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RELATIVE INVOLVEMENT OF DIFFERENT CNIDOCYTE
SUPPORTING CELL COMPLEXES AND EXTRACELLULAR
CALCIUM IN PREY CAPTURE BY SEA ANEMONE,
Haliplanella Luciae

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biology

by
Virginia Nenna Mc Auley

June 2001


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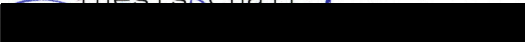
June 2001

Approved by:



Jeffrey Thompson, Dept. of Biology, CSUSB,
Thesis Chair


6/8/01
Date



David Hessinger, Dept. of Physiology, LLU,
Research Director



Nicole Bournias, Dept. of Biology, CSUSB



Ruth Wilson, Dept. of Biology, CSUSB

ABSTRACT

Nematocysts are the "stinging capsules" used by all cnidarians to kill prey. Nematocysts are contained in three functional types (Types A, B, and C) of cnidocytes supporting cell complexes (CSCCs). Nematocyst discharge from CSCCs is controlled by external stimuli, chemical and mechanical, which in turn initiate intracellular signaling pathways. Extracellular Ca^{2+} is required for nematocyst discharge. This study found that Types A, B, and C CSCCs are involved in prey killing at the ratio of 1:2:4 and that extracellular Ca^{2+} is required for prey killing by all three Types of CSCCs. The relative involvement of each CSCCs Type in the presence of various concentrations of extracellular Ca^{2+} was examined. Type A and C CSCCs are found to be actively involved in prey killing at low extracellular Ca^{2+} levels, whereas Type B CSCCs are not. Prey killing from Type A CSCCs peaked at 4mM Ca^{2+} and gradually declined at high extracellular Ca^{2+} levels. Type B and C CSCCs each peaked at 16mM Ca^{2+} and abruptly declined at high Ca^{2+} levels. Abrupt decline of prey killing observed at 18mM Ca^{2+} is due obviously to extracellular Ca^{2+}

entering as intracellular second messenger rather than electrogenetically.

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CHAPTER ONE

INTRODUCTION

For nearly two centuries cnidarians, including hydra, corals, jellyfish, and sea anemones, have been the object of great interest to naturalists and histologists. More recently, the cnidarians have been of keen interest to experimental biologists because they are the simplest metazoans having a nervous system (Muscatine and Lenhoff, 1974) and many species are easily reared in the laboratory (Hessinger and Hessinger, 1991). Cnidarians are diploblastic animals composed of three layers: the ectoderm, endoderm, and mesoglea. The mesoglea is a basement membrane containing nerve fibers and collagen. In addition to cementing ectoderm to endoderm, it provides a substratum over which cells can move. There are three acknowledged classes of cnidarians (Werner, 1973): the hydrozoa (e.g. hydra), the scyphozoa (e.g. true jellyfish), and the anthozoa (e.g. sea anemones and corals). A fourth class, the cubozoa (e.g. box jellyfish), has recently been proposed (Bridge *et al.* 1995).

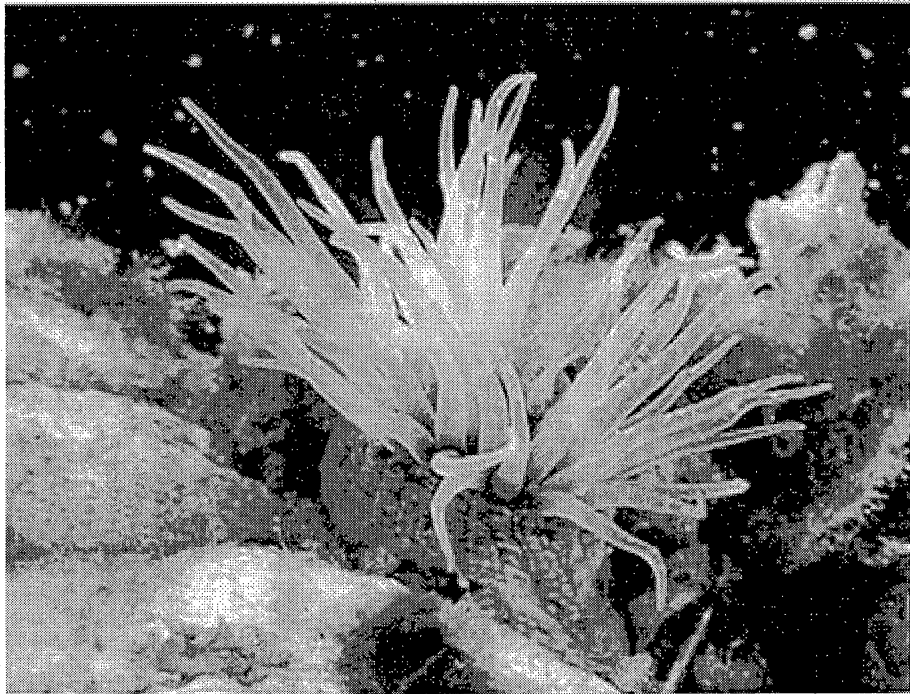


Figure 1. Sea Anemone *Haliplanella luciae* in its Natural Habitat. They are found under the rocks and gravel (Glen Watson, 1999 Computer image).

Like other cnidarians, sea anemones are aquatic predators possessing tentacles. The tentacles surround the oral disk. Anemones have no vision, and, therefore, rely on chemical and mechanical reception by the tentacle epidermis to detect prey.

Within the epidermis of the tentacles are specialized effector cells called cnidocytes (Hessinger and Ford, 1988). The cnidocytes are secretory cells that contain eversible capsules called cnidae. There are three major

types of cnidae: spirocysts, ptychocysts and nematocysts (Thorington and Hessinger, 1996).

Nematocysts are Used to Capture Prey

The nematocysts are double-walled capsules. The nematocyst capsule encloses and, at its apical end, is contiguous with a hollow inverted tubule of varying length and structure that is tightly coiled and folded (Ewer, 1947). The tubule bears arrays of tiny spines. Several distinct types of spines may be present on the tubule of a single nematocyst and spine type, size, and arrangement are used as diagnostic indicators in cnidarian taxonomy (Hand, 1954). The nematocyst capsules also contain a sophisticated and potent venom, which contains sulfur and phosphate compounds (Hessinger, 1988). The nematocyst's primary function is defense and prey capture. Upon receipt of appropriate stimuli, the cnidocyte is acted upon by unknown processes to cause the inverted tubule of the nematocyst to rapidly evert, a process called nematocyst discharge. The everting tubule attains sufficient force to penetrate the outer integument of prey and injects its fast-acting venom into the prey.

In the laboratory, the sea anemone, *Haliplanella luciae*, can be fed with brine shrimp (*Artemia salina*) nauplii. The surface of the shrimp is composed of chitin, a polysaccharide made of N-acetylated sugars. Prey capture is initiated by reception of chemical, vibrational, and contact stimuli from the swimming prey and culminates in nematocyst mediated retention of prey on the tentacles (Thorington and Hessinger, 1998). Through a series of neuromuscular movements, the captured prey is transferred to the oral disk, the mouth opens, and ingestion occurs (Lenhoff and Heagy, 1977).

Cell Complexes Control Nematocyst Discharge

Two types of cell complexes regulate nematocyst discharge from sea anemone tentacles (Thorington and Hessinger, 1998): the relatively rare sensory neuron supporting cell complexes (SNSCs), which consist of one sensory neuron and adjacent supporting cells; and the prevalent cnidocyte supporting cell complexes (CSCCs), which consist of a cnidocyte and two or more adjacent supporting cells (Watson and Hessinger, 1991). Both of these cell complexes have hair bundles that protrude into the surrounding seawater. These hair bundles consist of a

kinocilium and a bundle of surrounding stereocilia. In SNSCs, central sensory cells contribute a single kinocilium and 5-10 large diameter stereocilia and adjacent supporting cells contribute small diameter stereocilia (Westfall, 1965; Mariscal *et al.*, 1978; Bigger, 1982; Mire-Thibodeaux and Watson, 1994). In CSCCs, the cnidocyte contributes a single kinocilium to the center of the bundle, and the adjacent supporting cells each contribute several stereocilia to the periphery. While the kinocilium originates from the cnidocyte, the stereocilia and the chemoreceptors for N-acetylated sugars, such as N-acetylneuraminic acid (NANA), are located on the supporting cells that surround each cnidocyte (Watson and Hessinger, 1987). In sea anemone tentacles, the CSCCs are the primary functional and structural unit for nematocyst discharge (Thorington and Hessinger, 1990).

Cnidocyte Supporting Cell Complexes are of Three Types

There are three Types of CSCCs: Type A, B and C (Thorington and Hessinger, 1988a; 1989; and 1990). All three types of CSCC bear sensory receptors. Type C CSCCs bear only contact receptors and discharge in response to contact stimuli alone. Type B CSCCs bear both contact and

chemical receptors, and Type A CSCCs bear vibration, chemical, and contact receptors. All these different receptors function to detect prey. When stimulated, chemoreceptors and vibration receptors, predispose the contact receptors to trigger discharge in response to prey contact (Watson and Hessinger, 1991).

Type B Cnidocyte Supporting Cell Complexes use Ca^{2+} Channels

Lenhoff and Bovaird (1959) have shown that extracellular Ca^{2+} is required for discharge of nematocysts in hydra. Likewise, sea anemones have been widely used to study nematocyst discharge (Thorington and Hessinger 1988a; 1988b; 1990; 1992; 1996; 1998; Muir *et al.*, 1988; Watson and Hessinger, 1988; 1989; 1994b; Mire-Thibodeaux and Watson, 1993). Watson and Hessinger (1994b) report that extracellular Ca^{2+} is required for nematocyst discharge from Type A, B, and C CSCCs. Based upon single doses of N-acetylneuraminic acid (NANA) Watson and Hessinger, (1994) concluded that L-type calcium channel blockers (e.g. nifedipine, diltiazem) specifically block the nematocyst discharge from Type B CSCCs. More recent studies, however, by Thorington and Hessinger (unpublished) have shown that nifedipine dramatically shifts biphasic NANA dose response

to a much lower NANA concentration. The Ca^{2+} channel activator, S(-)Bay K 8644, totally blocks discharge from Type B CSCCs as does high (11 mM and above) extracellular calcium. Cadmium (Cd^{2+}), an inorganic calcium channel blocker, and low extracellular Ca^{2+} have also been shown to left-shift the NANA dose-response, thereby increasing the sensitivity to NANA like nifedipine (Thorington and Hessinger, 1988).

