CENTRAL SEROTONERGIC MODULATION OF HEART RATE IN *APLYSIA CALIFORNICA*

by

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Central Serotonergic modulation of heart rate
in Aplysia californica

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ABSTRACT

Studies have shown that sensitization is mediated in Aplysia californica by serotonin. This process, which causes both short-term and long-term enhancement of defensive behaviors such as gill withdrawal or tail withdrawal, can be mimicked by the application of serotonin to the nervous system. However, the cardiovascular response to sensitization has received little attention. In a previous study Litowitz demonstrated that in vitro heart rate increased immediately following sensitizing stimulation and remained elevated for at least 90 minutes (1994). This study explores the hypothesis that serotonin acts as a neuromodulator which promotes sensitization of heart rate, and when applied to the nervous system, duplicates the cardiovascular response produced by sensitizing noxious stimuli.

In this experiment, the cardiovascular response to serotonin was monitored in an in vitro preparation. Heart rate was recorded following three sequential applications of serotonin to the head ganglia ($5x10^{-5}$M or $5x10^{-6}$M). Significant short-term increases in heart rate lasting 10 minutes were observed after each application of $5x10^{-5}$M serotonin. These results indicate that serotonin, acting at the central nervous system, can promote short-term enhancement of cardiovascular function.

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I would like to thank Dr. Johanna Krontiris-Litowitz, Assistant Professor of Biological Sciences at Youngstown State University, for her expert leadership and invaluable guidance throughout this project. Her mentoring has caused me to reach beyond my perceived capabilities and to become much more than I ever believed I could be.

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Finally, I thank Ed Budde, M.S., from Youngstown State University, for all his help when I initially started this project. His advice and support in the lab has been invaluable.

DEDICATION

I would like to dedicate this research project to the memory of my father, Roland J. Schimmel, who always believed in me. Although he died much too early to ever see me realize my goals, I carry his strengths and ideologies with me and I am assured that he would have enjoyed sharing this experience with me.

I would also like to thank my husband, Charles L. Fulton and my children, Benjamin, Joshua, and Sara, for their love, patience, and support throughout this entire project.

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CENTRAL SEROTONERGIC MODULATION OF HEART RATE IN *APLYSIA CALIFORNICA*

INTRODUCTION

Use of Aplysia as a Model

Aplysia californica is an opisthobranch endemic to the shore environment of the California coast. It inhabits two areas - the intertidal zone which is alternately submerged and uncovered, and the sublittoral zone which is always submerged. It feeds on a diet of seaweed and has a one-year life span which peaks in the summer and ends shortly after spawning in the fall (Kandel, 1976).

Aplysia californica has become a model for biologists to use to study reflex behavior and synaptic plasticity associated with various types of learning (Kandel, 1976, Schwartz et al., 1983). The large, easily identifiable neurons and the ready accessibility of the nervous system has made this a popular animal model for cellular studies. Previous studies established pathways and mechanisms for defensive behaviors such as tail-siphon withdrawal (Cleary et al, 1991, Walters et al, 1983) and gill-siphon withdrawal (Castellucci et al, 1970, Schwartz et al, 1983, Glantzman et al, 1989, Mackey et al, 1989), and identified the neurotransmitter serotonin as the modulator of the resulting sensitization events in *Aplysia* (Hammer et al,1989, Byrne et al, 1990a,b).

Overview of the Nervous System

The nervous system of Aplysia californica consists of four paired ganglia surrounding the esophagus (collectively called the head ganglia or ring ganglia) and one unpaired abdominal ganglion. Each ganglion is composed of around 2,000 monopolar neurons organized into two distinct regions called zones. The cellular zone is comprised of all the cell bodies which are collected around the outside of the ganglion. Their axons give off dendritic trees, and are collected in a central area called the fibrous zone or neuropil. All the connections between neurons within the ganglion as well as connections with neurons from other ganglia occur in the synaptic field of this neuropil - there are no synapses in the cellular region. The rest of the neuropil is made of non-synaptic axon fibers passing through to connectives or commissures (Kandel, 1976).

The head ganglia are comprised of the cerebral, buccal, pleural, and pedal ganglia. The cerebral ganglia innervate the anterior wall, tentacles, mouth, and eyes; the buccal ganglia control the buccal mass, the proximal part of the alimentary canal, and the salivary glands; while most of the body wall, foot, and parapodial movements are controlled by the pedal ganglia. The pleural ganglia is considered to be mostly sensory in function, making monosynaptic reflex connections with motor neurons in the pedal ganglia and heterosynaptic connections through interneurons to the abdominal ganglion.

All the paired head ganglia communicate with their contralateral twin through commissures except for the cerebral ganglia, which are fused. They also connect to and communicate with the other ganglia on their same side through connectives. The link between the abdominal ganglia and the head is provided through the pleuroabdominal connective.

The abdominal ganglion is actually a composite of five smaller ganglia fused together and serves to control the functions of visceral organs in the mantle including circulation, respiration, excretion, and reproduction (Figure 1).

