonically active inhibitory interneuron. Red X is elimination of a synapse**

### *3.2.3 Cell 6*

#### *3.2.3.1 Reflex response to afferent stimulation of cell 6 is not inhibited during the centrally commanded motor program*

Cell 6 is a large phasically active cell and usually only fired in response to afferent stimulation (Kennedy and Takeda 1965 and Figure 18). Cell 6 was excited by the afferent stimulation before the onset of the motor program, and this excitation lasted for about 400ms and (Figure 18A). During the centrally commanded motor program, the overall activity of cell 6 was much higher than before the motor

program, average 16.39 ± 12.36Hz before the motor program *vs*.60.64 ± 19.15Hz during the motor program (Figure 21). Cell 6 responded with a brief excitation to the afferent stimulation, even when delivered during the motor program (Figure 18B). After the motor program, the cell was once again strongly excited in response to the afferent stimulation; however, the excitation only lasted about 150ms and was shorter in amplitude than before the centrally commanded motor program (Figure 18A *vs.* 18C). These data suggest that the postural reflex response to afferent stimulation of cell 6 was not modulated during the centrally commanded flexion motor program; however, the level of excitation was decreased during the motor program.

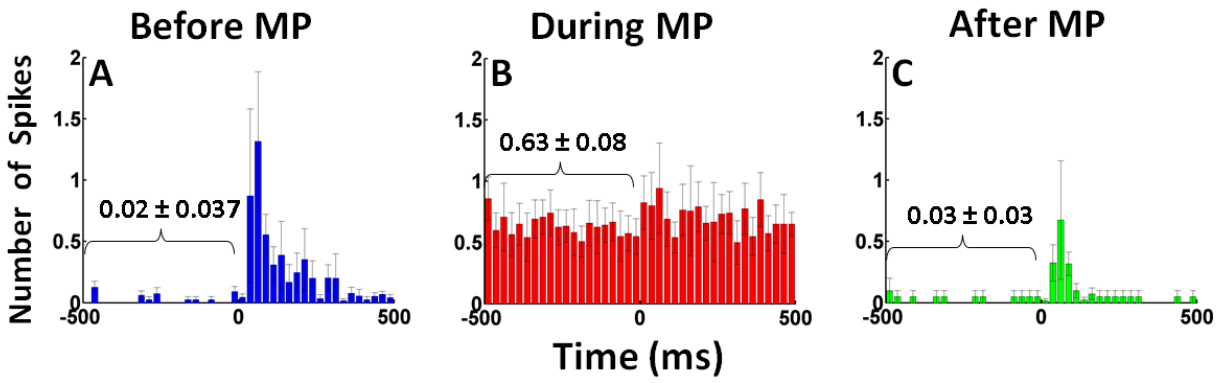


**Figure 18: Reflex response of cell 6 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2)**
**(A): Blue histogram is response to afferent stimulation before centrally commanded motor program. (B): Red histogram is response to afferent stimulation during the motor program. (C): Green histogram is response to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across 4 animals ± SEM. T=0=afferent stimulation at A6N2.**

*3.2.3.2 Hypothesized circuitry of response of cell 6 to afferent stimulation before, during and after the motor program*

Similar to cell 4, the reflex response to afferent stimulation was not inhibited during the centrally commanded motor program. Therefore, unlike cell 3 and similar to cell 4 there may not be descending

modulation directed at cell 6 during the centrally commanded motor program (Figure 19). This hypothesized circuitry does not rule out the possibility of a ceiling effect on the firing of cell 6. Similar to cell 4, it could be that case that the activation of cell 6 during the motor program was so high that the cell became incapable of responding to the afferent stimulation.

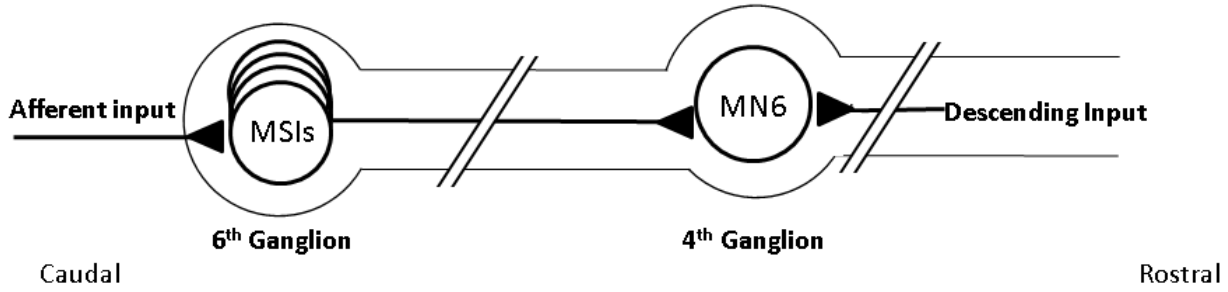


**Figure 19: Hypothesized circuitry of response of cell 6 to afferent stimulation before, during and after the centrally commanded motor program**
**Filled circles are inhibitory synapses and filled triangles are excitatory synapses. MSIs: Mechanosensory Interneurons (population). MN6: Motor Neuron 6.**

*3.2.3.3 Reflex response of cell 6 to afferent stimulation did not change with the application of picrotoxin at the six abdominal ganglion*

With the application of PTX, cell 6 was excited in response to the afferent stimulation before the motor program (Figure 20D). During the motor program, firing frequency of the neuron was higher than before the onset of the motor program, 0.18 ± 0.15 before the motor program *vs*. 39.16 ± 16.05 during the motor program (Figure 21) and the cell was briefly excited in response to afferent stimulation. After the motor program, cell 6 was excited by the afferent stimulation (Figure 20F). After the removal of PTX, cell 6 was excited by the afferent stimulation before and after the motor program, but the response to afferent stimulation was much smaller than before the application of PTX (compares Figures 20A, D and G). During the motor program the average firing frequency of cell 6 was higher than before the motor program, 0.50 ± .36Hz before the motor program *vs*. 48.31 ± 0.89Hz spikes during the motor program (Figure 21). After the motor program cell 6 was excited by the afferent stimulation (Figure 20I). These

data suggest that application of PTX to the sixth ganglion did not change the response of cell 6 to afferent stimulation.