Type A Cnidocyte Supporting Cell Complexes can be Specifically Inhibited

Many vertebrate vibration-sensitive mechanoreceptors are inhibited by aminoglycoside antibiotics, such as streptomycin. Discharge from vibration-sensitive Type A CSCCs has been shown to be inhibited by exposure to streptomycin or gentomycin, but discharge from Type B CSCCs and Type C CSCCs is not inhibited (Watson and Hessinger, 1994b).

The stereocilium bundles (SBs) of Type A CSCCs in sea anemone tentacles are filled with longitudinal bundles of microfilaments that control the shortening and elongation of the stereocilia. Cytochalasin B (CB) is a fungal metabolite that alters cell shape and inhibits a wide

variety of cellular movements by capping the plus end of actin microfilaments. The effects of CB on the nematocyst discharge have previously been tested. Cytochalasin B caused the SBs of Type A CSCCs to disappear. In the presence of NANA alone, Type A preferentially discharged nematocysts at frequencies of 5, 15, 30, and 40 Hz, but, when treated with CB, no discharge at any vibrational frequency occurred. Thus, CB inhibits discharge from Type A CSCCs, but not from Type B CSCCs (Watson and Hessinger, 1991).

When anemones were treated with proline (10^{-8} M), no significant effect on the shortening or elongation of stereocilia was observed. But, in the presence of both NANA (10^{-7} M) and proline (10^{-8} M), the elongation effect of NANA on the SBs was reversed and a net shortening of the stereocilia resulted. Furthermore, a combined NANA-proline treatment shifted maximal discharge of nematocyst to frequencies that were 60 Hz higher (65, 75, 90, and 100 Hz) than those of NANA alone (5, 15, 30, and 40 Hz) (Watson and Hessinger, 1991).

N-acetyl Neuraminic Acid Signaling Pathway Uses Cyclic Adenosine Monophosphate

Nematocyst discharge from Type A and Type B CSCCs in sea anemone tentacles is controlled by chemoreceptors activated by N-acetylated sugars, such as NANA. NANA stimulates adenylyl cyclase activity in the apical membrane of the supporting cells of sea anemone tentacles (Watson and Hessinger, 1992). Agents that increase intracellular cAMP levels (cholera toxin, forskolin, dibutyl-cAMP); (1) induce elongation of Stereocilia of the Type A CSCCs, (2) tune Type A vibration-sensitive mechanoreceptors to respond to lower frequencies, and (3) sensitize Type B CSCCs to discharge nematocysts (Watson and Hessinger, 1992). Ozacmak *et al.* (2001) reported that NANA specifically and dose-dependently increases the cAMP content of the anemone tentacle ectoderm. NANA stimulates *in situ* cAMP production in tentacles of the sea anemone (*Aiptasia pallida*), suggesting a possible role in chemosensitization of nematocyst discharge

Hypothesis

The previously discussed studies have shown that nematocyst discharge from sea anemone tentacles is under the control of extracellular stimuli that activate both

intracellular and intercellular signaling processes. Although, nematocysts are believed to be the principal means by which prey are captured, it is not known to what extent. These intracellular and intercellular processes affect prey capture and ingestion. The present proposed study is designed to extend our knowledge of signaling processes involved in the control of nematocyst discharge to include that of prey capture and ingestion and to reveal the relative roles of the different types of CSCCs and Ca^{2+} channels in prey capture.

This proposal stems from recent work on signaling pathways involved in nematocyst discharge in which defined chemicals and artificial mechanical probes were used to elicit nematocyst discharge. The focus of my proposed investigation will shift from nematocyst discharge *per se* to the capture and ingestion of prey. In this study, chemical stimuli are provided from the surface of live prey (shrimp) and mechanical contact stimuli are provided by the swimming movements of the prey.

The CSCCs inhibitors are a group of drugs, which block transduction pathways that control nematocyst discharge from respective CSCCs (Watson and Hessinger, 1987;

Thorington and Hessinger, 1990). These drugs are chosen in the present study because the signal pathways that control nematocyst discharge are suspected to govern prey capture and ingestion.

In considering the relative roles of different types of CSCCs, extracellular Ca^{2+} , and Ca^{2+} channels in sea anemone prey capture, the Null hypothesis states that three CSCCs Types extracellular Ca^{2+} , and Ca^{2+} channels are not involved in prey capture and ingestion. But, I reject the Null hypothesis and hypothesize that:

1. All three types of CSCCs are involved in prey capture.
2. Significant differences exist among the roles of three Types (A, B, and C) CSCCs in prey capture.
3. Ca^{2+} channels are involved in prey capture
4. Changes in extracellular Ca^{2+} levels affect prey capture.
5. Inhibitors of CSCCs, which block prey capture, are affected by extracellular Ca^{2+} levels, and extracellular Ca^{2+} affects Type B and C CSCCs by an intracellular effect.

CHAPTER TWO
MATERIALS AND METHODS

Materials

Monoclonal sea anemones, *Haliplanella luciae*, are derived from stock cultures, originally obtained from Dr. Richard Mariscal, Florida State University. Dessicated brine shrimp, *Artemia salina*, cysts were purchased from San Francisco Bay Brand (Hayward, CA). S(-)Bay K 8644 were purchased from Research Biochemicals, Inc. (Natick, MA). Streptomycin, cytochalasin B, and other reagents, unless otherwise specified, are purchased from Sigma Chemical Company (St. Louis, MO).

Cytochalasin B, nifedipine, and S(-)Bay K 8644 are dissolved in dimethylsulphoxide (DMSO), with a final concentration of DMSO on sea anemones not to exceed 1%. Filtered natural sea water (NSW) is obtained from Kerckhoff Marine Laboratory of California Institute of Technology (Corona del Mar, CA).

Artificial sea water (ASW) was prepared with deionized water and different concentrations of the following chemicals concentrations: NaCl (423 mM), KCl (10 mM), CaCl₂

(10 mM), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (24 mM), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (25 mM), and NaHCO_3 (1.2 mM) adjusted to pH 7.63.

Maintenance of Sea Anemones

Sea anemones, *H. luciae*, were placed individually in 35-mm diam. plastic culture dishes containing NSW covered with lids. The animals were fed three times a week (Sunday, Tuesday, and Friday) with freshly hatched brine shrimp nauplii (Hessinger and Hessinger, 1981). They were cleaned daily and maintained at room temperature ($23 \pm 1^\circ\text{C}$). Experiments were performed approximately 72 h after feeding for the purpose of maximizing prey capture (Thorington and Hessinger, 1988b).

Preparation of Brine Shrimp Nauplii

Dessicated brine shrimp cysts (11.0 g) were added to 300 ml of NSW at room temperature with gentle aeration. After 1h, the hydrated cysts were filtered (100 mesh nylon), rinsed with NSW, transferred to a brine solution (34.3 g rock salt in 300 ml NSW), and aerated for 24 h.

After 24 h, the shrimp and cyst suspension was allowed to settle for 5 min to separate the floating cysts from the swimming nauplii. The swimming nauplii were removed with a

basting syringe and transferred onto a nylon filter (100 mesh). The shrimp were gently rinsed on the filter and transferred to a beaker containing aerated ASW. Freshly hatched nauplii were prepared for standardization.

Shrimp Standardization

A 100-ml aliquot of the prepared shrimp suspension was diluted with an equal volume of ASW. Six 1-ml aliquots were transferred onto a 0.45-micrometer Millipore Type HA filter with suction. The nauplii on the filters were counted under a stereomicroscope. The mean of six replicate counts was used to calculate the volume to which the brine shrimp suspension was adjusted in order to be at a standard concentration of 332 shrimp per ml or 83 ± 1 per 250 microliter.

Standard Assays

Maximum Number of Shrimp Anemones are Capable of Capturing and Ingesting

Six groups of five plastic culture dishes each (35mm diameter) were used in each prey assay. Groups (1-6), the experimental dishes, contained 4.75 ml ASW and one adherent anemone per dish. The seventh group, the controls (C-2) also contained ASW, but no anemone. The C-2 was used to

measure the effect of treatments on brine shrimp viability. Prior to the experiment, all anemones were gently rinsed with ASW, and the dishes were filled with fresh ASW. Shrimp numbering (20, 40, 60, 80, 100, and 120) were counted respectively into the experimental groups (1, 2, 3, 4, 5, and 6), respectively. The C-2 for each experimental group contained the same number of shrimp as the experimental. Experimental and controls were incubated at room temperature in a wooden box that admits green light. Under the green light, the normally phototactic nauplii swim randomly and do not congregate. After 30 min, the dishes were removed from the box, and the number of shrimp ingested, killed uningested and total shrimp captured in each experimental group was counted under the stereoscope. The number of killed shrimp in controls was also counted. The result of this assay indicated that on average, an anemone captures and ingests a maximum of 83 ± 1 shrimp. This shrimp number was used in all further investigations to feed anemones.

Standard Starvation Time for Experimental Anemones

Seven groups of culture dishes (35mm diameter) were used with five dishes per group. Groups (1-6), the

experimental dishes contained 4.75 ml ASW and one adherent anemone per dish. The second group, the controls (C-2) also contained ASW, but no anemone. The anemones in experimental groups, (1-6) groups, were starved for various times (0, 12, 24, 48, 72, and 96 hours). At the end of these respective periods, the anemones were washed without feeding. On the test, all the anemones were gently rinsed with ASW and the dishes were filled with 4.75ml of fresh ASW. The 83 ± 1 shrimp were added to all the experimental and control dishes and incubated. In each experimental and control dishes, number of shrimp ingested, killed uningested and total shrimp captured was counted under the stereoscope. The result of this assay showed that maximum prey capture and ingestion occurred following 72 hr starvation. Therefore, all the experimental anemones were starved for 72 hr prior to each subsequent experiment.

Assay for Prey Capture and Ingestion

Four groups of culture dishes were placed on a flat surface. Group one, the experimental dishes, contained test solutions and one anemone per dish. Group two, untreated anemone control (C-1), contained ASW only and one anemone per dish. Group three, test solution control (C-

2), contained test solution without anemones. Group four, ASW control (C-3), contains ASW without sea anemones. The C-3 was used to determine the viability of brine shrimp in ASW. All anemones were gently rinsed with ASW prior to use.

The ASW in experimental and C-2 dishes was removed and replaced with test solutions. The ASW in C-1 dishes containing untreated control anemones was replaced with fresh ASW, and C-3 contained ASW only. All experimental dishes were then pre-incubated for various times, then, 0.25 ml of standardized shrimp suspension (83 ± 1 shrimp) was added to all four dishes. The dishes were then placed for incubation. The number of dead shrimp in C-2 and C-3 controls and the number of uningested dead and live shrimp in experimental and C-1 controls were counted under a stereomicroscope and the results recorded.