LOCATION OF THE GANGLIA IN

APLYSIA CALIFORNICA

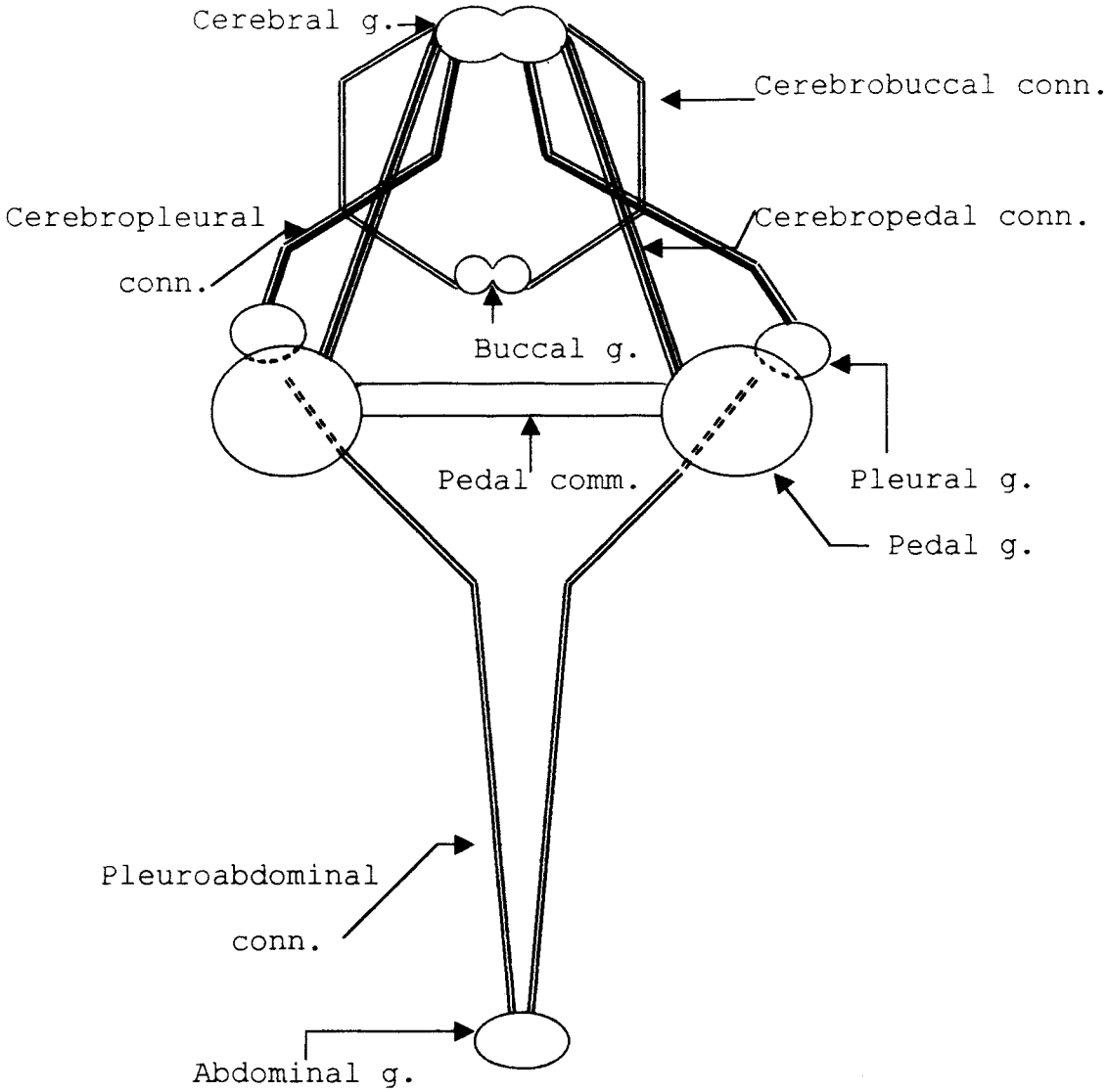


Figure 1

The Circulatory System and Related Neural Control

The circulatory system of Aplysia is considered to be a half-open one with a system of arteries distributing oxygenated blood (hemolymph) to the tissues after which it flows into the body cavity (hemocoel). It then returns to the two-chambered heart through one of two pathways - still unoxygenated blood through the renal vein system, and oxygenated blood through the branchial vein from the gill. These fluids mix in the auricle of the heart, pass through an atrioventricular valve into the ventricle, and then through a semilunar valve into one of three arteries for distribution to the body (Figure 2). Heart rate is maintained by a myogenic pacemaker which can be regulated by cardiovascular neurons located in the abdominal ganglion. When applied to cardiac tissue, the neurotransmitter acetylcholine is inhibitory, while serotonin is excitatory (Liebeswar et al, 1973; Liebeswar et al, 1975).

Several cells in the abdominal ganglion have been identified as cardioaccelerators or cardioinhibitors. One of the primary cardioacceleratory neurons, RB_{HE}, is known to be serotonergic and is excited by acetylcholine (Ach). It is considered to be a tonic excitor which increases the heart rate 50% after a 2-3 second stimulation, and that increase is sustained for several minutes. It fires at a slow, steady rate and slowly, smoothly depolarizes the cardiac fibers (Mayeri et

al, 1974). Although the neurotransmitters are not yet identified, LD_{HE} seems to have a phasic action on the heart and is usually silent. L10 is cholinergic and also responds to Ach; it is sensitive to blood pressure, blood flow to the abdominal ganglion, and oxygen and carbon dioxide levels. When blood flow is interrupted or when oxygen supply is suppressed to the ganglion, L10 is excited and its firing pattern changes from a tonic to a bursting mode. It then mediates large excitatory postsynaptic potentials (EPSP's) of its follower neurons, including RB_{HE}, to increase cardiac output (Mayeri et al, 1974; Furgal and Brownell, 1987).

Cardioinhibitory neurons LD_{HI1} and LD_{HI2} of the abdominal ganglion are also located in the LD cluster. Their action is identical and they are cholinergic but are themselves inhibited by Ach. The apparent neural circuit for increasing cardiovascular activity seems to begin with the activation of L10 by interneurons from the head ganglia or by local events in the ganglion. Excitation of this cholinergic neuron produces EPSP's in the heart excitor neurons and IPSP's in the inhibitory neurons, thus causing acceleration of the heart rate (Mayeri et al, 1974). Another inhibitory neuron is Interneuron II, which makes the same synaptic contacts as L10, but has actions exactly opposite those of L10.

Other neurons regulate vessel diameter. Neurons in the LB cluster (LB_{VC1}, LB_{VC2}, and LB_{VC3}) cause vasoconstriction by

releasing Ach at the gastroesophageal artery and the abdominal aorta (Mayeri et al, 1974).

Recently, two more classes of cells that synapse on the heart have been identified. One is the multimodal neuron, L7, initially identified as the primary motor neuron responsible for actions of the gill and siphon. It has now been found to send branches to the auricle of the heart where it can initiate or increase heart beat (Koester and Koch, 1987; Alevizos et al., 1989). Koester suggests that its role is to restart the heart following respiratory pumping and/or to help increase blood flow to muscle tissue following noxious stimuli. Its neurotransmitter is not known, but it is not acetylcholine or serotonin (Alevizos et al, 1989).

A second group of neurons that synapse on the heart are the R7 and R8 neurons, located in the neurosecretory R cluster of the abdominal ganglion. The cardiovascular function of these cells has not been clearly defined, however, neurons in this cluster use glycine as a neurotransmitter, and glycine is known to increase heart rate and frequency (Koester and Koch, 1987).

Finally, while performing feeding/arousal studies, an additional neuron, the cerebral-pedal regulator (CPR) was identified in the cerebral ganglion. This neuron affected the activity of neurons in the pleural and pedal ganglia, and also

caused an increase in heart rate of from 10 to 20% by exciting cardiovascular command neuron L10 and RB_{HE} (Teyke et al, 1990).