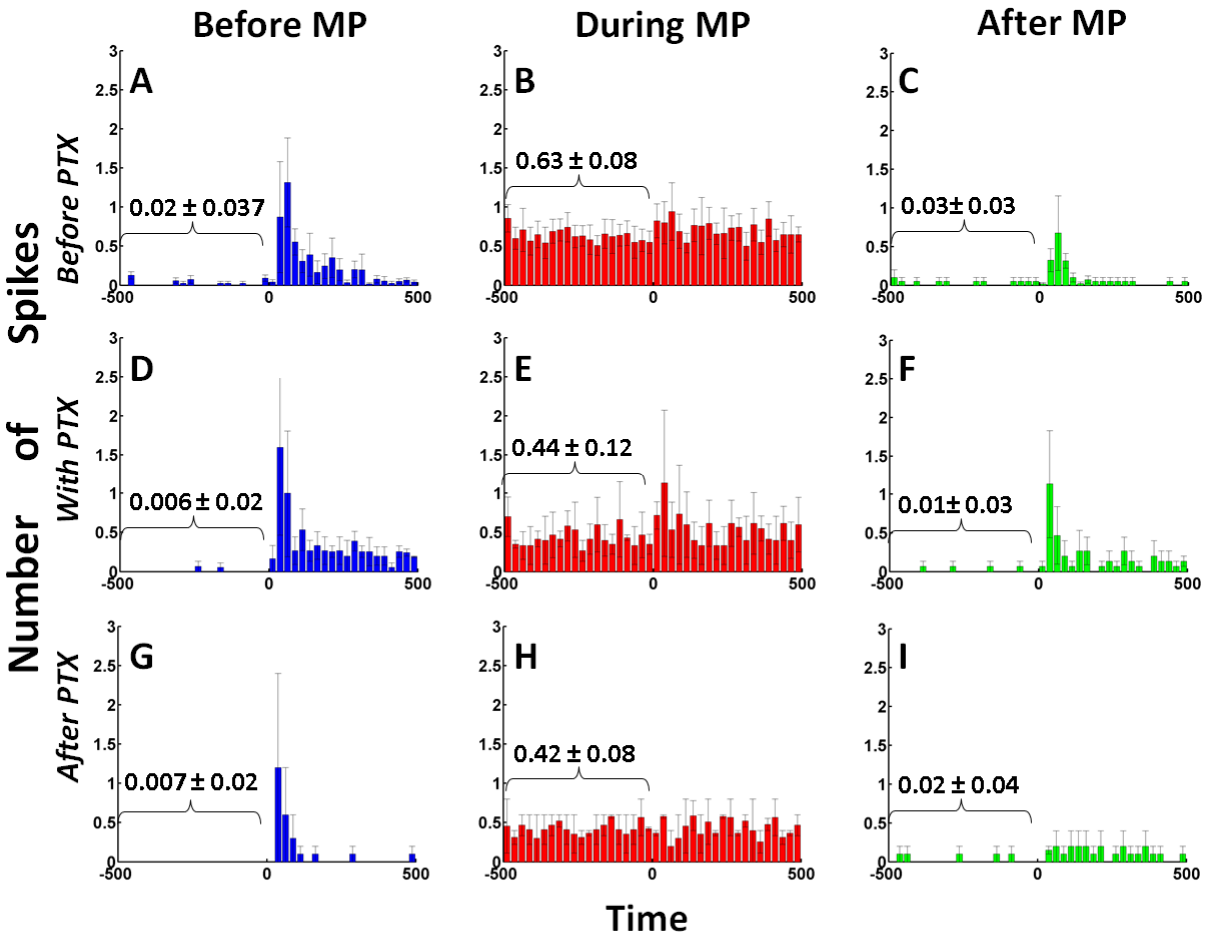


**Figure 20: Reflex response of cell 6 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2) before, with and after the local application of picrotoxin**
**(A), (D), and (G): Blue histograms are responses to afferent stimulation before centrally commanded motor program. (B), (E), and (H): Red histograms are response during the motor program. (C), (F), and (I): Green histograms are responses to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across multiple animals ± SEM. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. T=0=afferent stimulation at A6N2.**

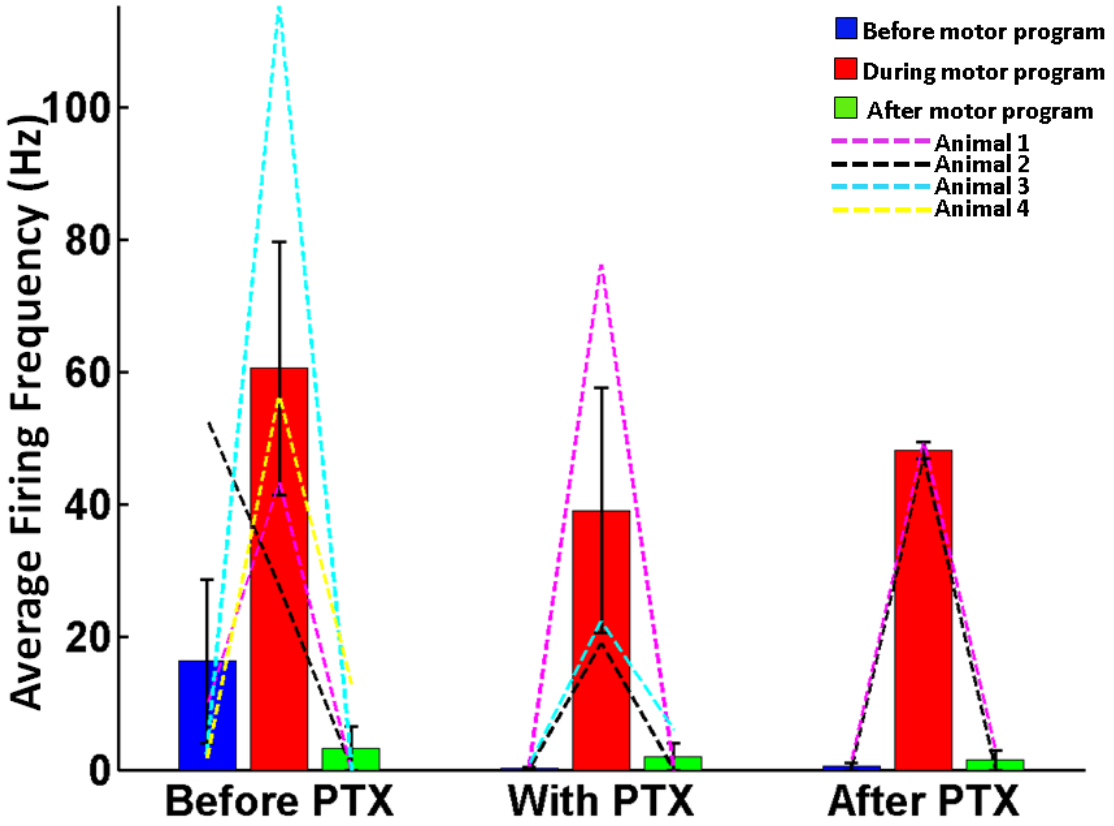


**Figure 21: Average firing frequencies of cell 6 of superficial flexor nerve 3 of the fourth abdominal ganglion (A43s)**
**Average firing frequency is average over entire 500ms before afferent stimulation. Blue bars are averages across animals before the centrally commanded motor program, red are averages across animals during the motor program and green are averages across animals after the motor program. Dotted lines are averages within a single animal. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. Error bars are ± SEM.**

### *3.2.4 Cell 1*

#### *3.2.4.1 Cell 1 does not have a robust reflex response to afferent stimulation at the sixth abdominal ganglion.*

In response to the afferent stimulation the cell may have been initially briefly excited then inhibited between 25ms and 75ms after the afferent stimulation (Figure 22A). The response to afferent stimulation is not very robust; therefore, it cannot be concluded with the present sample size (4 animals) whether this mixed excitation and inhibition would still occur with a larger sample size. During the cen-

trally commanded motor program the cell did not respond to the afferent stimulation (Figure 22B). After the termination of the motor program the cell may be slightly excited in response to the afferent stimulation, however, once again the sample size is not large enough to be certain (Figure 22C). These data suggest that cell 1 may not participate in the reflex response to afferent stimulation, and therefore may not be modulated during the centrally commanded motor program.