Determination of Shrimp Killed by Anemones

To compensate for the effect of treatment on shrimp viability in tests, the number of shrimp killed by treatment alone (C-2) was subtracted from the number of uningested killed shrimp to give the adjusted number of uningested killed. The total number of ingested shrimp was

determined by subtracting the number of live uningested shrimp plus the number of killed uningested shrimp from number of added shrimp (83 ± 1). The total number of shrimp killed in experimentals was determined by adding the adjusted uningested killed shrimp to total ingested shrimp.

To adjust for the effect of ASW on shrimp viability in controls, the number of dead shrimp in ASW alone (C-3) was subtracted from the number of killed uningested shrimp in C-1 dishes to give the adjusted number of uningested killed in controls, killed shrimp and the number of shrimp alive. The total ingested shrimp in controls was determined by subtracting number of killed uningested shrimp plus shrimp alive from shrimp added (83 ± 1). The total shrimp killed in controls was determined by adding adjusted uningested killed shrimp to total ingested shrimp. The percentages of the mean total shrimp killed were determined by dividing the mean total killed in the experimentals by the mean total killed in the controls and multiplying by 100. The percentages of the mean total shrimp ingested were determined by dividing the mean total ingested in the experimentals by the mean total ingested in the controls and multiplying by 100.

Experimental Protocols

Inhibition of Prey Capture From Type A Cnidocyte Supporting Cell Complexes

Proline Treatment. Twenty culture dishes were divided evenly into four groups. The first group contained experimental anemones, one anemone per dish. The second, third and fourth groups were the C-1, C-2, and C-3 controls. The experimental anemones were pre-incubated in 5ml 10^{-7} M NANA in ASW for five min at room temperature. The ASW in experimental dishes was replaced with 4.75ml 10^{-8} M proline and pre-incubated for ten min. The C-1 dishes contained one anemone per dish and 4.75ml ASW, the C-2 dishes contained 4.75ml 10^{-8} M proline solutions in ASW, while the C-3 dishes contained only 4.75ml ASW. After ten min pre-incubation, 0.25ml of standardized shrimp solution is added to all dishes. The dishes were incubated, after which data was collected and recorded.

Streptomycin Treatment. Twenty culture dishes were divided evenly into four groups. The first two groups, experimental and C-1 control, contain one anemone per dish; the third and fourth groups, were the C-2 and C-3 controls, and do not contain anemones. The experimental anemones were pre-incubated with 5ml 10^{-4} M streptomycin in ASW for

ten min. C-2 controls contain 4.75ml 10^{-4} M streptomycin in ASW. The C-1 and C-3 controls contain 4.75ml ASW. After the ten min pre-incubation, 0.25ml of standardized shrimp solution was added to all dishes, and data was obtained after incubation.

Inhibition of Prey Capture From Type B Cnidocyte Supporting Cell Complexes

There were four groups of dishes, five dishes per group. Group one was the experimental dishes and contained a 4.75ml of 10^{-4} M S(-)Bay K 8644 in ASW. Groups two, three, and four were the C-1, C-2, and C-3 controls, respectively. C-1 and C-3 dishes contained 4.75 ml ASW and C-2 dishes contained 4.75 ml 10^{-4} M S(-)Bay K 8644 in ASW. The experimental dishes were pre-incubated for ten min, after which 0.25ml standardized shrimp solution was added to all dishes. Dishes were then incubated and data collected. This protocol was repeated using 10^{-6} M Methoxyverapamil (D-600), another known blocker of Type B CSCCs. In addition, this protocol was repeated using 10^{-6} M A23187, a Ca^{2+} ionophore and a known blocker of Type B CSCCs in *A. pallida* (Thorington and Hessinger, 1998).

Inhibition of Prey Capture From Type A, B and C Cnidocyte Supporting Cell Complexes

There were ten groups of dishes, five dishes per group. The experimental groups 1-4 and C-1 controls contained one anemone per dish, groups 6-9 were the C-2 controls and group ten was the C-3 controls, and did not contain anemones. Anemones in the first of the experimental dishes were treated in 10^{-7} M tetrodotoxin in ASW, which inhibits Type A and B CSCCs, but not Type C (Watson and Hessinger, 1994). The second group was treated with 10^{-4} M streptomycin, which inhibit Type A CSCCs, but not Type B and C CSCCs. The third group was treated with 10^{-6} M amiloride, which inhibits Type B and C CSCCs, but not Type A CSCCs. The fourth group was treated with 40mM extracellular KCl which blocks nematocyst discharge from all three CSCC Types (A, B, and C) (Watson and Hessinger, 1994). Each of the four groups of C-2 dishes contained one of the four different experimental solutions; and C-1 and C-3 controls contained ASW. All of the dishes were pre-incubated for ten min at room temperature, after which 0.25ml of standardized shrimp solution was added to each dish followed by incubation.

Positive Modulation of Type B Cnidocyte Supporting Cell Complexes by Blocking Ca²⁺ Influx

There was one group of experimental dishes and three control groups (C-1, C-2, and C-3), five dishes per group. The experimental and C-1 dishes contained one anemone per dish, but C-2 and C-3 dishes contained no anemones. The experimental group contained 10⁻⁵ M nifedipine in ASW. The C-2 group contained 10⁻⁵ M nifedipine in ASW; C-1 and C-3 controls contained ASW. The experimental dishes were pre-incubated for ten min, after which 0.25ml standardized shrimp solution was added to all samples and incubated for 30 min.

Effect of Different Extracellular Ca²⁺ Levels

There were sixteen groups of experimental dishes and three groups of controls (C-1, C-2, and C-3), five dishes per group. The experimentals and C-1 groups contained one anemone per dish. C-2 and C-3 dishes contained no anemones. Each of the experimental groups 1-16 contained 0, 0.5, 1, 2, 4, 6, 7, 8, 10, 12, 14, 16, 17, 18, or 20 mM CaCl₂ in ASW respectively. All the C-1 and C-3 dishes contained ASW. The experimental and control dishes were pre-incubated for ten min, after which 0.25ml of

standardized shrimp solution was added to all dishes and incubated under green light.

Inhibition of Types A, B, and C Cnidocyte Supporting Cell Complexes in the Presence of Different Extracellular Ca²⁺ Levels

There were fifteen groups of experimental dishes and three sets of controls (C-1, C-2 and C-3). The C-1, C-2 and C-3 dishes were treated as described earlier. The fifteen groups of experimental dishes each contained either 0, 0.5, 1, 2, 4, 6, 7, 8, 10, 12, 14, 16, 17, 18, or 20 mM CaCl₂ respectively. The anemones in first group of experimentals were incubated in 10⁻⁶ M amiloride (inhibitor for Types B and C CSCCs), the second group in 10⁻⁷ M tetrodotoxin (blocker of Types A and B CSCCs). After ten min incubation, the anemones were washed with ASW, and then 4.75 ml of each Ca²⁺ concentration was added to the designated group. Shrimp (83±1 in 0.25 ml) was added to all the experimental and control dishes, and further incubated for 30 min. The total shrimp killed and ingested were counted and recorded.

The Statistical Analysis

Experimental data are expressed as means ± S.E.M. ANOVA and t-tests were performed using SPSS. Differences

among groups were determined by *post hoc* ANOVA tests (Scheffe). Differences between the groups were considered statistically significant at probability values $p < 0.05$, 95% confidence interval for mean.

CHAPTER THREE

RESULTS

Involvement of all Three Types of Cnidocyte Supporting Cell Complexes (A, B, and C) in Prey Killing and Ingestion

High extracellular K^+ inhibits discharge from all Types of CSCCs (Watson and Hessinger, 1994). After ten minutes of pre-incubation in ASW containing 40mM KCl followed by 30 min with 83±1 shrimp, only 4.3% ingestion and 12.2 % killing occurred (Figure 2, Table 1, and Table 2)). Since 40mM extracellular K^+ blocks all nematocyst discharge, the 12% "killing" was attributed to either entanglement in discharging spirocysts or the stickiness of the tentacles mucus layer (Thorington and Hessinger, 1998). The statistical analysis with a T-test showed that the p values for both ingested and killed were less than 0.05 (Table 1).

Table 1. Statistical Analysis of 40mM KCl

	Eq of Variances	Eq of Means		
	p	t	df	p
Ingested	0.42	-149.11	28	<0.001
Killed	0.08	-155.39	28	<0.001

Inhibition of Type A, B, and C CSCCs by 40mM KCl. Levene's test was used for equality of variance. $P > 0.05$ in each case. A T-test was used for equality of means. $P < 0.001$ in each case.

Table 2. Mean and Relative Percentage of Prey Killing and Ingestion by Each CSCC Type

CSCCs Inhibited	Inhibitors	Experimentals				Controls	
		Mean Ingestion	Relative Percent Ingestion	Mean Total Killed	Relative Percent of Total Killed	Mean Ingestion	Mean Total Killed
All Three	KCl	2.9±0.4	4.3	8.7±0.2	12.2	67.4±0.2	71.9±0.3
A	Streptomycin	52.7±0.3	81.1	62.9±0.3	87.1	64.9±0.4	72.3±0.3
A	Proline	57.4±0.3	87.6	65.5±0.4	91.1	65.5±0.6	71.9±0.5
B and C	Amiloride	20.1±0.3	29.9	25.1±0.2	34.8	67.3±0.2	71.9±0.4
B	Verapamil	40.7±0.3	59.2	47.9±0.3	65.5	68.7±0.4	73.1±0.3
B	A23187	48.9±0.3	71.5	54.5±0.2	74.6	68.3±0.2	73.1±0.2
B	Bay K	26.1±0.4	38.3	56.9±0.4	77.6	68.3±0.2	73.3±0.2
A and B	TTX	26.9±0.5	39.8	32.1±0.8	44.4	67.6±0.2	72.2±0.4

Mean values are expressed as plus or minus the Standard Error of the Mean (SEM). The relative percent of the prey ingested and captured were calculated by dividing the mean ingested and captured in the experimental by the mean ingested and captured in the control, and multiplying by 100.