OVERVIEW OF THE CIRCULATORY SYSTEM IN APLYSIA

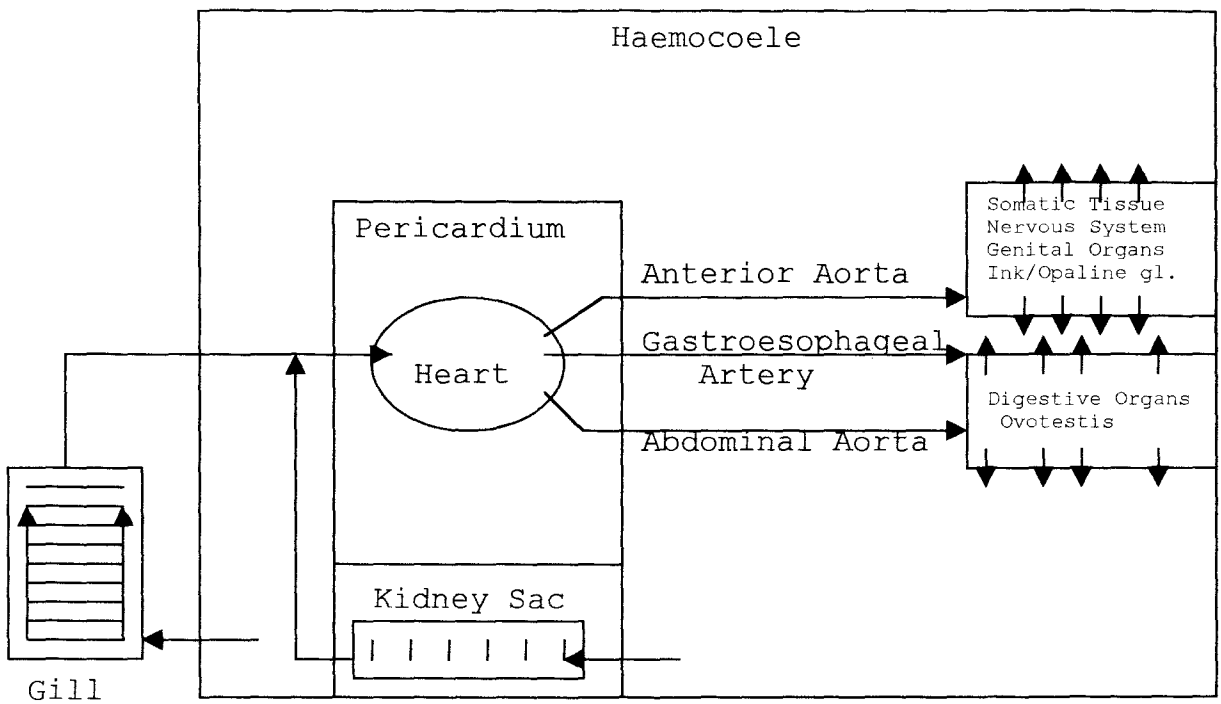


Figure 2

The Role of Serotonin in the Nervous System of Aplysia

Serotonin (5-hydroxytryptamine, 5-HT), is a monoamine derived from the amino acid tryptophan. In the central nervous system of Aplysia, 5-HT acts at sensory-to-motor synapses to promote presynaptic facilitation, and thus serves as a modulatory neurotransmitter (Kandel and Schwartz, 1982). Serotonergic neurons have been located in all the ganglia of Aplysia with the exception of the pleural ganglia (Hernardi et al,1992). However, many axons in the pleural neuropil originate from other ganglia, and their branches send many varicosities around the pleural cell bodies. These contacts occur directly with the plasma membrane - no glial cells intervene - and the synapses are either at the cell body or on the axon hillock. All of these findings suggest that there is some release of 5-HT onto these cell bodies (Byrne, et al., 1990a).

Serotonin applied to the gill sensory neuron-motor neuron synapses results in an increased amplitude of EPSP's and enhanced duration of the action potential, modulated through activation of second messenger systems (reviewed by Kandel, 1976). Analyses of the cellular mechanisms involved in synaptic facilitation have focused on the effect serotonin has on K+ conductance (Schwartz et al 1983, Baxter and Byrne, 1989, 1990). Two channels are affected by serotonin through cAMP: a 5-HT-sensitive K+ channel (K_S) and a calcium-activated

K+ channel (K_{Ca})(Baxter and Byrne, 1989). A third voltage-dependent K+ channel (K_V) is affected by serotonin through activation of protein kinase C (PKC)(Baxter and Byrne, 1990; Sacktor and Swartz, 1990).

The S channel is voltage independent, noninactivating, active over a wide range of action potentials, and increases K+ efflux with time during depolarization. It therefore allows for the slow, continuous efflux of K+ through the cell membrane. Serotonin, through cAMP-dependent phosphorylation of protein kinase A (PKA), suppresses this channel, slowing the efflux of K+ from the cell (Klein et al, 1982; Pollock et al, 1985). Elevated cAMP and PKA levels are also associated with the closure of K_{Ca} channels - the combined effect being heightened excitability and a slower, longer depolarization of the sensory neuron (Baxter and Byrne, 1990).

The K_V channel opens as membrane potential rises and allows K+ to flood out of a depolarized cell to rapidly repolarize it. Byrne et al, 1989, proposed that serotonin modulates this channel through PKC. Closing this channel slows the outward K+ current, prolonging repolarization and increasing the duration of the action potential (Baxter and Byrne, 1990). Sacktor and Schwartz examined the involvement of PKC as the other second messenger activated by 5-HT and found that in the presence of serotonin PKC was translocated to the

cell membrane of sensory neurons following serotonin release (1990).

It appears, then, that serotonin modulates sensory neurons using two different second messenger systems. Sacktor and Swartz suggest that there are possibly two receptors for 5-HT - one that activates adenylate cyclase and thus cAMP-dependent phosphorylation of PKA, and the other which activates PKC. These two protein kinases, although acting independently through seperate mechanisms, are responsible for both heightened excitability and increased action potential duration following serotonergic stimulation (1990).

This response occurs not only in the sensory neuron soma but in the synaptic terminals between sensory neurons and motor neurons. Data from a sensory neuron (SN) body in the pleural ganglion, a motor neuron (MN) body in the pedal ganglion, and a SN axon between them in the pleural-pedal connective revealed that 5-HT broadened the action potential in both of the somata as well as in the axon. Identical results following the severing of the pleural-pedal connective produced the same response, indicating that 5-HT can act on axons independent of the soma. This may be possible due to the presence of 5-HT receptors on the axons or possibly there are serotonin-sensitive channels in the synaptic terminals (Hammer et al, 1989).

Learning and Sensitization

Learning has been defined as, "the modification of behavior by experience" (Hawkins et al, 1993). As such, two major types of learning have been described, associative and non-associative. Associative learning is by far the more complex, requiring the subject to form relationships between stimuli, or between a stimulus and a response. Once thought to require complex circuitry, studies of Aplysia in the 1980's showed associative learning capabilities within this relatively simple model (Carew et al, 1983;, Hawkins et al, 1986; Hawkins et al, 1989; Walters, 1989).

Non-associative learning is defined as learning that occurs in response to a single stimulus. One example of non-associative learning is habituation, where a subject learns through repetition to recognize and ignore harmless stimuli. This is accomplished through a decrease in neurotransmitter release which causes homosynaptic depression of excitatory synapses (Castellucci et al, 1970). Another example that has been well-documented in Aplysia studies is sensitization, in which reflex responses are strengthened by strong, noxious, or threatening stimuli. In these cases, noxious stimuli excite interneurons to increase neurotransmitter release from sensory neurons - a heterosynaptic process called presynaptic facilitation (Hawkins et al, 1981; Hawkins and Schacher, 1989; Mackey et al, 1989). The identified neurotransmitter in this

process is serotonin, and the behaviors most studied in Aplysia are two withdrawal reflexes, the gill-siphon withdrawal and the tail-siphon withdrawal (Hawkins et al, 1993).

Two types of sensitization have been observed: short-term sensitization lasting for minutes, and long-term sensitization lasting for hours or days. To produce sensitization a train of weak shocks or even a single weak shock producing a weak withdrawal reflex is followed by a strong stimulus. A subsequent weak stimulus produces a much enhanced withdrawal when compared with the response prior to training. In the tail-siphon withdrawal reflex, the neural circuit involved begins with sensory fibers from the tail and/or body wall which transmit the impulse to their cell bodies in the left or right pleural ganglion. For tail withdrawal there is a monosynaptic circuit straight to the appropriate motor neurons in the pedal ganglion which, when excited, conducts a motor response directly to the tail musculature. Concurrently the sensory neuron synapses with interneurons that excite motor neurons in the abdominal ganglion to withdraw the siphon (Abrams et al, 1984; Cleary and Byrne, 1986; Walters et al, 1983).

Long-term sensitization is similar to short-term except that it requires a longer training period. In studies that investigated if the location and cellular mechanisms for

short-term sensitization were the same or similar to that of long-term sensitization, pleural and pedal ganglia were removed after long-term training and the membrane properties and cAMP effects on trained cells were compared with ganglia from short-term sensitization. Results confirmed that the same sensory neurons were involved, indicating that the same cell body can have both short-term and long-term memory (Scholz and Byrne, 1987).