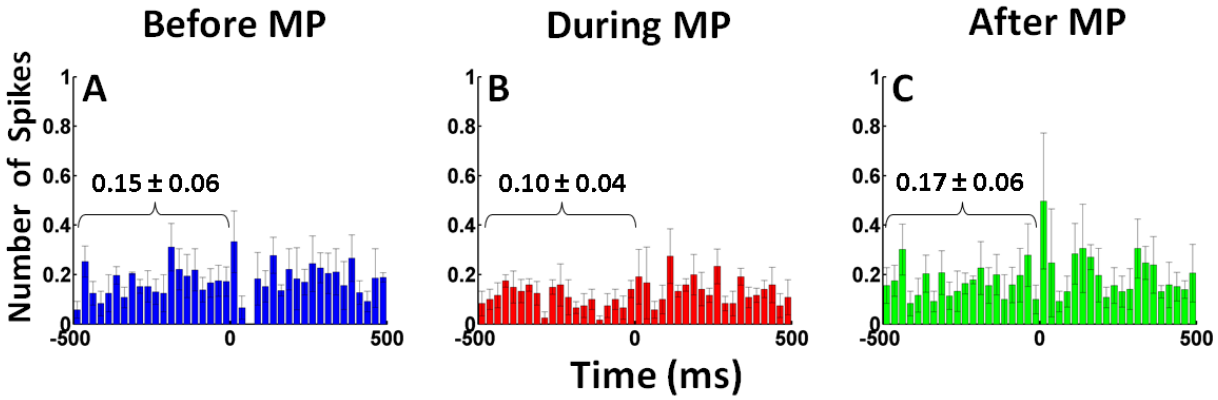


**Figure 22: Reflex response of cell 1 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2)**
**(A): Blue histogram is response to afferent stimulation before centrally commanded motor program. (B): Red histogram is response to afferent stimulation during the motor program. (C): Green histogram is response to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across 4 animals ± SEM. T=0=afferent stimulation at A6N2.**

*3.2.4.2 Application of picrotoxin increases the baseline firing frequencies of cell 1*

With the application of PTX the overall firing frequency of cell 1 was much higher than before PTX application, 11.31 ± 4.72Hz before PTX *vs.* 26.23 ± 4.40Hz with PTX (Figure 24). Cell 1 responded with a brief inhibition in response to afferent stimulation before the motor program (Figure 23D). During the motor program, the average firing frequency levels of cell 1 was lower than before the motor program, 26.23 ± 4.40 Hz before the motor program *vs.* 13.87 ± 1.59Hz during the motor program (Figure 24). During and after the motor program cell 1 did not respond to the afferent stimulation.

After the removal of PTX the firing frequency of cell 1 returned to the same level as before PTX, 11.31 ± 4.72 Hz before PTX vs. 13.06 ± .38 Hz after PTX (Figure 24). After the removal of PTX, cell 1 was excited in response to afferent stimulation before the motor program and did not respond to the afferent stimulation during or after the motor program (Figures 23G-I).

These data suggest that the application of PTX at the terminal abdominal ganglion caused the baseline activity levels of cell 1 before the motor program to increase; after the removal of PTX the baseline returned to the same levels as before PTX. Due to the lack of a strong reflex response even before PTX, nothing can be presently determined about any reflex modulation during the centrally commanded motor program.

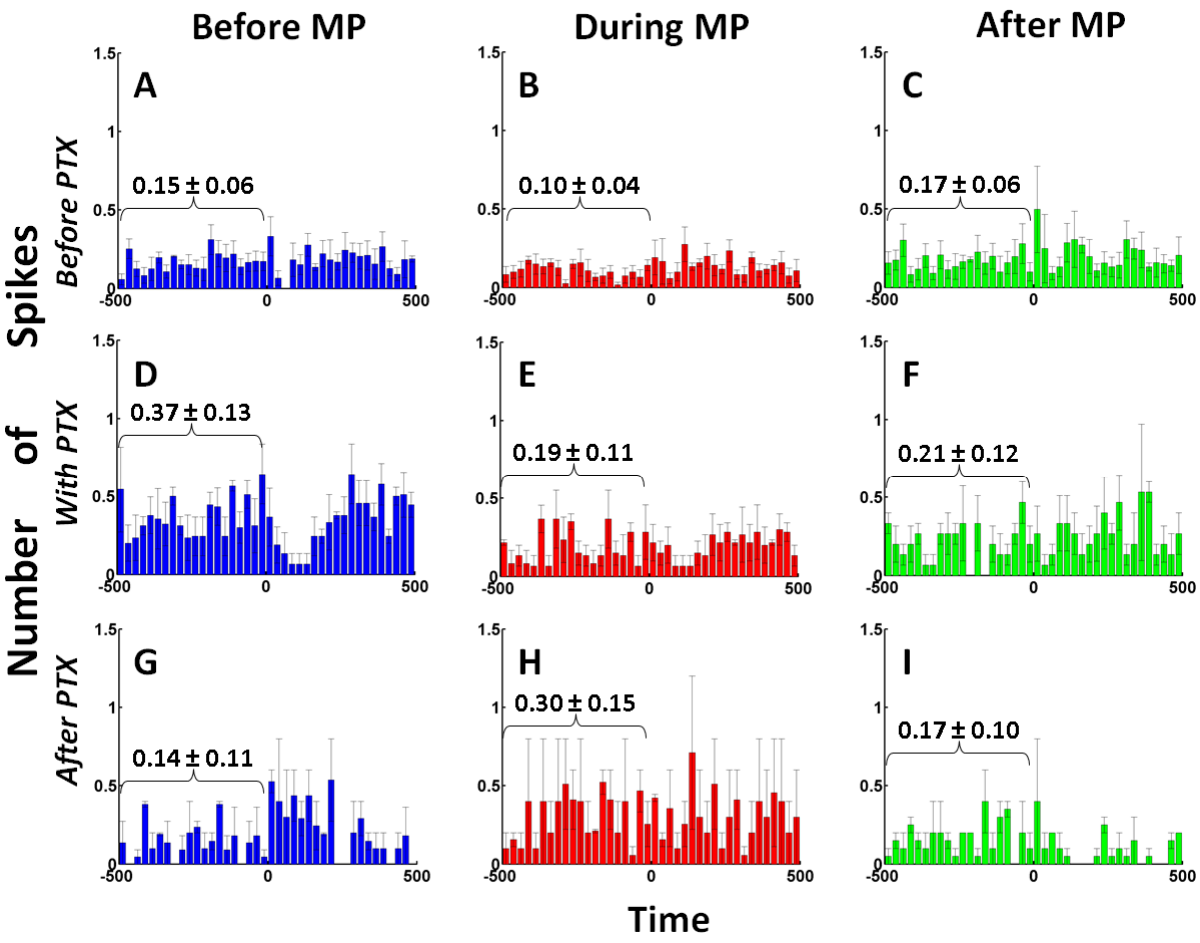


**Figure 23: Reflex response of cell 1 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2) before, with and after the local application of picrotoxin**

**(A), (D), and (G): Blue histograms are responses to afferent stimulation before centrally commanded motor program. (B), (E), and (H): Red histograms are response during the motor program. (C), (F), and (I): Green histograms are responses to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across multiple animals ± SEM. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. T=0=afferent stimulation at A6N2.**