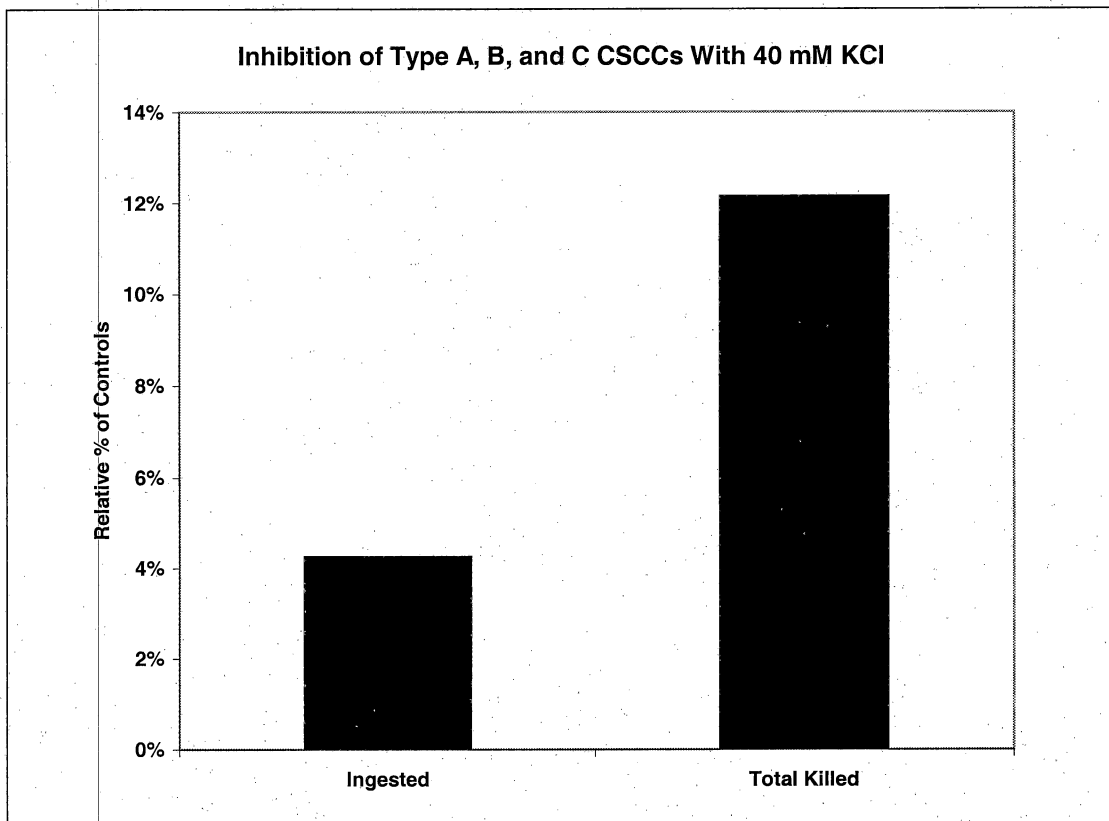


Figure 2. Inhibition of Type A, B, and C CSCCs With 40mM KCl. The contribution of all the three Types of CSCCs were analyzed by treating the experimental anemones with 40mM KCl which was shown to inhibit nematocyst discharge in all three Types of CSCCs (Waston and Hessinger). The result of this investigation suggested 95.7% inhibition in prey ingestion and 87.2% inhibition in prey capture. The result was complementary to the previous studies in other species of sea anemones (Thorington and Hessinger).

Involvement of Type A Cnidocyte
Supporting Cell Complexes
in Prey Killing
and Ingestion

The extent to which Type A CSCCs are involved in prey killing and ingestion of prey in *H. luciae* was evaluated by inhibiting Type A CSCCs with 10^{-4} M streptomycin or with 10^{-8} M proline. The experiment was run 3 times with five replicates each. N=15 anemones were studied and incubated in the 10^{-4} M streptomycin and 10^{-8} M proline respectively. In both cases, only Type B and C CSCCs were available to capture prey. Numbers of shrimp killed and ingested by experimental anemones were compared to those of the controls in both streptomycin and proline treatments. Results were expressed as percentages of controls. Streptomycin decreased killing and ingestion to 87.5% and 81.5% of control (Figure 3). Thus, streptomycin sensitive Type A CSCCs contribute to 12.5% prey killing.

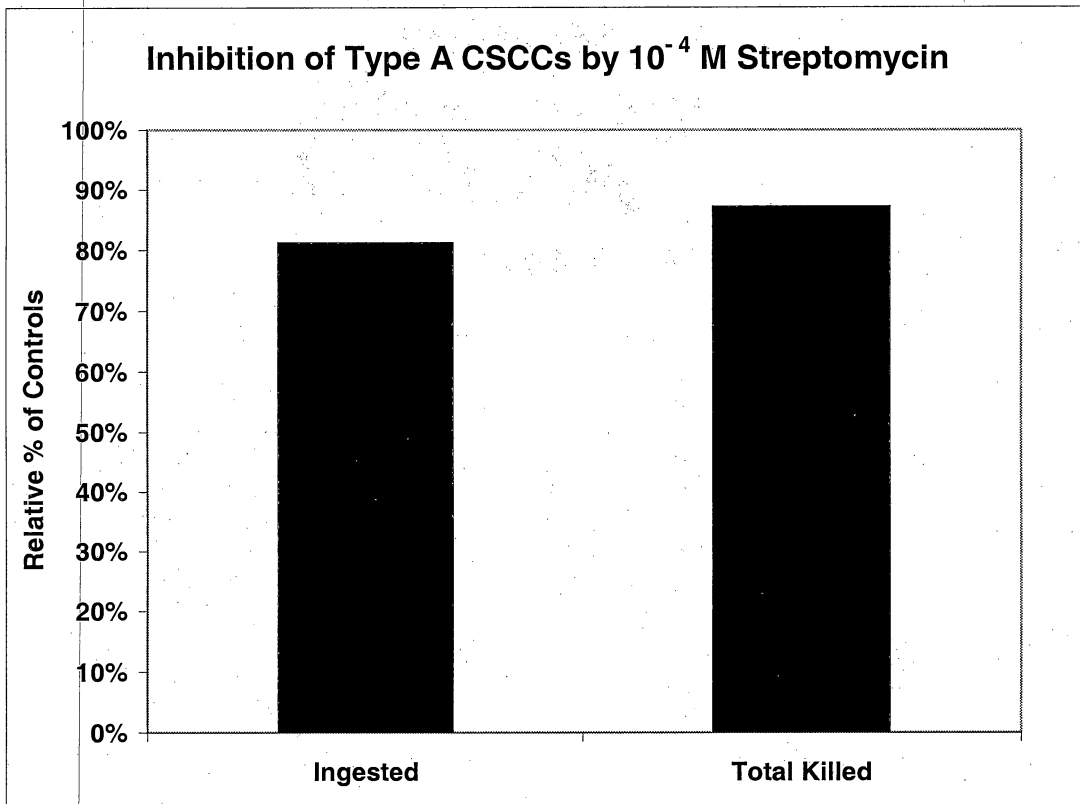


Figure 3. Inhibition of Type A CSCCs by 10^{-4} M Streptomycin. The inhibitory effect of 10^{-4} M streptomycin on prey ingestion and prey capture from Type A CSCCs of anemones was evaluated. Shrimp killed and ingested in experimental anemones was divided by shrimp killed and ingested in controls, and multiplied by 100. Results suggest that Type A CSCCs was involved in 12.5% prey killing.

Inhibition of Type A CSCCs by 10^{-8} M proline also decreased prey killing and ingestion relative to controls. Proline decreased killing to 91.1% of control. In the inhibition of proline, Type A CSCCs contribute 8.9% in prey killing (Figure 4, Table 2).

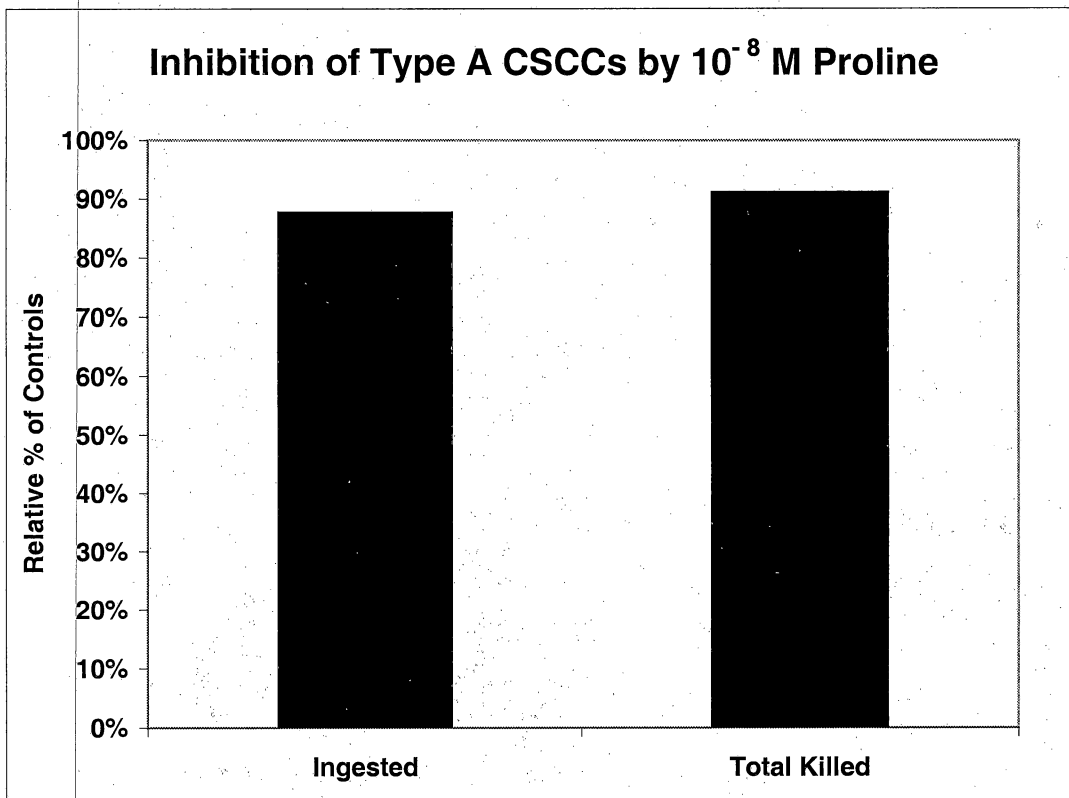


Figure 4. Inhibition of Type A CSCCs by 10^{-8} M Proline. The Type A CSCCs in this investigation contributed less than 10% total prey capture. This result was expected of 10^{-8} M proline inhibitory effect.

Cytochalasin B, which inhibits discharge only from Type A CSCCs by depolymerizing actin bundles within the vibrational sensitive hair bundles (Watson and Hessinger, 1991), was also tested. However, cytochalasin B treatment caused gross shortening of the tentacles. This data was not included in the results.

Involvement of Type B Cnidocyte Supporting Cell Complexes in Prey Killing and Ingestion

The extent to which Type B CSCCs are involved in prey killing and ingestion were determined by inhibiting Type B CSCCs with either methoxyverapamil (D-600), A23187 or S(-) Bay K8664. In each case, Type A and C CSCCs are unaffected and are available to capture prey.

Using 10^{-5} M D-600, experimentals exhibited 65.5% killing and 59.2% ingestion compared to controls. Thus, the data indicated that Type B CSCCs contributed 33.5% of killing compared of control (Figure 5, Table 4).