Later studies by Byrne et al showed that 5-HT and cAMP can produce both effects (1990b). It was found that in both short-term and long-term sensitization, serotonin modulates the withdrawal response by activating both adenylate cyclase (and thus cAMP) and protein kinase C (PKC). As mentioned earlier, cAMP initiates a phosphorylation cascade which closes K_S channels, leading to depolarization and enhanced excitability. The longer action potential duration from the effect of 5-HT on K_V channels also allows time for increased influx of Ca^{++} which heightens neurotransmitter release through the activity of PKC (Hawkins et al, 1993). Morphological changes observed in cells subjected to long-term training such as increased varicosities and branching suggested long-term changes in neuronal protein synthesis. Exposure to radioactive methionine during training indicated the possibility of synthesis of both induction proteins and maintenance proteins (Noel et al, 1989, 1991). In comparing

studies, protein synthesized after training electrical shocks were found to be similar to protein synthesis following exposure to 5-HT and cAMP at the soma (reviewed by Byrne et al.,1990a).

Intracellular injections of cAMP into sensory pleural somata doubled the number of varicosities, and branch points increased by 50% when compared with cells injected with the inactive metabolite, 5'-AMP (Nazif et al, 1991). The problem with this mechanism is that cAMP is degraded too rapidly to account for such long-term changes. Cleary et al (1991) have reported that there must be two pathways activated by cAMP - a rapid one modulating short-term sensitization, and a slower one leading to alteration of protein synthesis or gene expression. The mechanism suggested for this slower pathway involves a decrease in the regulatory subunit of protein kinase A (PKA), which allows phosphorylation to persist after cAMP is degraded (Greenburg et al, 1987; Bergold et al, 1990; Bergold et al 1992). Recent research confirmed degradation of these regulatory subunits by an ATP-dependent ubiquitin protosome pathway (Hegde et al, 1993). Differences observed following long-term sensitization training might ultimately be due to phosphorylation of DNA regulatory proteins that affect gene expression and thus influence protein synthesis (Montalaro et al, 1986; Bergold et al, 1990; Dash et al, 1990). The expressions of these genetic changes might be

increases in the number of synapses made within ganglia (increased varicosities), increased numbers of synaptic terminals, increased numbers of postsynaptic receptors, or permanent changes in the K_S channel (Bailey and Chen, 1988a,b).

Development of the Hypothesis

The studies cited have furthered our understanding of sensitization and synaptic plasticity but have not included visceral responses to sensitization stimuli. In 1994, Litowitz used *in vitro* preparations to determine if sensitization training would produce a long-term increase in heart rate and whether or not that increase was neurally induced. Applications of noxious stimuli to the body wall were found to cause an immediate increase in heart rate which persisted for 90 minutes in most animals, and in some animals continued for 6 hours. Also, by severing nerve connections to the head ganglia and the abdominal ganglion, it was demonstrated that the CNS played an important role in cardiovascular response to sensitization.

Early experiments had implicated serotonin as the modulatory neurotransmitter involved in sensitization, and had shown that applications of serotonin to the head ganglia was as effective as sensitization training in eliciting withdrawal reflex behavior (reviewed by Byrne, et al, 1989). Therefore, this study was developed to determine if applications of serotonin to the head ganglia could produce the same effects on heart rate as sensitization training by noxious stimuli. The hypothesis proposed in this project is that serotonin, when applied to the head ganglia, produces the same

cardiovascular response as that produced during sensitization training.

MATERIALS AND METHODS

Aplysia californica used for this study were obtained from Alacrity Marine Biological Services, Redondo Beach, California. They weighed 300 to 600 grams, and were maintained in a tank of artificial seawater (ASW) at 15°C. Day/night lighting was maintained artificially by a timer set on a 12 hour cycle. The animals were fed a diet of leaf lettuce. Immediately after removal from the tank, subjects were anesthetized by injecting them with isotonic $MgCl_2$ equal to ½ of the animal's body weight. During surgery, the anesthetized state was maintained by bathing the prep in ½ ASW and ½ isotonic $MgCl_2$. During this time there was no heart activity. Experiments were conducted (20°-22°C) in pure ASW. Application protocol was rotated so that animals in each shipment were used in each phase of the experiment.

Procedure

Animals were opened using a ventral incision to expose the aorta and the peritoneal cavity. The digestive system was retracted to expose the heart in the pericardial cavity. Slits were placed through the connective sheaths around the pleural and pedal ganglia to assure the exposure of serotonin to synapses in the neuropil. These slits were placed uniformly at a depth and length to assure adequate access of test substance to the ganglia (Figure 3). A canula placed in the gill delivered a constant flow of aerated ASW to the heart, maintaining intrapericardial pressure and promoting continuous heart activity. A second canula was inserted in the anterior aorta and monitored pressure and pulse rate during the experiment.

Of major concern in the development of the preparation was the delivery of serotonin to the head ganglia without allowing leakage. This posed a significant problem because the entire procedure was conducted in a bath of ASW and the neurotransmitter was applied in diluted form. Previous work had used a raised well which housed the ganglion/ganglia in question above the level of ASW around the animal, thereby isolating it from other fluids in the preparation. The disadvantages of this system were the damage caused by disturbing the ganglia as they were dissected free from the animal and the stretching of nerves and pulling on the ganglia

that came from placement in the well. To maintain as non-invasive a preparation as possible, a waterproof collar big enough to include all the head ganglia was placed around the ganglia. Grooves were cut on the underside to allow nerves to exit from the ganglia without being compressed by the collar. The isolation collar used in this set of experiments was made from two plumbing gaskets (1¼" diameter) fastened together with waterproof glue to be ½" high. A thick layer of petroleum jelly was then applied to the grooves and the entire bottom of the collar to provide a watertight seal. The collar was placed carefully over the ganglia, the nerves were placed in the grooves, and the collar was pinned through the animal into the wax base of the dissecting tray. Patency of the isolation collar was checked by filling it with ASW and checking for leakage. After the surgery was completed the $MgCl_2$ bath was aspirated and replaced with pure ASW. The preparation was then perfused with aerated ASW to remove any residual $MgCl_2$, and normal heart rhythms were restored either spontaneously or following coaxing using soft compressions or increased ASW perfusion volumes to stimulate myocardial stretch receptors (Figure 4).