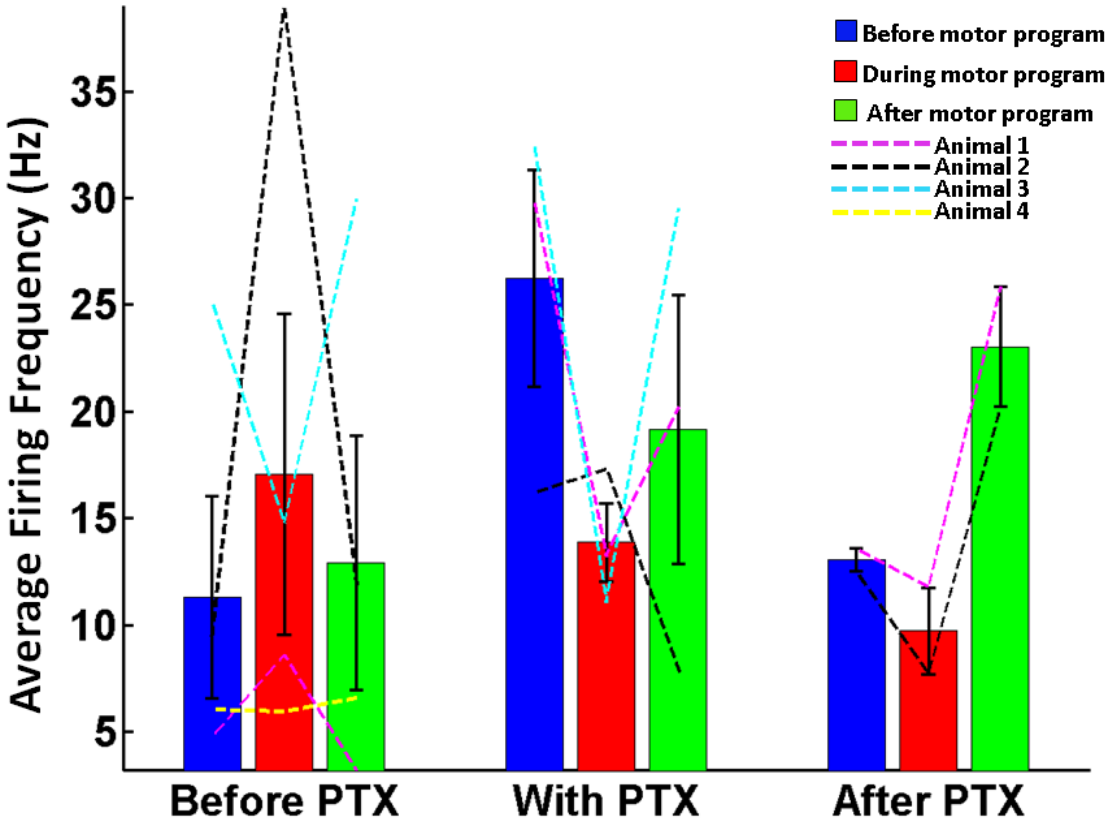


**Figure 24: Average firing frequencies of cell 1 of superficial flexor nerve 3 of the fourth abdominal ganglion (A43s)**
**Average firing frequency is average over entire 500ms before afferent stimulation. Blue bars are averages across animals before the centrally commanded motor program, red are averages across animals during the motor program and green are averages across animals after the motor program. Dotted lines are averages within a single animal. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. Error bars are ± SEM.**

*3.2.4.3 Hypothesized circuitry explaining the increase in baseline firing of cell 1 with the application of PTX*

With the application of PTX there was an increase in the baseline firing of cell 1. This could be explained by a disinhibition by PTX at the sixth ganglion, as proposed in figure 25. Under this hypothesis the motor neurons tonic firing rate is mediated by tonically active ascending interneurons (AIs) that project from the sixth ganglion to the fourth ganglion where they excite the motor neuron. The AIs are themselves tonically inhibited by tonic inhibitory interneurons (TIs) in the sixth ganglion, thereby reduc-

ing the AI activity and the motor neuron activity levels as observed prior to the activation of a motor program. With the application of PTX at the 6th ganglion, much of the local inhibition onto the AIs is blocked and the tonic activity of the AIs increase and therefore the baseline firing of cell 1 is also increased (Figure 23 A and D and Figure 25).

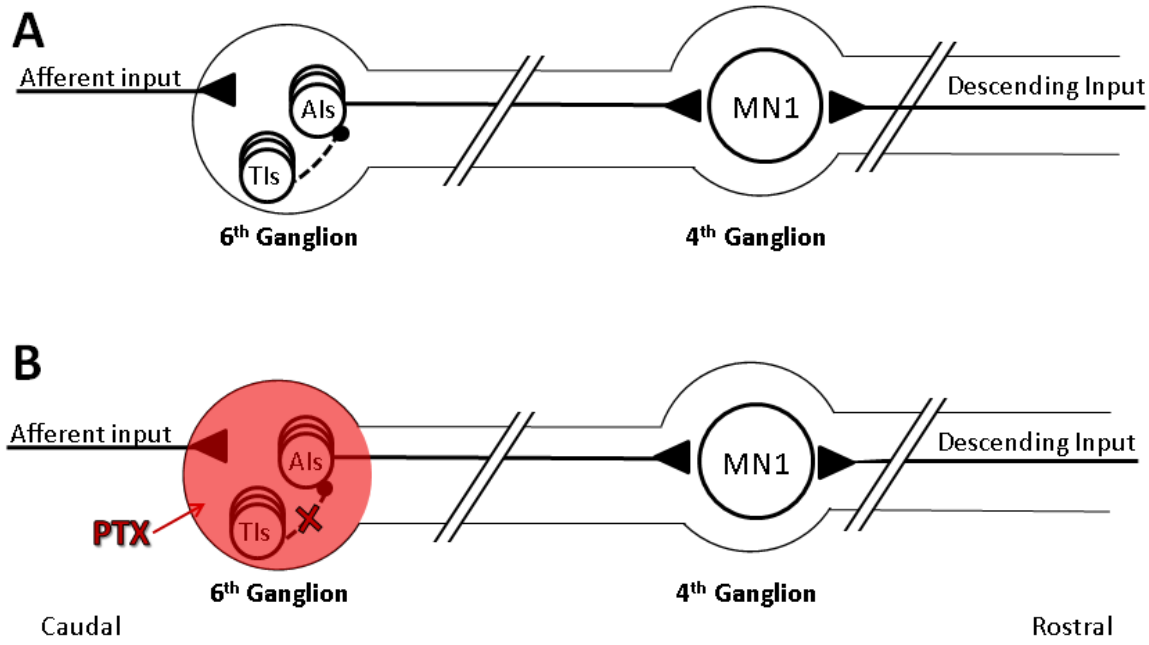


**Figure 25: Hypothesized circuitry explaining the increase in baseline firing of cell 1 with the application of PTX**
**Filled circles are inhibitory synapses and filled triangles are excitatory synapses. AIs- ascending excitatory interneurons. MSIs: Mechanosensory Interneurons (population). MN1: Motor Neuron 1. TI: tonically active inhibitory interneuron. Red X is elimination of a synapse**

### *3.2.5 Cell 2*

#### *3.2.5.1 Cell 2 does not have a robust reflex response to afferent stimulation at the sixth abdominal ganglion*

In response to the afferent stimulation cell 2 may have responded with a brief excitation then inhibition between 50ms and 75ms (Figure 26A). During the centrally commanded motor program the

average firing frequency of cell 2 slightly increased, 8.14 ± 2.56Hz before the motor program vs. 14.79 ± 2.55Hz during (Figure 28) and the cell did not respond to the afferent stimulation (Figure 26B). After the termination of the motor program the cell responded with a brief excitation to the afferent stimulation but the inhibition at 50-75ms after the afferent stimulation did not occur (Figure 26C). The baseline activity of cell 2 is increased and the reflex response of the cell to afferent stimulation may be modulated during the centrally commanded motor program. Similar to cell 1, however, the reflex response of cell 2 to afferent stimulation is not very robust, and it cannot be concluded whether the centrally commanded motor program has any effect on the minimal reflex response.