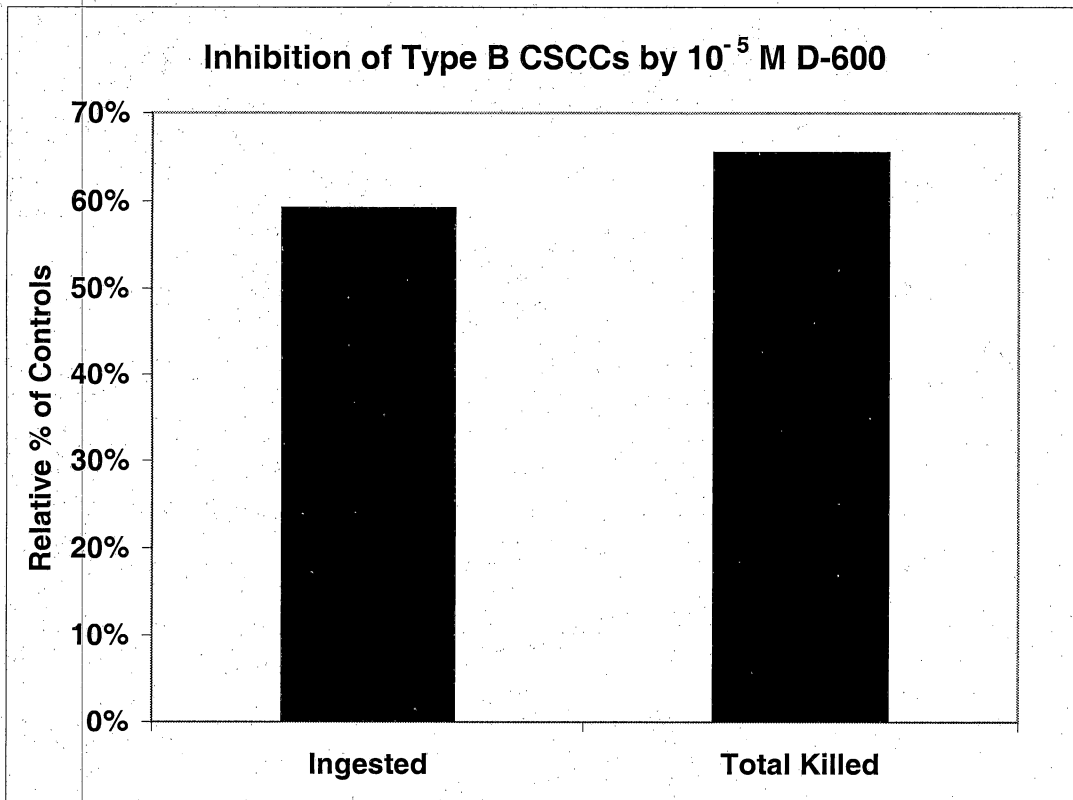


Figure 5. Inhibition of Type B CSCCs by 10^{-5} M D-600. The data revealed 33.5% prey killing involvement of Type B CSCCs.

Type B CSCCs were further tested by blocking with 10^{-6} M A23187. The inhibition of Type B CSCCs by A23187 indicated that Type B CSCCs contributed 26% of prey killing (Figure 6, Table 2).

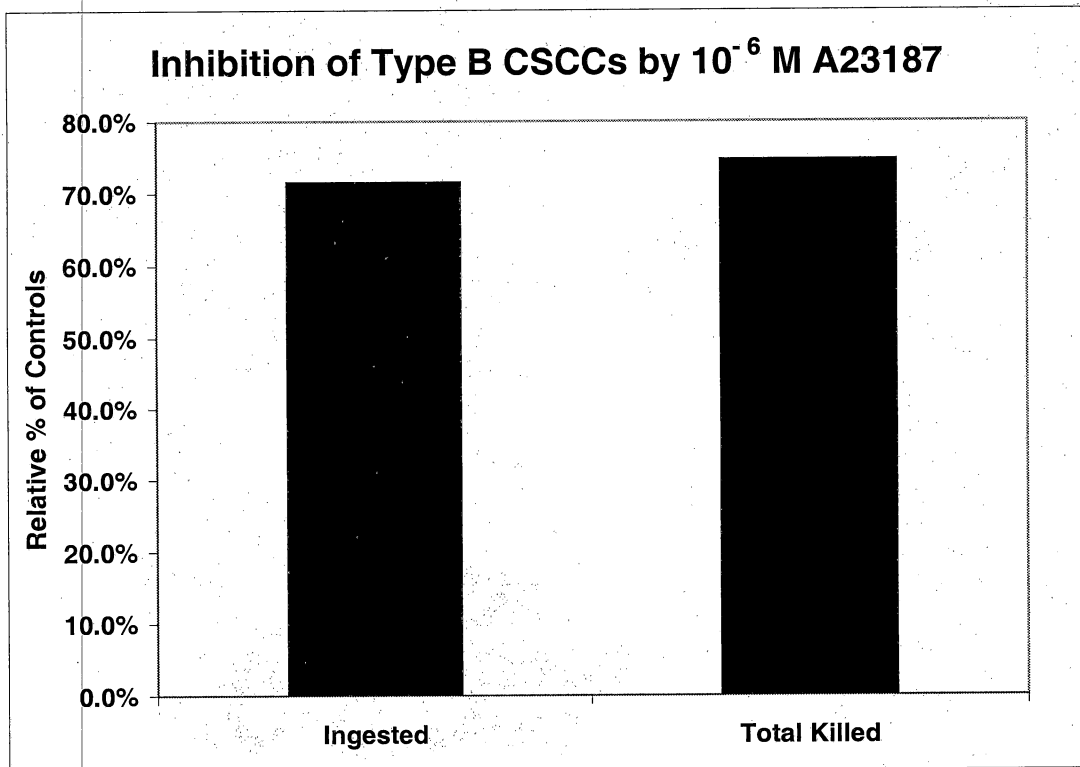


Figure 6. Inhibition of Type B CSCCs by 10⁻⁶ M A23187. Further analysis showed that Type B CSCCs contributed 25.4% in prey capture.

The extent of Type B CSCCs involvement in prey killing and ingestion was further estimated by using third Type B specific drugs 10⁻⁴ M Bay K 8664. The data indicated that Type B CSCCs contribute 22.4% of control prey killing (Figure 7, Table 4).

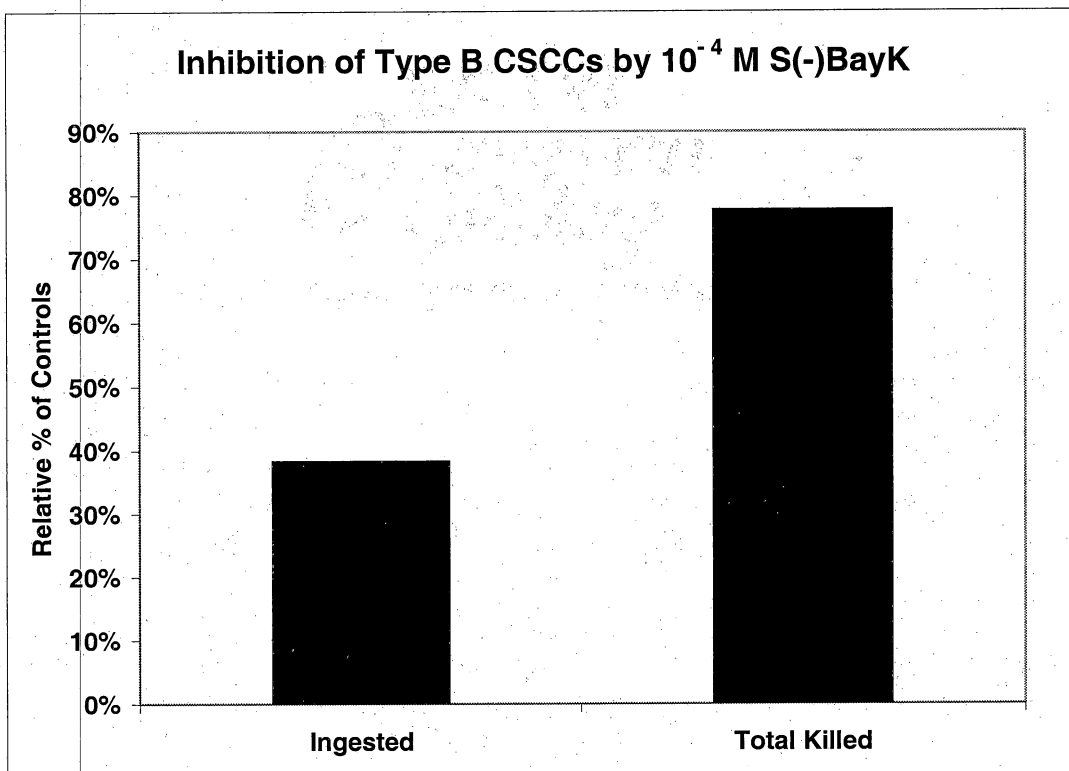


Figure 7. Inhibition of Type B CSCCs by 10^{-4} M S(-)BayK. Analysis of the role of Type B CSCCs in prey capture was determined by inhibiting Types B CSCCs with 10^{-4} M Bay K 8664. The inhibitory effect suggested that Type B CSCCs contributed 22.4% of total prey capture. The percentage was obtained by subtracting 77.6% (The % of total prey captured by Types A and C CSCCs) from 100% (% of total shrimp added).

Involvement of Type C Cnidocyte Supporting Cell Complexes in Prey Killing and Ingestion

To estimate the extent of involvement of Type C CSCCs in prey killing and ingestion, Type A and B CSCCs were blocked with 10^{-7} M tetrodotoxin (TTX). The data indicate

that Type C CSCCs kill 44.4% of control prey and ingest 39.8% of controls (Figure 8, Table 4).