POSITION OF PLEURAL/PEDAL SLITS

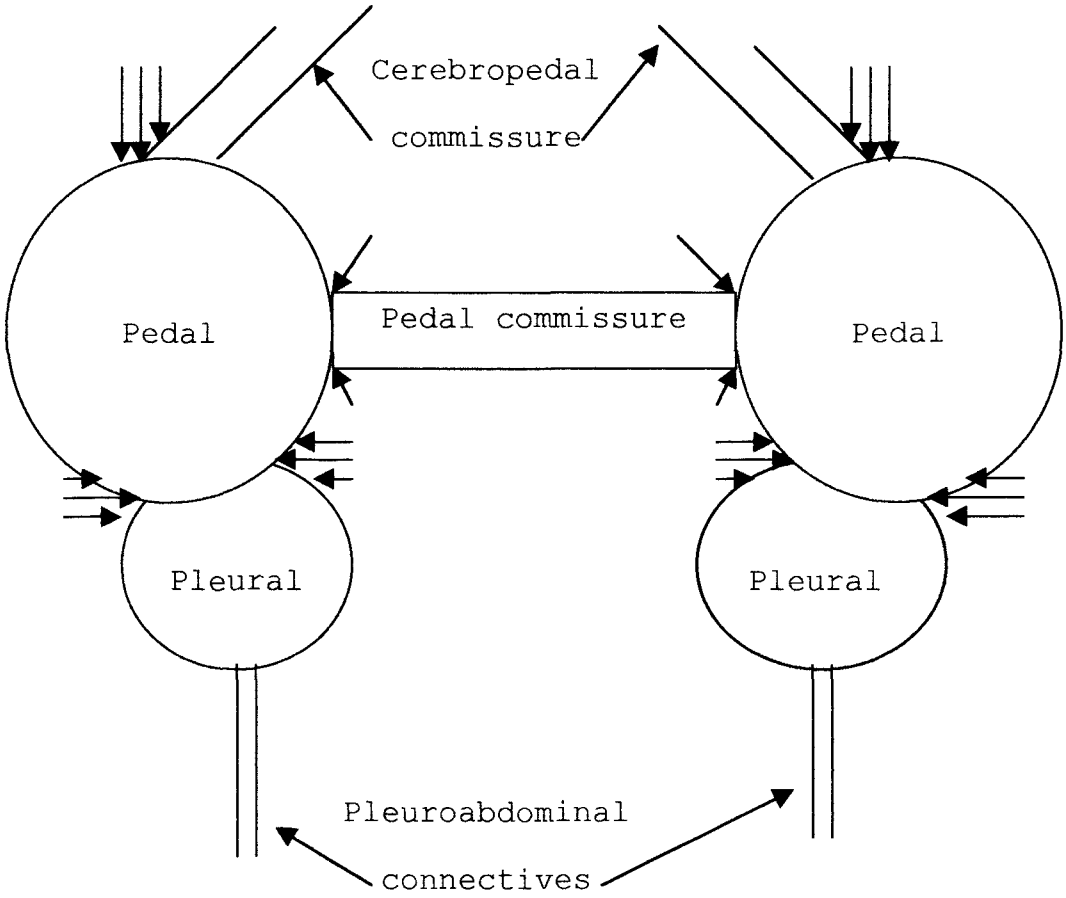


Figure 3

PREPARATION FOR MEASURING HEART RATE BY APPLYING SEROTONIN TO THE HEAD GANGLIA

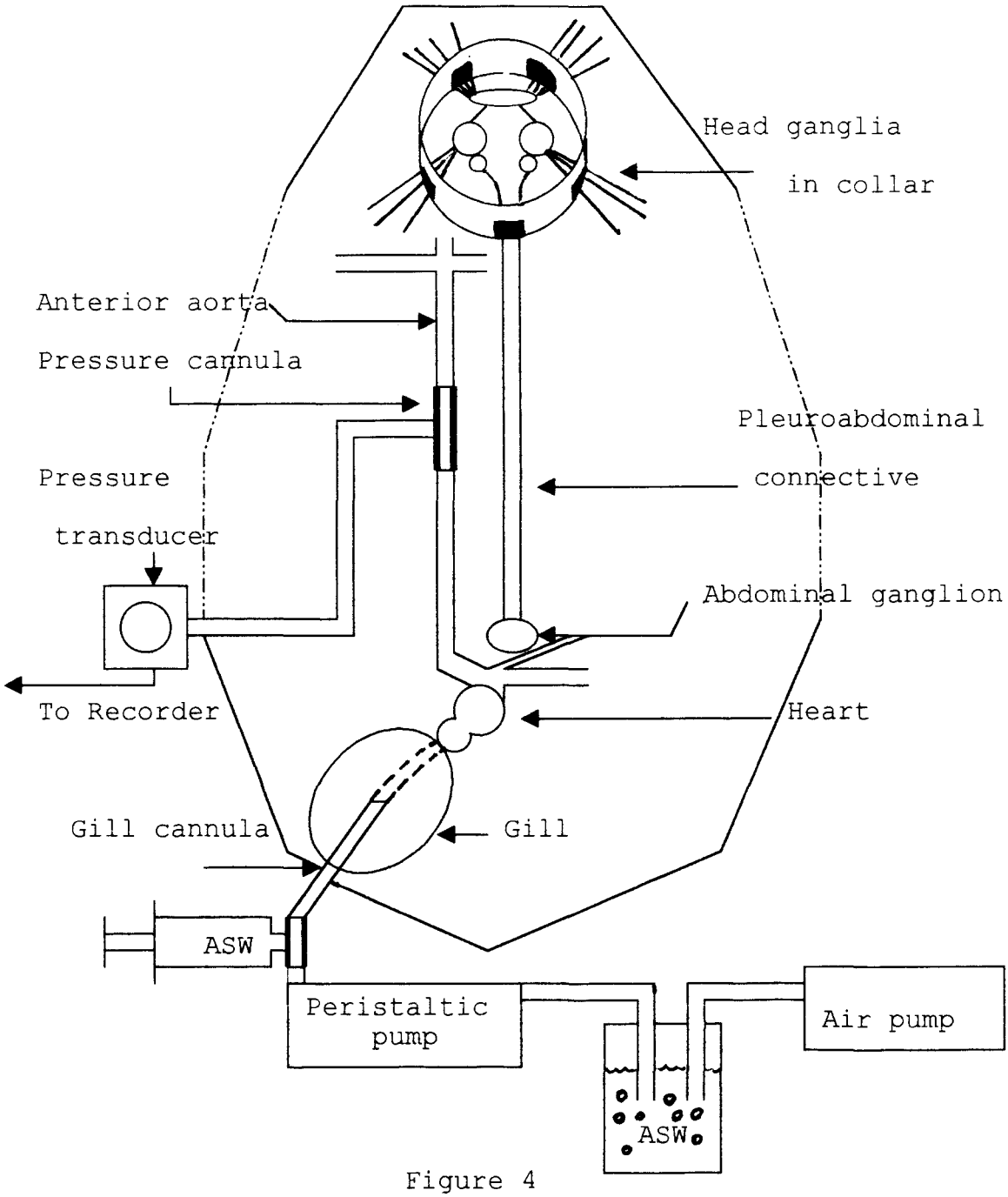


Figure 4

Protocol

When stable heart rhythm was established and had continued uninterrupted for at least 15 minutes, a baseline heart rate was calculated by averaging the number of beats in two-minute intervals at 15 minutes, 10 minutes, and 5 minutes prior to the application of the first dose of serotonin. At time 0, three ml of serotonin was applied to the ganglia and allowed to remain for 3 minutes, after which it was aspirated out, rinsed with ASW and refilled with ASW. Two minutes were allowed to go by to minimize readings affected by the physical disruption of the preparation followed by a two-minute count that actually represented the affected heart rate. The serotonin application was repeated after the tenth minute following the above protocol until serotonin had been applied three times. After the heart rate following the third application had been recorded, the prep was allowed to continue undisturbed for an additional 90 minutes to parallel the sensitization experiment done previously. Two-minute readings were taken at 10-minute intervals through and including that 90-minute time period and entered as raw data. In control experiments ASW was substituted for serotonin throughout the protocol. Data is reported as a percentage of baseline heart rate which was designated in each animal to be 100%. For each group of animals, mean, standard deviation, and standard error of the mean were calculated at each interval.

Statistical significance within groups was determined using a 1-way ANOVA for repeated measures, followed by a Dunnett's test for multiple comparisons. Between-group comparisons were made using a 1-way ANOVA.