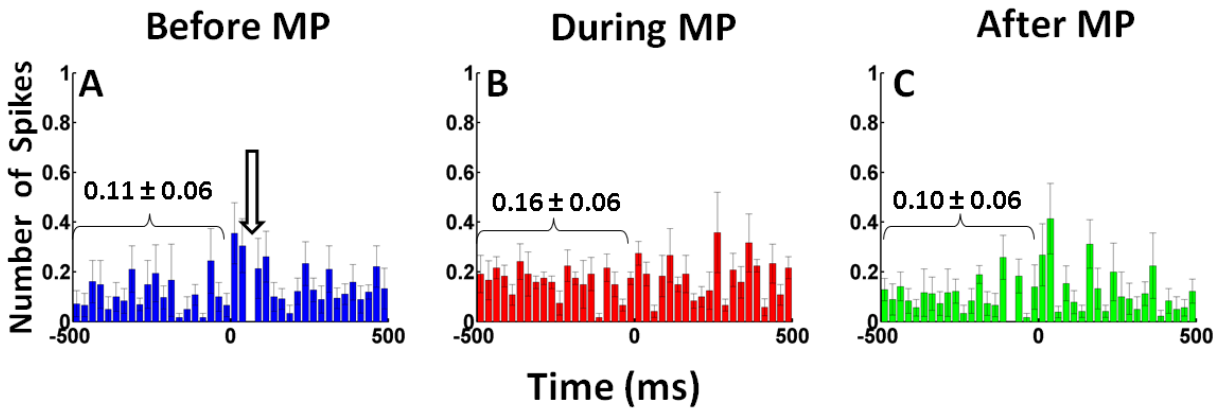


**Figure 26: Reflex response of cell 2 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2)**
**(A): Blue histogram is response to afferent stimulation before centrally commanded motor program. (B): Red histogram is response to afferent stimulation during the motor program. (C): Green histogram is response to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across 4 animals ± SEM. T=0=afferent stimulation at A6N2.**

*3.2.5.2 Local application of picrotoxin at the sixth abdominal ganglion increases the baseline firing frequency of cell 2*

With the application of PTX, the overall firing rate of cell 2 increased before the motor program from 8.14 ± 2.56 Hz before PTX to 30.32 ± 12.89 Hz with PTX (Figure 28). It should be noted that there

was a large amount of variation across animals with the application of PTX on the baseline firing frequencies and the effect of the motor program on cell 2 with the application of PTX (Figure 28- With PTX, dotted lines). Cell 2 did not respond to the afferent stimulation before, during or after the motor program with or after the application of PTX. After the removal of PTX, the baseline firing frequency before the motor program once again decreased, 9.50 ± .94 Hz (Figure 28). These data suggest that the baseline activity of cell 2 was increased with the application of PTX. Cell 2 may not participate in the reflex response to afferent stimulation, and with the present number of samples it cannot be concluded whether the reflex response of cell 2 is modulated during the centrally commanded motor program.

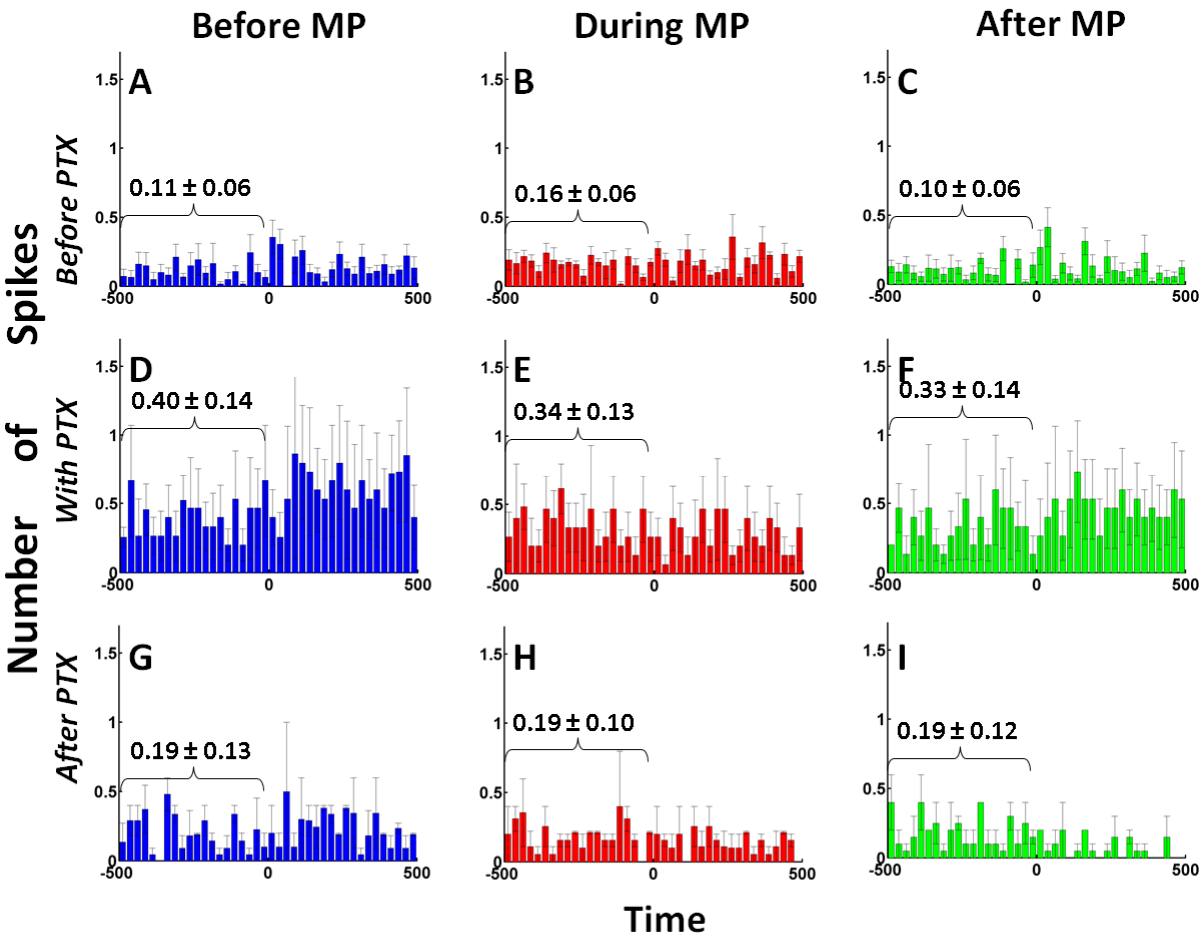


**Figure 27: Reflex response of cell 2 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2) before, with and after the local application of picrotoxin.**
**(A), (D), and (G): Blue histograms are responses to afferent stimulation before centrally commanded motor program. (B), (E), and (H): Red histograms are response during the motor program. (C), (F), and (I): Green histograms are responses to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across multiple animals ± SEM. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. T=0=afferent stimulation at A6N2.**