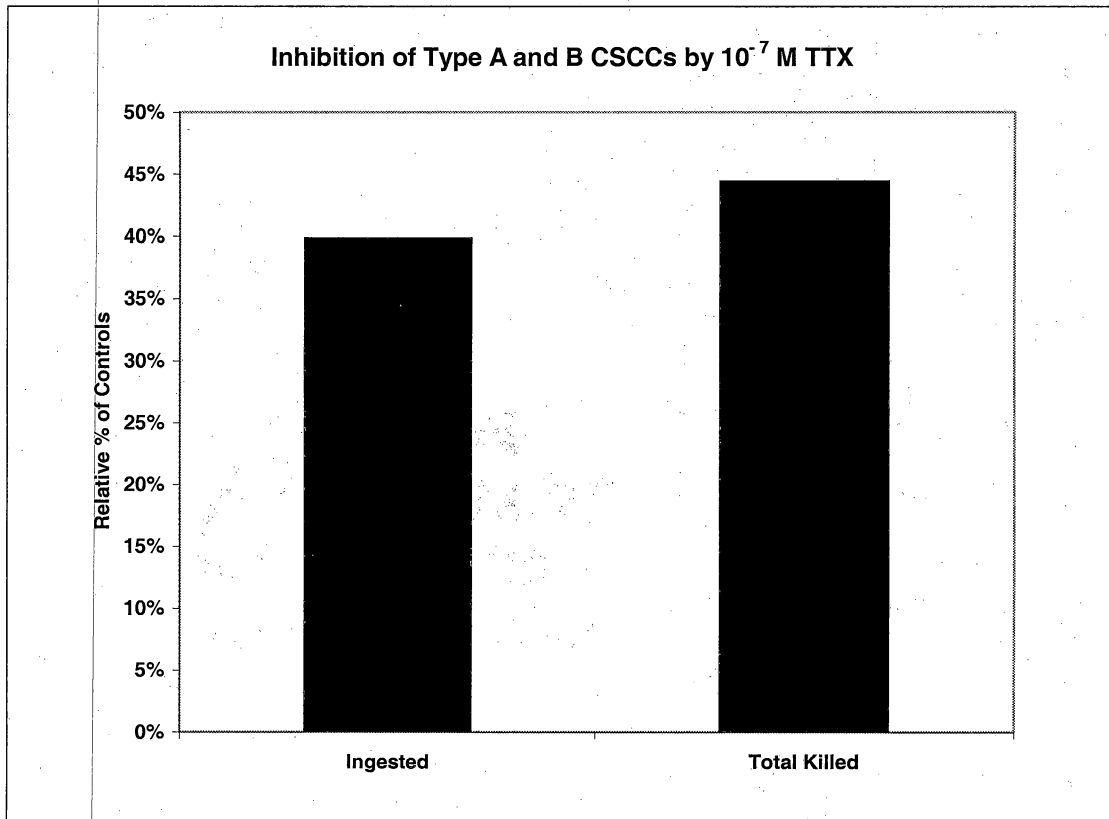


Figure 8. Inhibition of Type A and B CSCCs by 10⁻⁷ M TTX. The figure represents the result of Type C CSCCs involvement in prey capture. The computed analysis showed that Type C CSCCs were involved 44.4% in prey capture and 39.8% ingestion.

The statistical analysis of relative involvement of the three Types of CSCCs indicated that there is a significant difference between prey captured and ingested by Type A, B, and C ($p < 0.001$) (Table 3).

The summary of the percentage of each Type of CSCC suggested that the average contribution of Type A when inhibited with 10^{-4} M Streptomycin and 10^{-8} M Proline was 10.7%. The average contribution of Type B was 27.6 % and Type C was 44.4% (Table 4).

Statistical Analysis of Type A, B, and C Cnidocyte Supporting Cell Complexes

Table 3: The Statistical Analysis of Prey Killing by Each CSCC Type

Type A vs B	P<0.001
Type B vs C	P<0.001
Type A vs C	P<0.001

The data was analyzed using *post hoc tests* (Scheffe). Prey captured by each CSCC Type (A, B, and C) was statistically compared. The data indicated the significant difference ($p < 0.001$, 95% confidence interval) exist among prey killing by Type A, B, and C CSCCs

Table 4: The Summary of Percentage Contribution of Each CSCC Type

Type A	10.7%
Type B	27.6%
Type C	44.4%

The summary of each CSCC type percentage contribution in prey captured and ingested. The result showed Type C CSCCs greater involvement than Types A and B CSCCs.

Effects of Extracellular Ca^{2+} on Prey Killing and Ingestion.

The role of extracellular Ca^{2+} on the anemone prey killing and ingestion was examined. The anemones were incubated at various extracellular Ca^{2+} concentrations. The Ca^{2+} dose-response was biphasic in shape (Figure 9). The dose response showed that there is complete inhibition of prey killing in both Ca^{2+} -free ASW and 18, and 20 mM Ca^{2+} -containing ASW. Maximum prey killing and ingestion occur over the range of 10mM to 16mM Ca^{2+} (Figure 9). Natural sea water normally contains about 10mM free Ca^{2+} .

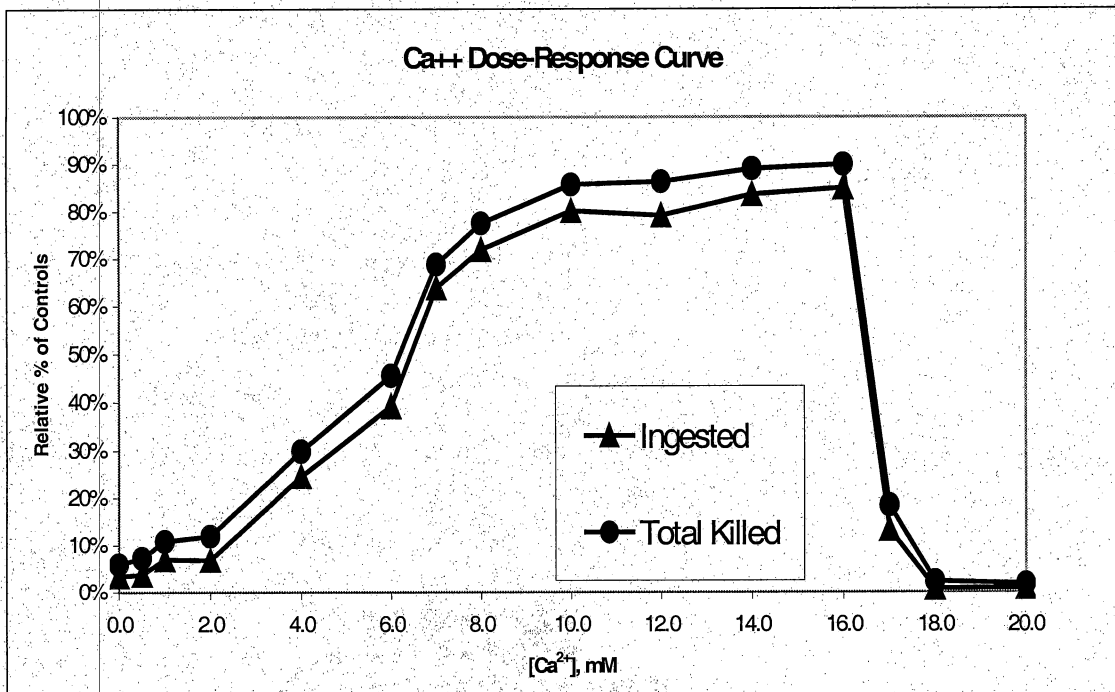


Figure 9. Ca²⁺ Dose-Response Curve. The effect of various concentrations (0, .5, 1, 2, 4, 6, 7, 8, 10, 12, 14, 16, 18, and 20mM) of extracellular Ca²⁺ in prey ingestion and prey capture was determined. The experimental and control (C-2) anemones were incubated in extracellular Ca²⁺ with 83±1 shrimp for 30 min at room temperature. The data suggested that extracellular Ca²⁺ were involved in prey ingestion and prey capture. The Maximum prey capture and ingestion occurred at 16mM Ca²⁺, but complete inhibition was observed at 0,18, and 20mM Ca²⁺.

Effects of Specific Cnidocyte
Supporting Cell Complexes
Inhibitors in the Presence
of Various Extracellular
Ca²⁺ Concentrations

In different extracellular Ca²⁺ concentrations prey killing and ingestion were determined in the presence of these different CSCCs inhibitors. 10⁻⁶ M amiloride, which inhibits Types B and C CSCCs and leaves Type A CSCCs was tested, 10⁻⁷ M Tetrodotoxin, which blocks Types A and B CSCCs and leaves Types Cs was examined. 10⁻⁴M streptomycin, which blocks Type A CSCCs and leaves Type B and C CSCCs, was also used. In the presence of amiloride or TTX, the involvement of Type A and C CSCCs respectively can be directly determined. The involvement of Type B CSCCs was estimated by subtracting the results in the presence of TTX (*ie* Type C CSCCs) from the results in the presence of streptomycin (*ie* combined Type B and C CSCCs). The results (Figure 10) indicated that Type A CSCCs appears most involved in prey killing at Lower Ca²⁺ level, being partially active at 1mM Ca²⁺ and maximal action at 4mM Ca²⁺. At Ca²⁺ concentration above 4mM, the involvement of Type A CSCCs gradually declines. On the other hand, both Type Bs and Type Cs gradually displayed increased prey killing

which peaks at 16mM Ca²⁺ and then abruptly decline to complete inhibition at 18mM Ca²⁺. Although, Type C CSCCs begin to be involved at 2mM Ca²⁺, Type Bs do not become involved until 4 or 6mM Ca²⁺ levels. While Type A CSCCs predominate below 6mM Ca²⁺, Type C CSCCs predominate at concentrations above 6mM Ca²⁺. Summing individual CSCCs contribution at each Ca²⁺ concentrations yields a combined Ca²⁺ dose-response curve (Figure 10) that showed progressively increased killing with a maximum attained at 16mM Ca²⁺ and then dropping precipitously at slightly higher concentration. Although the general features of this "summed" dose-response match those of the combined dose-response in Figure 9, the plateau of maximum killing from 10 to 16 mM Ca²⁺ in the combined dose response is lacking in the "summed" dose-response and the steepness of the "summed" dose-response were at lower Ca²⁺ concentration. It is less than third of the combined curve.