RESULTS

In the first set of experiments, the head ganglia were exposed to three 3-minute applications of $5x10^{-6}$ M serotonin in the collar. Heart rate remained robust throughout but was not significantly changed. Following the first application the mean heart rate was 101% (SD=9.6, N=8). Following the second application ten minutes later the heart rate was 99%, SD=13.7; and after the third application heart rate was 92%, SD=92% (Figure 5).

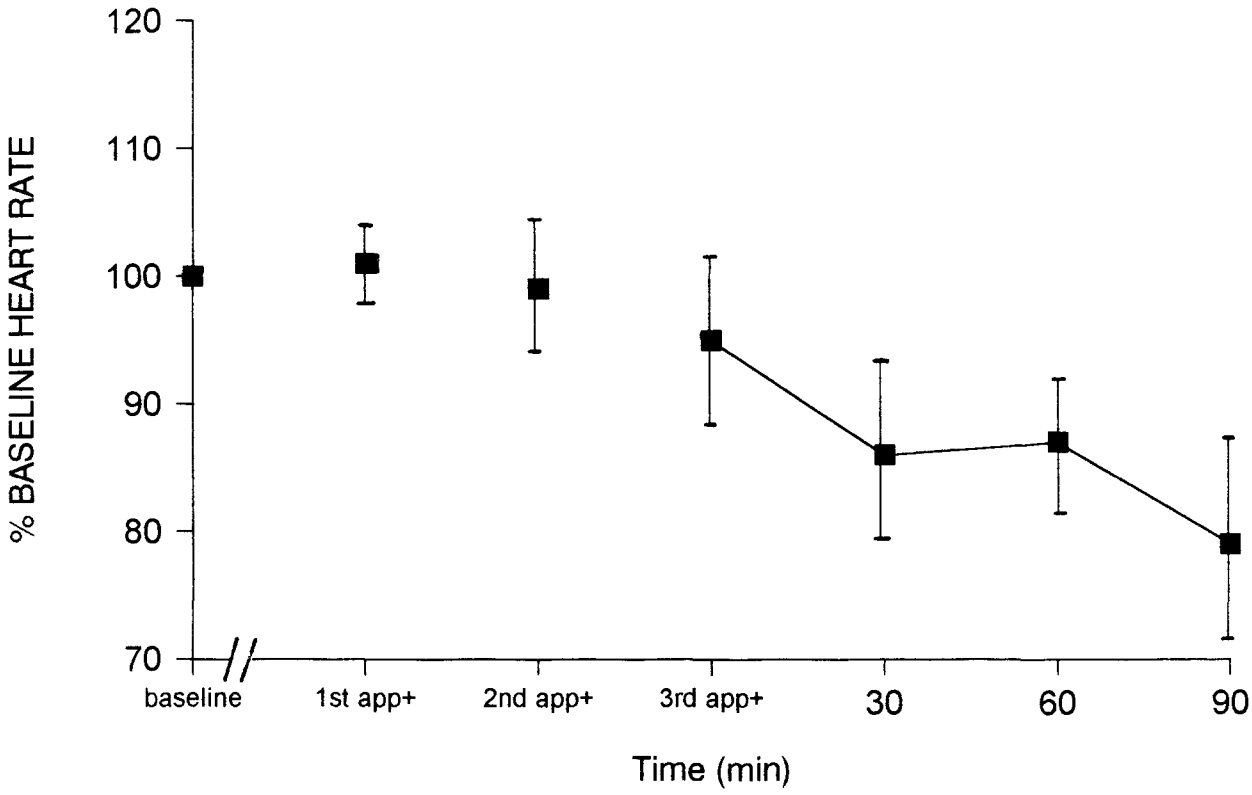
In the second set of experiments, the head ganglia were exposed to three applications of $5x10^{-5}$ M serotonin in the collar. Heart rate after these applications were increased significantly. Following the first application heart rate increased to 110% (SD=11.2, N=10). After the second application heart rate was 113% (SD=16.8), and after the third it was 110% (SD=14.2). At 10 minutes after the third reading heart rate was not significantly different from baseline (101%, SD=13.3), but continued to be strong and robust throughout the experiment. At 60 minutes heart rate declined to 89% (SD=13.7) but by 90 minutes it had recovered to 92% (SD=18.5)(Figure 6).

Control animals were exposed to three 3-minute applications of ASW in place of serotonin. No significant

increase in heart rate was observed for the duration of the experiment (Figure 7). A comparison of controls and serotonin applications revealed a significant difference in heart rate after the first and second exposure to $5x10^{-5}M$ serotonin. Comparison of the third exposure to $5x10^{-5}M$ serotonin produced a p value of 0.054. (Figure 8).

Figure 5. Serotonin ($5x10^{-6}$ M) applied to the pedal-pleural ganglia. ⧳ = mean ± SE (N=8)

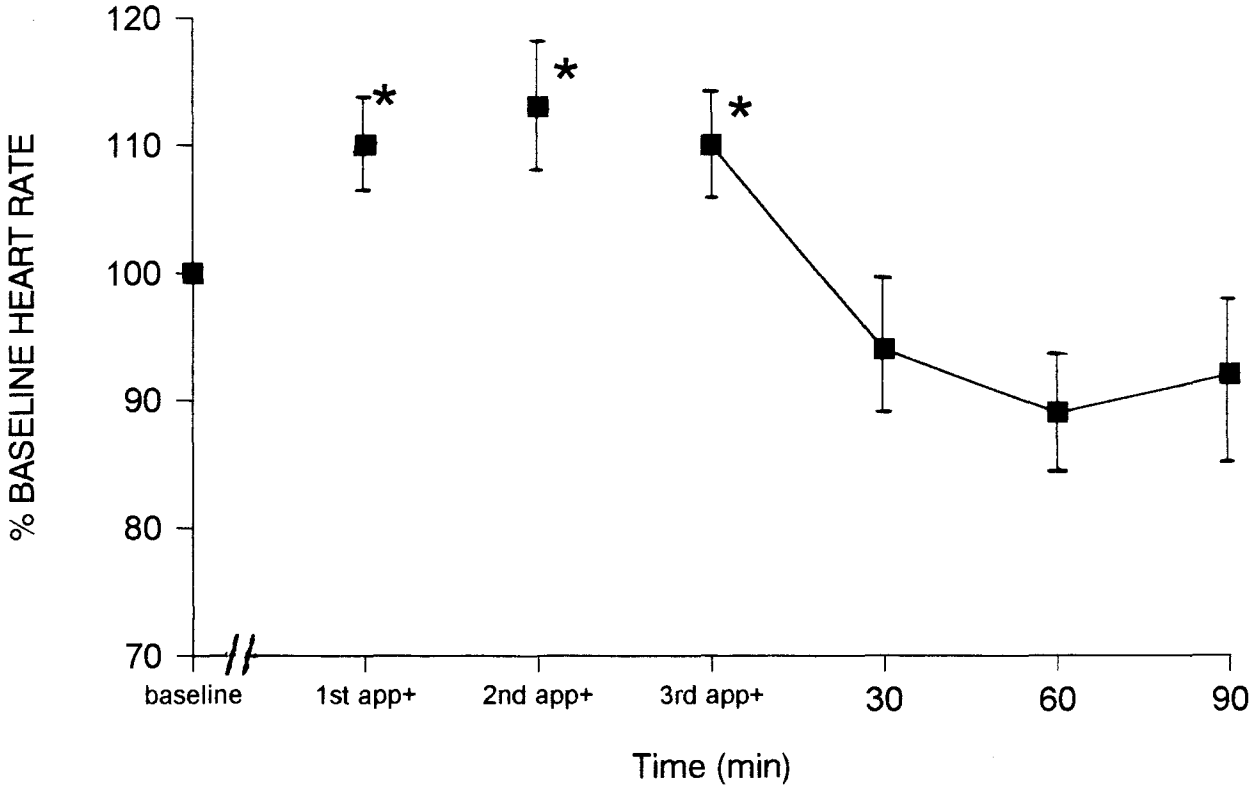
SEROTONIN 5x10-6M



(+ applications @ 10-min intervals)