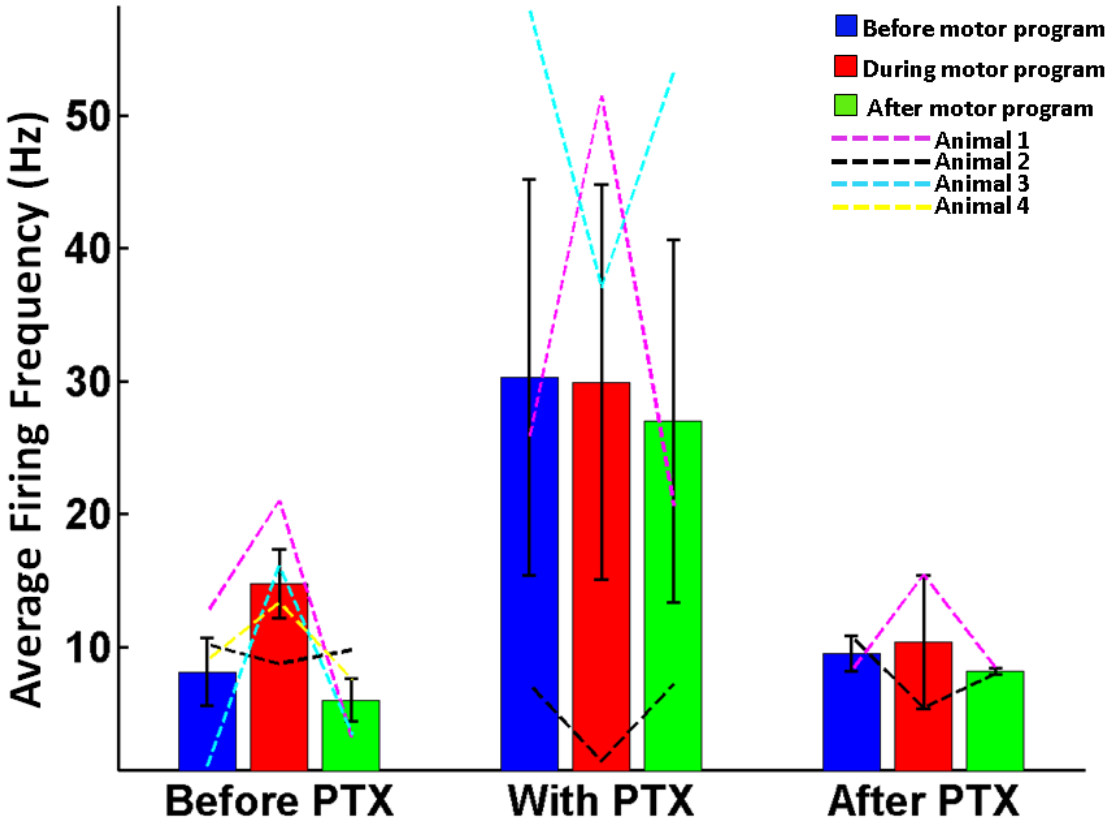


**Figure 28: Average firing frequencies of cell 2 of superficial flexor nerve 3 of the fourth abdominal ganglion (A43s)**
**Average firing frequency is average over entire 500ms before afferent stimulation. Blue bars are averages across animals before the centrally commanded motor program, red are averages across animals during the motor program and green are averages across animals after the motor program. Dotted lines are averages within a single animal. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. Error bars are ± SEM.**

*3.2.5.3 Hypothesis explaining the increase in baseline firing of cell 2 with the application of picrotoxin at the sixth ganglion*

Similar to cell 1, the baseline activity of cell 2 increased with the application of PTX. This increase in activity could be explained with the exact same mechanism as cell 1. The tonic activity of cell 2 may be set by tonically inhibited ascending interneuron and the application of PTX would disinhibit them. This disinhibition would lead to an increase in baseline firing the ascending interneurons and of cell 2.

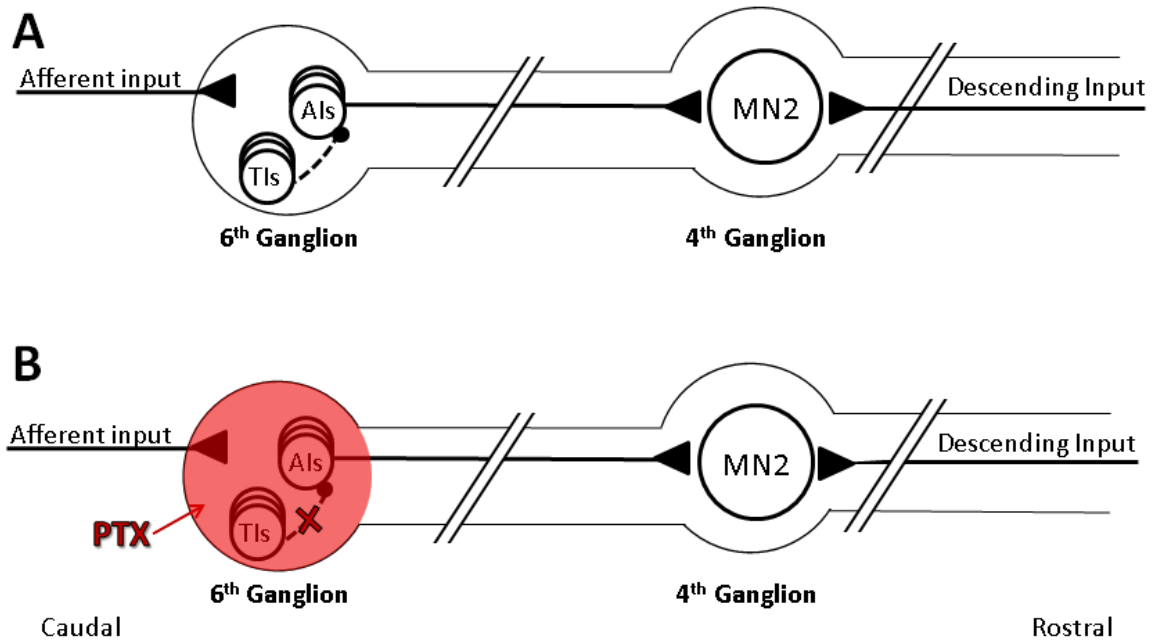


**Figure 29: Hypothesized circuitry explaining the increase in baseline firing of cell 2 with the application of picrotoxin at the sixth ganglion**
**Filled circles are inhibitory synapses and filled triangles are excitatory synapses. AIs- ascending excitatory interneurons. MSIs: Mechanosensory Interneurons (population). MN2: Motor Neuron 2. TI: tonically active inhibitory interneuron. Red X is elimination of a synapse**

### *3.2.6 Cell 5*

#### *3.2.6.1 Cell 5 fires little throughout the trials*

Cell 5 is an inhibitory motor neuron and fired much less than the excitatory motor neurons throughout the trials (Figure 30). Before the onset of the centrally commanded motor program, cell 5 was slightly excited by the afferent stimulation (Figure 30A). During the motor program the baseline firing frequency of cell 5 was higher than it was before the motor program 0.12 ± 0.12 Hz before the motor program vs. 2.06 ± 1.22 Hz during the motor program (Figure 32). Cell 5 did not respond to the afferent stimulation during the centrally commanded motor program (Figure 30B). After the motor program the activity levels of cell 5 returned to the same baseline as before the motor program, .02 ± 0.02 (Figure 32). Cell 5 was once again slightly excited by the afferent stimulation after the motor program (Figure 31C). These data suggest that the reflex response of cell 5 to afferent stimulation may be modulated

during the centrally commanded flexion motor program. Since the cell responds very little to the afferent stimulation and the baseline firing of the motor neuron is low, it cannot be concluded for certain whether the cell is participating in the reflex response at all. There is also a large amount of variation across animals on average firing frequencies of the cell before any afferent stimulation (Figure 33).