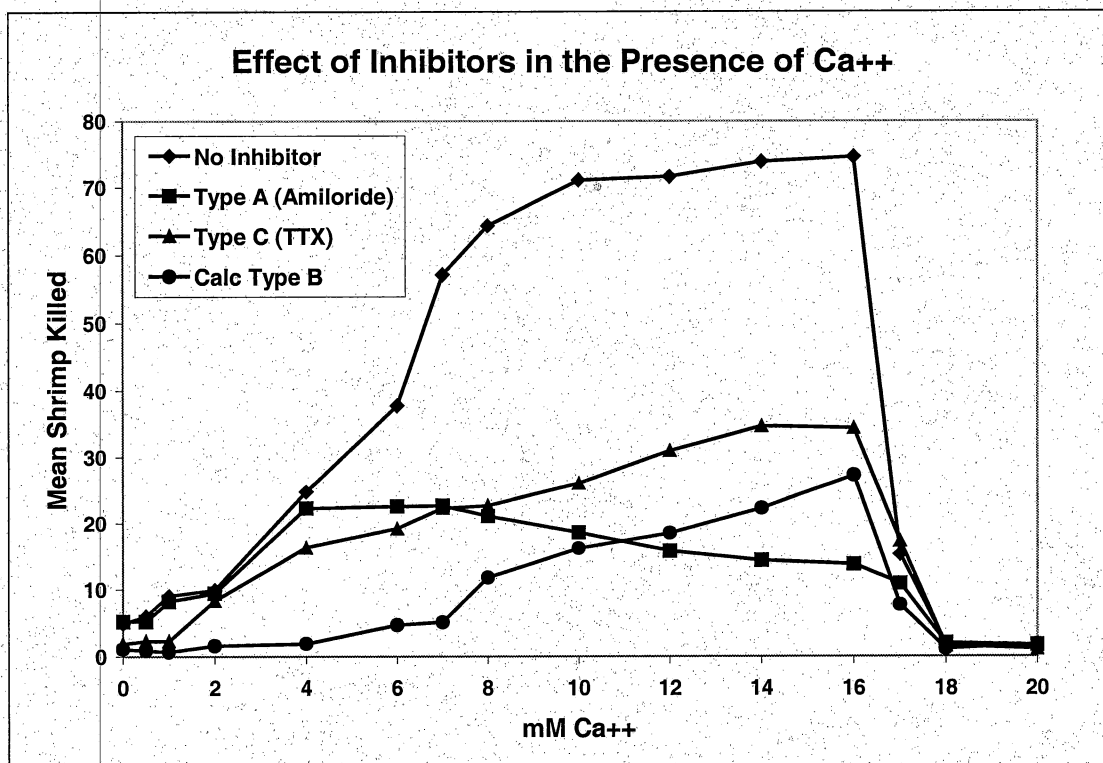


Figure 10. Effect of Inhibitors in the Presence of Ca²⁺. The inhibitory effect of Amiloride (inhibits Types B and C CSCCs), Tetrodotoxin (inhibits Types A and B CSCCs), and Streptomycin (inhibits Type A CSCCs) were examined in the presence of various concentrations of Ca²⁺ (0, 1, 2, 4, 6, 7, 8, 10, 12, 14, 16, 17, 18, and 20mM). The four graphs represent: Ca²⁺ mM with no inhibitors (diamond ♦), TTX in Ca²⁺ (triangle ▲), Amiloride in Ca²⁺ mM (square ■), and calculated Type B CSCCs effect in the presence of Ca²⁺ (circle ●). The calculated B CSCCs was obtained by subtracting the TTX data from Streptomycin data (Streptomycin in Ca²⁺ - TTX in Ca²⁺). That is (Types B+C CSCCs - Type C CSCCs).

The Abrupt Decline in High
 Ca^{2+} Levels is Due to
Intracellular
Effect

The inhibition of prey killing in high levels of extracellular Ca^{2+} was investigated. If the effect is due to an intracellular effect of influxing Ca^{2+} then, BAPTAM-AM, a membrane permeant form of BAPTA should chelate intracellular Ca^{2+} and block its intracellular effect. Thus, BAPTA-AM should increase killing at inhibiting level of extracellular Ca^{2+} (eg 18mM). If extracellular Ca^{2+} acts electrogenically, then intracellular BAPTA-AM should have no effect on killing. The experimental attempt to rescue anemone killing from the inhibitory effect of 18mM Ca^{2+} acting intracellularly was confirmed with intracellular BAPTA-AM increasing prey killing by nearly 40-fold over high Ca^{2+} inhibited controls and ingestion approximately 60-fold (Figure 11).

Table 5: Statistical Analysis of BAPTA-AM and A23187.

	Case	N	Mean	Std. Deviation	Std. Error Mean	p
BAPTA 18mM Ingested	Exp	10	59.20	2.15	0.68	<0.001
	Control	10	0.90	0.88	0.28	
BAPTA 18mM Killed	Exp	10	61.60	2.12	0.67	<0.001
	Control	10	1.60	0.97	0.31	
A23187 16mM Ingested	Exp	10	38.30	2.41	0.76	<0.001
	Control	10	68.10	1.10	0.35	
A23187 16mM Killed	Exp	10	46.30	2.16	0.68	<0.001
	Control	10	73.10	1.91	0.61	

The results show that BAPTA-AM blocked the inhibitory effect of 18mM Ca²⁺ on prey capture. A23183 inhibited prey capture at the peak of prey killing (16mM Ca²⁺). There is a significant difference (p<0.001) in prey capture between the control and BAPTA-AM treated anemones. Also A23187 and its controls showed significant difference of p<0.001.

If an abrupt decrease of prey killing at high Ca²⁺ levels is due to an intracellular effect of Ca²⁺, but not due to an electrogenic effect of entering Ca²⁺, then the non electrogenic calcium ionophore, A23187 should cause a decrease in killing and ingestion at 16mM Ca²⁺. In fact, A23187 decreased peak values for prey killing and ingestion at 16mM in controls by 27% and 30%, respectively (Figure 12).

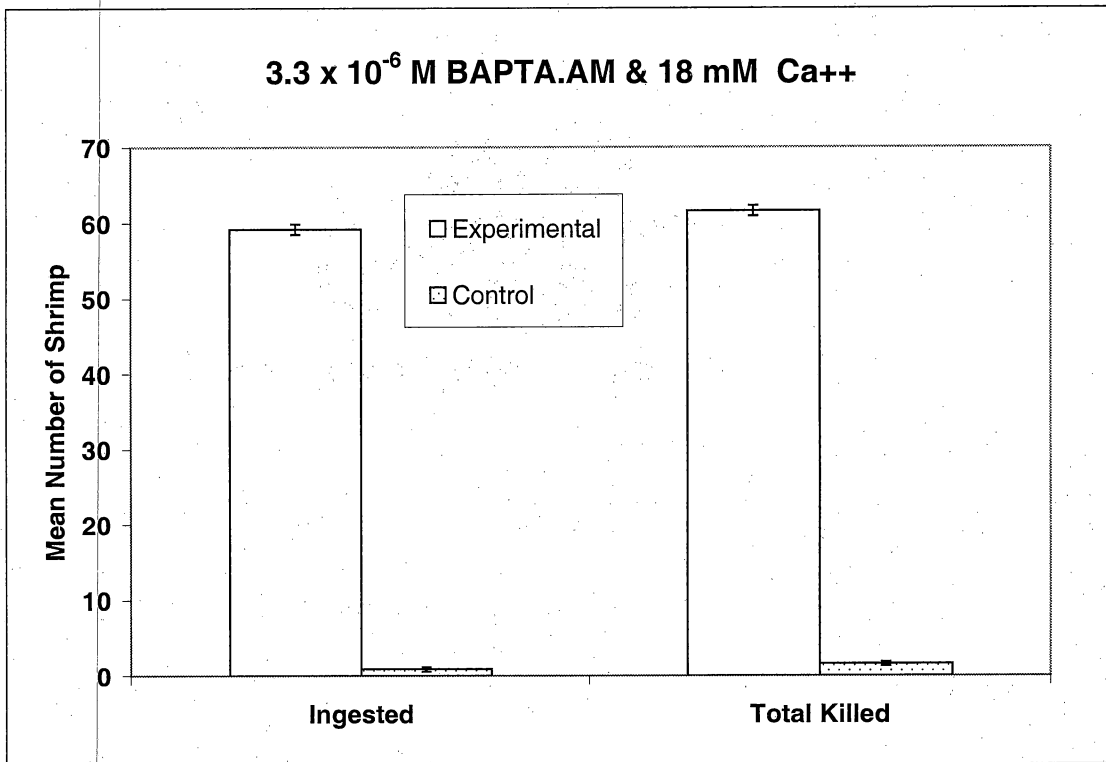


Figure 11: 3.3×10^{-6} M BAPTA-AM and 18 mM Ca²⁺. Effects of BAPTA.AM on high Ca²⁺ level inhibition. The inhibition of ingestion and prey capture by BAPTA.AM suggested that BAPTA.AM blocked the inhibiting effect of 18mM Ca²⁺ on prey killing and ingestion (see Figure 9)

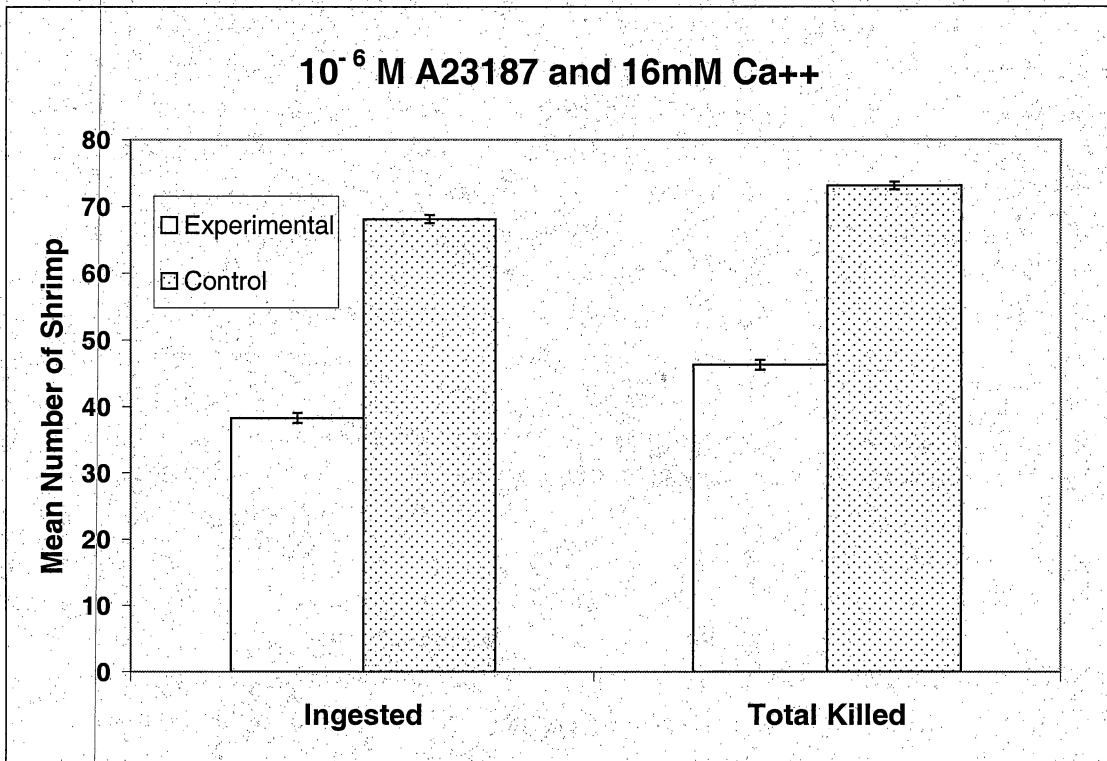


Figure 12. 10^{-6} M A23187 in 16mM Ca²⁺. The data indicated that 10^{-6} M A23187 decreased prey capture and ingestion at 16 mM Ca²⁺ (see Figure 9).