Figure 6. Serotonin ($5x10^{-5}$ M) applied to the pedal-pleural ganglia. $\overline{\blacksquare}$ = mean ± SE (N=10)

SEROTONIN 5x10-5M



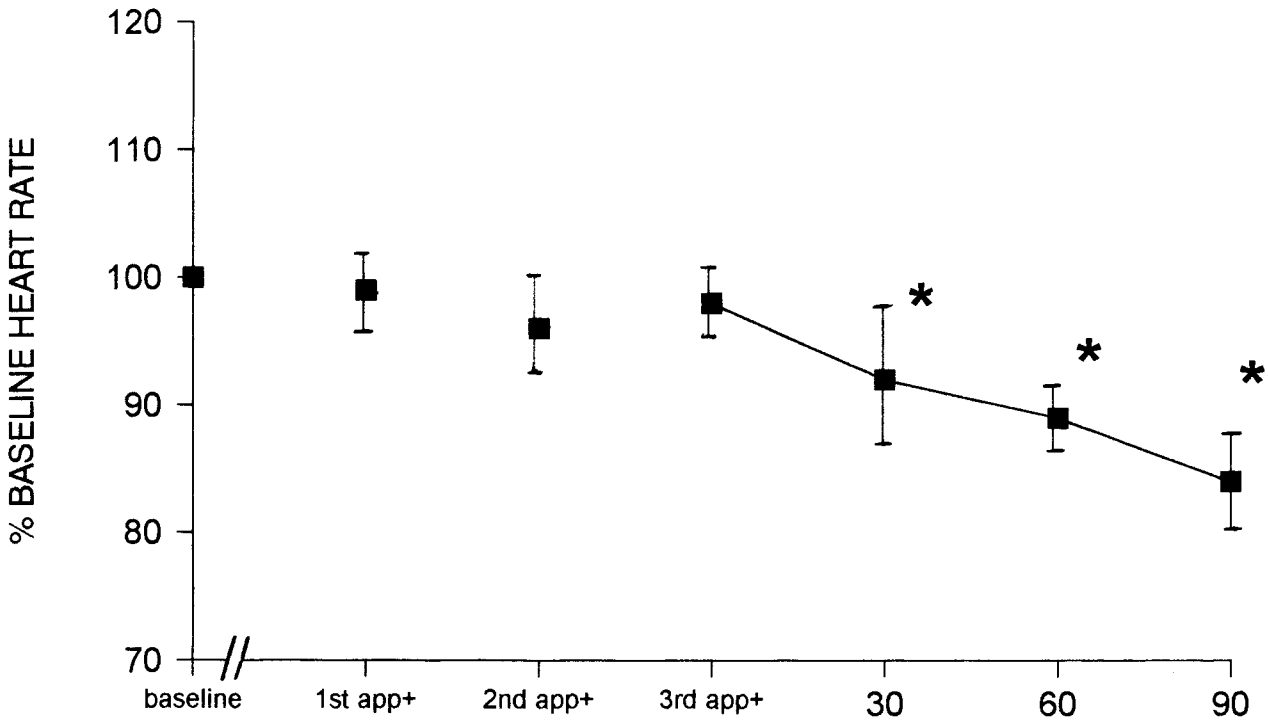
(+ applications @ 10-min intervals)

* $p<0.05$

Figure 7. ASW applied to the pedal-pleural ganglia.

 = mean ± SE (N=10)

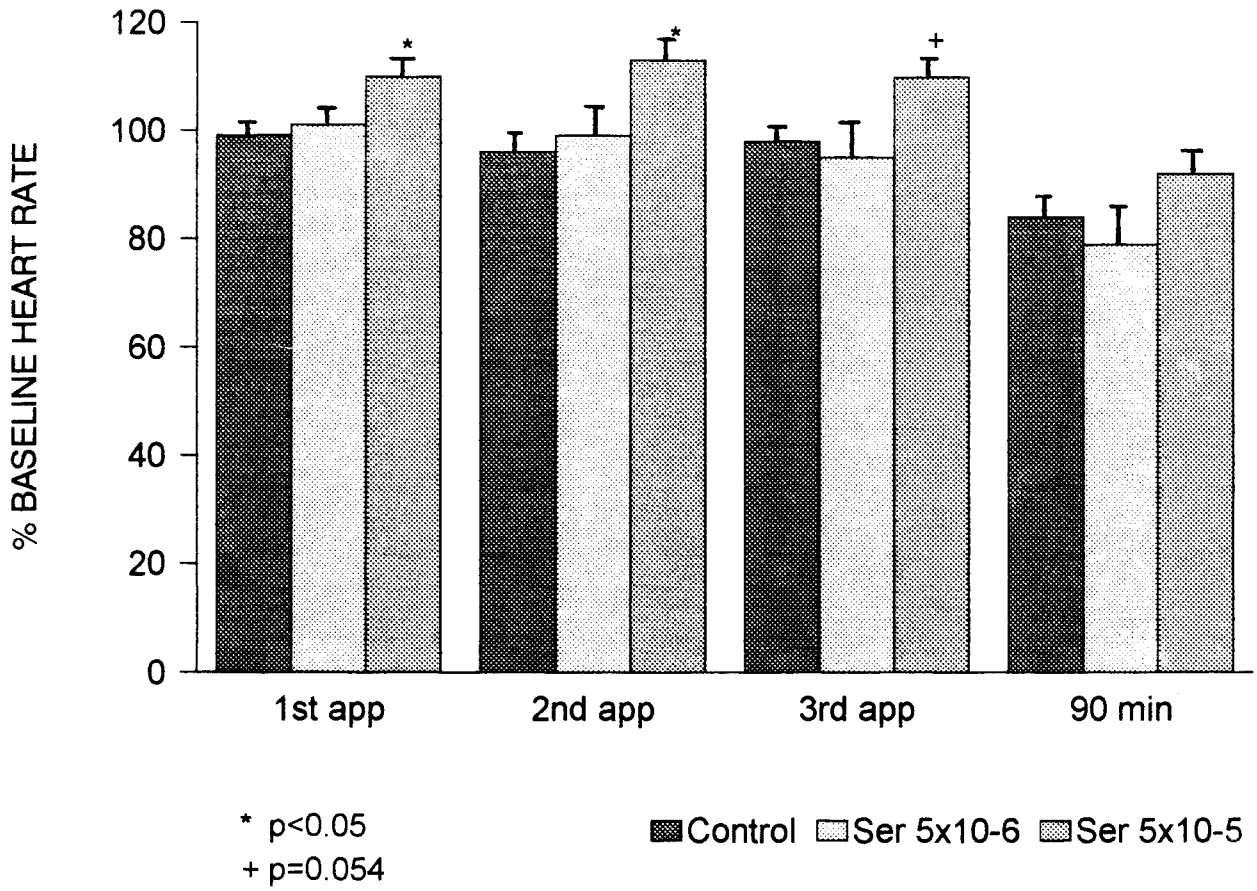
CONTROL



(+ applications @ 10-min intervals)

*p<0.05

Figure 8. A comparison of the % change in heart rate following 3 applications of serotonin to the pedal-pleural ganglia. Serotonin ($5x10^{-5}$ M, $5x10^{-6}$ M) was applied to the ganglia at three 10-minute intervals (1^{st} appl, 2^{nd} appl, 3^{rd} appl); also shown at 90 minutes post-serotonin.