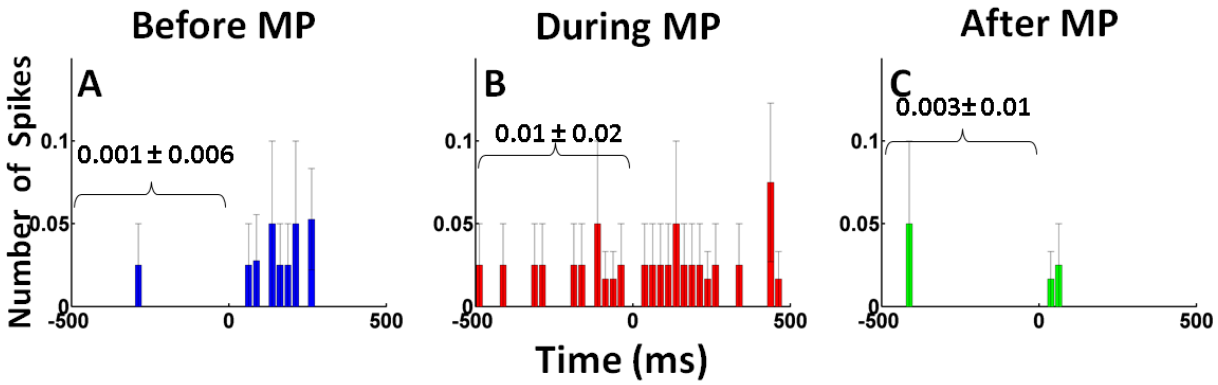


**Figure 30: Reflex response of cell 5 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2)**
**(A): Blue histogram is response to afferent stimulation before centrally commanded motor program. (B): Red histogram is response to afferent stimulation during the motor program. (C): Green histogram is response to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across 4 animals ± SEM. T=0=afferent stimulation at A6N2.**

*3.2.6.2 Local application of picrotoxin at the sixth abdominal ganglion does not change the response of cell 5 to afferent stimulation.*

With the application of PTX the overall responses of cell 5 did not change. The overall activity levels of cell 5 are very low and no conclusion can be made about its reflex response or possible modulation during the motor program (Figure 31).

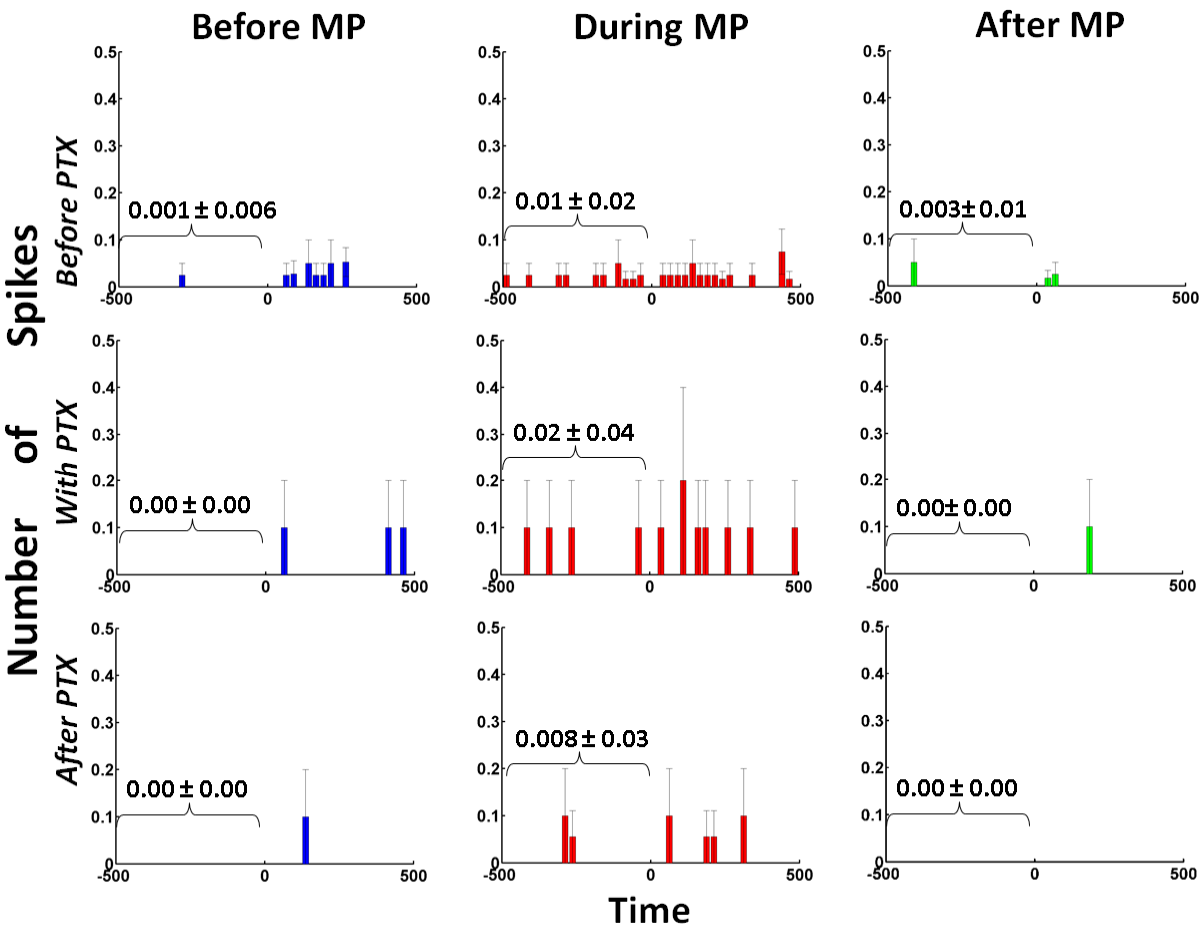


**Figure 31: Reflex response of cell 5 of superficial flexor nerve 3 of the fourth ganglion (A43s) to afferent stimulation of the ipsilateral second nerve of the sixth ganglion (A6N2) before, with and after the local application of picrotoxin**
**(A), (D), and (G): Blue histograms are responses to afferent stimulation before centrally commanded motor program. (B), (E), and (H): Red histograms are response during the motor program. (C), (F), and (I): Green histograms are responses to afferent stimulation after the motor program. Numbers presented on the left of each histogram are average number of spikes per 25ms bin over entire 500msbefore afferent stimulation ± STD. 25ms bins are averages across multiple animals ± SEM. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. T=0=afferent stimulation at A6N2.**