% BASELINE HEART RATE
120
100
80
60
40
20
0
*
*
+
1st app
2nd app
3rd app
90 min
* p<0.05
+ p=0.054
Control
Ser 5x10-6
Ser 5x10-5

DISCUSSION

The purpose of this study was to determine whether serotonin, when applied to the head ganglia, could have the same effect on heart rate as sensitization training. Previous studies in intact animals have shown that sensitizing stimuli also produced an increase in heart rate which persists for at least 90 minutes and continued in some for up to 6 hours (Litowitz, 1994). In this study, serotonin was applied to the head ganglia of intact animals following the same sequence and time intervals as sensitizing stimulation. At higher concentrations of serotonin ($5x10^{-5}M$) heart rate increased significantly. This group also showed a mean rise in heart rate from the 60-minute reading of 89% to the 90-minute reading of 92%. At lower concentrations of serotonin ($5x10^{-6}M$) heart rate was not significantly different from controls. However, although all groups showed progressive decrease in heart rate following the effect of the final application, the heartbeat in serotonin-treated preparations was always more robust and the heart rate higher than in untreated controls.

Several observations indicate that the tachycardia observed in this study was solely in response to treatment with serotonin. First, the heart responded in a dose-dependent manner to serotonin applications. While a $5x10^{-6}M$ concentration of serotonin appeared to improve cardiac response over the response of the control group, $5x10^{-5}M$ serotonin induced

significant tachycardia following each application to the head ganglia. Although it is known that serotonin excites the heart, this is the first evidence that it can do so via the head ganglia.

Second, in both groups of serotonin-treated animals the heart continued to respond to stimulation of the head ganglia during the intervals between applications. Following each three-minute serotonin application, when the collar was filled with only ASW, heart rate was either maintained or fell only slightly. In no case did it return to baseline before the next application, indicating a continuous response for at least ten minutes following exposure of the head ganglia to serotonin. This was not the case in the control animals, whose heart rate showed steady decline throughout the procedure. Litowitz (1994) produced immediate and persistent tachycardia with noxious stimuli to the body wall (as in sensitization training). This study has produced immediate and persistent tachycardia with applications of serotonin to the head ganglia. In this study, the maintenance of these short-term increases in heart rate have occured independent of the presence of serotonin and so might reflect the same neural response to serotonin as seen during short-term sensitization. In both studies, the firing pattern of the neurons involved in accelerating the heart rate must have been altered - in this experiment that alteration was due to three-minute exposures

of the head ganglia to serotonin. In keeping with previous studies of serotonin as a modulator of sensitization events, these short-term responses may be attributed to serotonin's known effect on sensory cell membranes - that it activates two second-messenger systems through two different receptors. One of these receptors activates adenylate cyclase causing cAMP-dependent phosphorylation of PKA and ultimately heightened excitability along with a longer depolarization; the other activates PKC, which is responsible for increasing the duration of the action potential.

A final observation was that heart rate in the $5x10^{-5}M$ serotonin-treated group remained above baseline with no further stimulation until the 30-minute reading, when it fell to 94% of baseline. From that point the rate slowly fell to a low of 89% at 60 minutes - exactly the same as the heart rate of the control group at 60 minutes. This similarity suggests a return to normal of the firing pattern of the neurons involved in this response. Continual decline was expected in all groups and was observed in all but the $5x10^{-5}M$ serotonin-treated group, whose heart rate began to spontaneously return to baseline. This correlates interestingly with the data from previous work which also reported an increase in heart rate from 60 minutes following stimulation to 90 minutes following stimulation (Litowitz, 1994). Since this increase only occurred in $5x10^{-5}M$ serotonin-treated animals, it can be

assumed that it is attributable to this concentration of serotonin, and that there is some lasting effect associated with the neural pathway stimulated in this manner - possibly prolonged increases in cAMP leading to permanent intracellular changes. This observation, although not statistically significant in this study, deserves more research. It can be stated, however, that the hypothesis proposed in this study is true - that serotonin, acting at the head ganglia, can produce a short-term increase in cardiovascular function.

The question still arises as to the site of action of serotonin in this protocol since it is being applied to the head ganglia and the cardioaccelerator neurons are located in the abdominal ganglion. The cardioaccelerator RB_{HE} is a known serotonergic neuron located in the abdominal ganglion. It's major synergists in speeding the heart rate, LD_{HE} and L7, either release acetylcholine or an unidentified neurotransmitter which is not serotonin. However, all these neurons are excited only by acetylcholine. This raises an interesting point. Serotonin is being released by RB_{HE} in the abdominal ganglion to increase heart rate during this experiment but RB_{HE} itself responds to Ach. If the head ganglia is treated only with serotonin, then there must be a cholinergic interneuron modulating cardioacceleration in the head ganglia. The fibers of this interneuron would pass

through the pleuroabdominal connectives and excite the cardioaccelerator neurons.

Modulation of those interneurons from the head ganglia is under investigation - this study postulates that one key neurotransmitter is serotonin. Serotonergic neurons have been found throughout the head ganglia with the exception of the pleural ganglia. Slits to allow for serotonin access were intentionally placed around the pleural-pedal region due to its established role in producing withdrawal reflexes in response to sensitization studies. The assumption has been that serotonin is acting at the sensory-to-motor synapse between these ganglia to modulate sensitization, yet the source of that serotonin cannot be the pleural ganglion somata. One explanation is that serotonin is merely acting at these synapses and is being released from another ganglion. One must remember that the pleural cell bodies are surrounded by varicosities of the branches of axons in the neuropil from serotonergic neurons in the other head ganglia. The cerebral ganglia have already been implicated as a reliable source of serotonin in reflexive activities, therefore it is quite possible that these might be the source of serotonin release throughout the head ganglia. A good candidate for involvement in this pathway would be the CPR neuron, already known to modulate heart activity through stimulation of L10 and RB_{HE} (Teyke et al, 1990). One must also consider that there may be

serotonin receptors on axons in this region, including axons from the pleural ganglia, or serotonin-sensitive K^+ channels in the pleural synaptic terminals. This could explain an EPSP of increased amplitude in the sensory - to - motor synapses between pleural and pedal ganglia in the absence of serotonergic pleural neurons and the response to serotonin applications in this experiment. Of course, the neurotransmitter applied to the head ganglia also could have diffused through any of the neural sheaths within the head ganglia to effect the recorded responses. In either case, it is clear that serotonin is an important neurotransmitter within the head ganglia and is responsible for stimulating interneurons around the pleural-pedal ganglia whose fibers are in communication with cardioaccelerators in the abdominal ganglion. The cardioaccelerator neurons then, become the final targets of sensitization in this pathway.

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