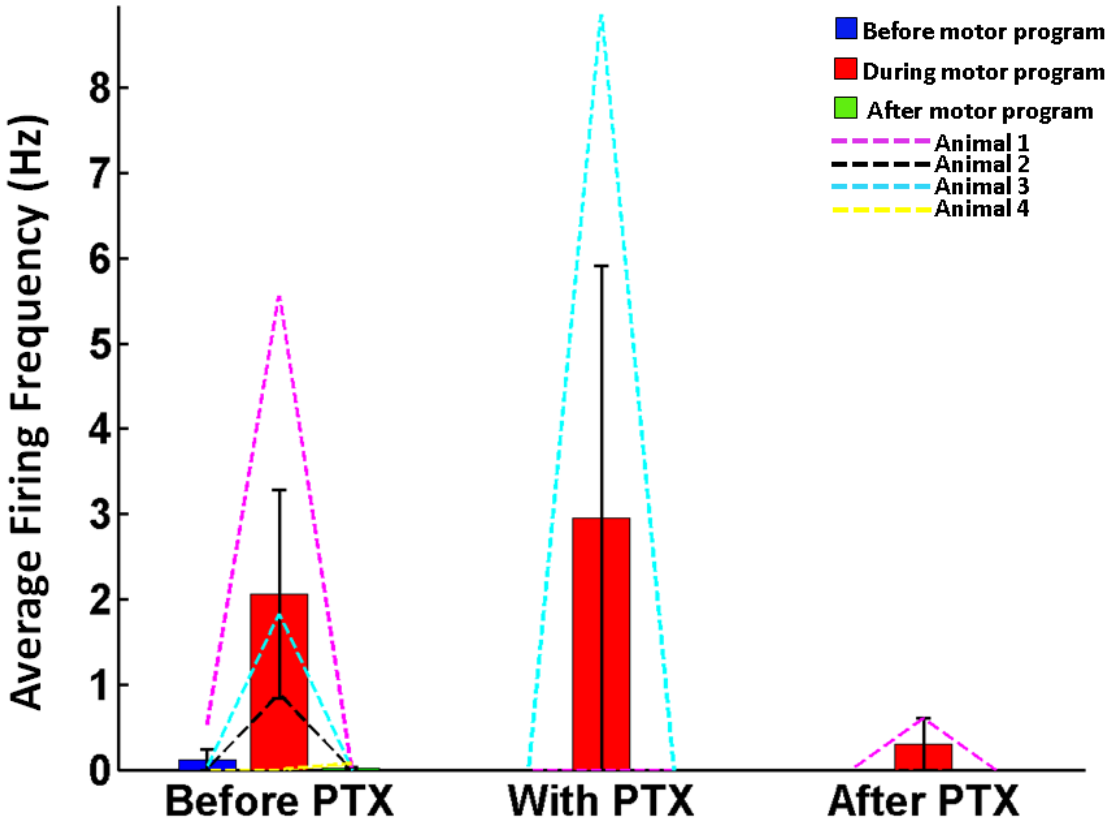


**Figure 32: Average firing frequencies of cell 5 of superficial flexor nerve 3 of the fourth abdominal ganglion (A43s)**
**Average firing frequency is average over entire 500ms before afferent stimulation. Blue bars are averages across animals before the centrally commanded motor program, red are averages across animals during the motor program and green are averages across animals after the motor program. Dotted lines are averages within a single animal. N=4 animals before PTX, N=3 animals with PTX and N=2 animals after PTX. Error bars are ± SEM.**

**Table 2: Summary of results of all motor neurons**

| Cell | Type of motor neuron | Reflex response to afferent stimulation | Modulation of reflex response during centrally commanded motor program | Effect of Picrotoxin |
|---|---|---|---|---|
| 3 | Medium tonic | Strong excitation | Reflex is inhibited | No effect |
| 4 | Medium tonic | Strong excitation | Reflex is reduced | Longer response to afferent stimulation |
| 6 | Large phasic | Strong excitation | Reflex is reduced | No effect |
| 1 | Small tonic | No response | N/A | Increase in baseline firing frequency |
| 2 | Small tonic | No response | N/A | Increase in baseline firing frequency |
| 5 | Inhibitory phasic | Possible excitation | Reflex may be inhibited | No effect |

# 4 CONCLUSIONS

## 4.1 Modulation of the local postural flexion reflex response of cell 3 to afferent stimulation during the centrally commanded flexion motor program

The superficial flexor motor nerve contains the axons of six motor neurons. Only the medium tonic and large phasic of these motor neurons responded to the afferent stimulation. The excitatory reflex response of cell 3 was shown to be inhibited during the centrally commanded motor program. However, the reflex responses of cells 4 and 6 were not inhibited during the centrally commanded motor program. In crayfish, it has been shown that during the rapid flexion response of escape behavior, walking, and tailfan movements, local reflexes are modulated during the centrally commanded movements (Bryan and Krasne 1977 a and b; Cattaert et al. 1992; Kirk and Wine 1984; El Manira and Clarac 1991; Cattaert et al. 1992). The data from this study suggest that responses to afferent stimulation of the motor neurons of the superficial flexor nerve are differentially modulated based on the motor neuron itself.

There could be a number of recruited elements responsible for this modulation during the motor program and they could in turn modulate the motor neurons differently; this would explain the inhibition of the response to afferent stimulation of cell 3 during the motor program and not cells 4 and 6. As stated previously (see Results) the present experiments do not rule out the possibility of a ceiling effect during the motor program.

### 4.2 Effect of picrotoxin on the modulation of the local postural flexion reflex response during the centrally commanded flexion motor program

Application of PTX to the sixth ganglion did not prevent the modulation of the response to afferent stimulation of cell 3 during the motor program. If descending GABAergic modulation during the centrally commanded flexion motor program was directed at the sixth ganglion synapse between the primary sensory afferents and MSIs during the motor program, then application of PTX at the 6$^{th}$ ganglion would have prevented this inhibition and the modulation by the motor program. This is not what the data suggest. Under PTX application, cell 3 was excited by the afferent stimulation before and after the motor program and did not respond to the afferent stimulation during it. Therefore, the application of PTX at the sixth ganglion did not prevent the modulation during the centrally commanded motor program and the modulation must be directed elsewhere.

For a behaving animal, this could mean that the local reflexes may be modulated based on the type of the centrally commanded movement. For example, during the escape tailflip behavior of the crayfish the descending modulation is directed at the sixth ganglion synapse preventing response and habituation to its own movements. Tailflip consists of a series of very rapid and powerful flexions and extensions of the abdomen. The centrally commanded postural flexion motor program is much slower and mediated by a different set command fibers and motor neurons for postural flexion. During the postural flexion motor program the inhibition of the incoming sensory information seems to be modulated at a site postsynaptic to the sixth ganglion. This would allow the sensory afferent input to excite the

same population of MSIs at the same levels before, during or after the motor program and would allow those same MSIs to continue to mediate a number of other reflexes throughout the animal during the motor program; including those of motor neurons 4 and 6. Directing the modulation during the motor program at the second synapse in the circuit (MSI to motor neuron) would allow the ability to specifically modulate the local postural flexion reflex during the centrally commanded flexion motor program.

### 4.3 Notes about statistical testing

In order determine statistical significance I would need to perform a number of one-way ANOVA comparisons. Table 2 summarizes the comparisons I would make and the tests I would use.

To determine the effect of the afferent stimulation on each of the six cells I would need to compare the average number of spikes before afferent stimulation to average number of spikes after afferent stimulation of each animal. I would determine the time that I would compare before and after the afferent stimulation based on the cell itself. For example, cell 3 responds to the afferent stimulation for about 300ms, whereas cell 1 may be inhibited by the afferent stimulation for 50-75ms.

To determine the effect of the command fiber stimulation on each of the 6 cells I would compare the average number of spike before the afferent stimulation before, during and after the centrally commanded motor program. I would need to use a one-way ANOVA on all three states and then if a pair was found to be significantly different I would run a post-hoc test to determine which pair. For example, for cell 3, I would expect the baseline firing of the cell to be the same before and after the motor program but statistically different during the motor program.

To determine the effect of picrotoxin on the baseline firing of the cells, I would need to perform a one-way ANOVA with post-hoc test to compare the average number of spikes before afferent stimulation before, with and after the local application of picrotoxin. For example, for cell 2, I would expect the average number of spikes before afferent stimulation before the application of PTX to be the same as the after the application of PTX but different than with the application of PTX.

**Table 3: Summary of statistical tests**

| Test question | Comparison | Conditions of the test | Test | Post-hoc test |
|---|---|---|---|---|
| Is there an effect of the afferent stimulation on the motor neuron? | Average number of spikes before afferent stimulation vs. after afferent stimulation | Before the motor program, during the motor program, after the motor program | One-way ANOVA | No |
| Is there an effect of the command fiber stimulation on the motor neuron? | Average number of spikes before afferent stimulation before motor program vs. during the motor program vs. after the motor program | Before PTX, with PTX, after PTX | One-way ANOVA | Yes |
| Is there an effect of the application of PTX on the baseline firing of the motor neuron? | Average number of spikes before afferent stimulation before PTX vs. with PTX vs. after PTX | Before the motor program, during the motor and after the motor program | One-way ANOVA | Yes |

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