CHAPTER FOUR

DISCUSSION

One of the main goals of this study was to determine the relative extent to which each of the three Types of CSCC (A, B, and C) contribute to prey killing and ingestion by *H. luciae*. To accomplish this goal, it is necessary to estimate the relative proportions of the different Types of CSCC in the tentacle of *H. luciae*. The different Types of CSCC are functionally distinguished rather than morphologically defined. Thus, it is on the basis of functional differences that the relative proportions of CSCCs in the anemone tentacle must be established. The relative proportions of different functionally mature CSCCs in the feeding tentacles can be estimated from the measured extent of nematocyst discharge under optimal conditions for triggering each Type. Discharge from Type C CSCCs is solely dependent upon physical contact. Contact triggered discharge in the absence of other chemical and vibrational stimuli only occurs from Type C CSCCs. Type B CSCCs discharge only in the presence of a predisposing chemosensitizer, such as N-acetylated sugars in response to non-vibrating mechanical contact. Type A CSCCs are

distinguished from Type B and C CSCCs by their requirement for specific vibration frequencies to elicit discharge.

The total number of CSCCs per tentacle in *H. luciae* have been estimated at about 41,000 (Watson and Hessinger, 1994). Nematocyst discharge studies, suggest that the ratio of Type B to C is 1:1. In the case of Type A CSCCs, however, the situation is complicated by the possible existence of four different vibration-specific sub-types, each with characteristic optimal discharge frequencies at 5, 15, 30 and 40 Hz (Watson and Hessinger, 1989). If these specific vibrational optima characterize distinct sub-populations of Type A CSCCs, then the ratio of Type A: B: C CSCCs is 4:1:1. If, however, the four different vibrational optima represent four functional states of a single population of Type A, then the ratio of Type A: B: C is 1:1:1.

The results of the present study indicate that the ratio of prey killing from Type A, B, and C CSCCs is approximately 1:2:4. Contrary to the original hypothesis Type A CSCCs used for capture of *Artemia* nauplii are either 1/10 or 1/40 that of their numerical occurrence, depending on the relative proportion of Type A CSCCs to other Types

in the tentacles. Thus, discharge from Type C CSCCs predominate in the capture of live *Artemia* by *H. luciae* under the condition of the present experiment. Type B CSCCs are employed about half as much as Type C CSCCs, with Type A CSCCs the least employed.

The unexpectedly high involvement of Type C CSCCs in the capture and killing of *Artemia* nauplii may be due to the fact that the surface of the nauplii is chitin, a very insoluble form of polymerized N-acetylated sugars. The extreme insolubility of chitin makes it poor for stimulating the primary chemoreceptors that regulates the sensitization of Type A and B CSCCs. It is possible that other Types of CSCCs would predominate for prey with more soluble surface molecules, such as the mucins of small fish, which abound in conjugated N-acetylated sugars.

Type A CSCCs utilize mechanically gated ion channels located at the tip of hair bundles (Watson and Hessinger, 1994). The channels are sensitive to vibrating stimuli at various frequencies (Watson and Mire, 1999). Vibration from swimming prey presumably open the mechanically-gated ion channels. Streptomycin, an aminoglycoside antibiotic blocks influx of ions through this mechanically-gated ion

channels and thus inhibits prey killing in *Hydra* (Wiley, 1968; Gitter and Thurm, 1993). Streptomycin also blocks nematocyst discharge from Type A CSCCs, but not from Type B or C CSCCs in *H. luciae* (Watson and Hessinger, 1994). If, in fact, streptomycin affects nematocyst discharge through this mechanically-gated ion channel, it would be expected that there would be inhibition of prey killing from Type A CSCCs in anemones treated with streptomycin. Indeed there was a 12.5% decrease in prey killing. This implied that Type A CSCCs contributed to 12.5% of prey killing. There was no significant difference between the inhibition of prey killing by proline or by streptomycin ($p > 0.05$). Proline selectively tune NANA stimulated Type A CSCCs to frequencies higher than that of swimming *Artemia nauplii* (Watson and Hessinger, 1994) and therefore, would be expected to block killing by Type A.

Each of the three Types of CSCCs requires extracellular Ca^{2+} (Watson and Hessinger, 1994) for nematocyst discharge. Based upon the published studies, which indicate that only Type B CSCCs use dihydropyridine-sensitive L-Type Ca^{2+} channels (Watson and Hessinger, 1994). Nematocyst discharge from Type B CSCCs was inhibited with

the Ca^{2+} channel agonist, Bay K 8664 and methoxyverapamil (Thorington and Hessinger, 1998). In the present study, prey capture was blocked from Type B CSCCs with Bay K 8664 and methoxyverapamil. The inhibition of prey capture from Type B CSCCs, indicate that Ca^{2+} channels are indeed involved in *H. luciae* prey killing. Type B CSCCs are the least involved in prey capture compared to Type C CSCCs, the increase and/or decrease in Ca^{2+} channel sensitization may account for this observation. Indeed, extracellular Ca^{2+} is required for both live prey killing and ingestion (Figure 9). While killing and ingestion require Ca^{2+} at lower Ca^{2+} concentrations, Ca^{2+} levels above 16mM dramatically inhibited killing and ingestion. The abrupt fall-off of killing at higher extracellular Ca^{2+} levels with or without Ca^{2+} channels were determined. Anemones were treated with A23187, a Ca^{2+} ionophore and decrease in prey killing and ingestion was observed. This result confirmed that extracellular Ca^{2+} is involved in prey capture.

Extracellular Ca^{2+} levels seem to affect the three Types of CSCCs differently. The normal Ca^{2+} levels in natural seawater is 10 mM. Reduction of Ca^{2+} level below the normal seawater level decreases prey killing along a

sigmoidal dose response, while increases above 16mM cause complete inhibition of discharge (Figure, 10). However, the extent to which increases or decreases affect nematocyst discharge depends on the species of anemone. For example, in NANA-sensitized *A. pallida*, the effect of extracellular Ca^{2+} on nematocyst discharge from Type B CSCCs is hyperbolic up to 10mM Ca^{2+} , while all concentrations greater than 10mM cause complete while all concentrations greater than 10mM cause complete inhibition of discharge (Thorington and Hessinger, 1992).

The studies on prey capture by *H. luciae* did not indicate which CSCC type is involved and at what concentrations of extracellular Ca^{2+} . To accomplish this each CSCC type was inhibited at different levels of extracellular Ca^{2+} . These results indicated that prey killing by each CSCCs Type is indeed affected differently at various Ca^{2+} concentrations. Type A CSCCs are predominantly active in prey killing at lower levels of Ca^{2+} peaking at 4mM Ca^{2+} and gradually declining at 18mM Ca^{2+} . Type B CSCCs are not involved in prey killing at lower Ca^{2+} levels from 0 to 4mM, but gradually increased killing and, peaked at 16mM Ca^{2+} only to abruptly drop at 18mM Ca^{2+} . On

the other hand, Type C CSCCs are active in prey killing beginning at 1mM Ca^{2+} and gradually increasing with discharge peak at 16mM and then abruptly declining at 18mM Ca^{2+} . Thus, there are significant differences in the responsiveness of the three Types of CSCCs to various levels of extracellular Ca^{2+} . The different response of the CSCCs to extracellular Ca^{2+} may suggest either different transduction pathways involved in the control of nematocyst discharge and prey killing or may indicate the involvement of different kinds of Ca^{2+} channels.

Stimulation of receptors for N-acetylated sugars sensitizes nematocyst discharge through an adenylyl cyclase-cAMP signaling pathways (Watson and Hessinger, 1992; Ozacmalc, 2001). Chitin in the surface of crustacean (prey) polymerized N-acetylgalactosamine. The level of extracellular Ca^{2+} affects the ability of N-acetylated sugars to sensitize and desensitize nematocyst discharge from Type A and B CSCCs. In *A. pallida*, abrupt inhibition of nematocyst discharge was observed at high Ca^{2+} levels (Thorington and Hessinger, 1988b). In *H. luciae*, abrupt inhibition of prey killing was observed at high Ca^{2+} (16 to 18mM Ca^{2+} , Figure 9). Is the sudden drop of prey killing at

the high Ca^{2+} concentrations due to electrogenic or second messenger effects of Ca^{2+} ?

If the abrupt decline in prey killing by *H. luciae* observed at 18mM Ca^{2+} is due to an increase in intracellular Ca^{2+} , then Ca^{2+} , possibly, BAPTA-AM, a membrane-permeant, calcium chelator, should block the inhibitory effect of high extracellular Ca^{2+} and thus, increase prey killing. In addition, A23187, a calcium ionophore, which facilitates Ca^{2+} entry without affecting the membrane potential, should inhibit prey killing at 16mM Ca^{2+} , which is the peak of prey killing for Type B and C CSCCs. Indeed, BAPTA-AM blocked the inhibitory effect at 18mM Ca^{2+} and significantly increased prey killing (Figure 12). Likewise, at 16mM Ca^{2+} A23187 inhibited prey killing. The present study suggests that the inhibition of prey killing by high levels of extracellular calcium is mediated by some intracellular effect of calcium.

Such an effect of increased intracellular calcium may involve activation of calmodulin and consequent stimulation of cAMP-specific phosphodiesterase. This activation would lower cAMP levels in NANA sensitized CSCCs and thereby desensitize nematocyst discharge and effectively inhibit

prey capture. While other calcium-mediated inhibitory effects are possible, calmodulin is likely involved as W-7, a calmodulin inhibitor, sensitizes nematocyst discharge in *H. luciae* (Thibodeaux-Mire and Watson, 1994).

CHAPTER FIVE

CONCLUSION

The present study has established several lines of evidence indicating that three Types of CSCCs are involved in *Artemia* nauplii killing by *Haliplenella luciae*. Type C CSCCs are most involved in prey capture and Type A CSCCs are the least involved. In addition, extracellular Ca^{2+} is required for anemone prey killing at low and normal levels, and Ca^{2+} functions as a second messenger. At high levels of Ca^{2+} (*i.e.* $>16\text{mM}$) Ca^{2+} is inhibitory. Experiments indicate that the inhibitory effects of high Ca^{2+} are due to influxing Ca^{2+} . Further studies are required to investigate the functions of Ca^{2+} at lower concentrations (*i.e.* $<10\text{ mM}$). The possibility that Type C CSCCs may have additional means of prey killing besides the chemical depended contact receptor pathway also warrants further investigation.

REFERENCES

- Ewer R. F. (1947) On the functions and mode of action of the nematocysts of hydra. *Proc Zool Soc London* 117, 365-376.
- Gardner et al (1995) *Pharmacology*, Churchill Livingstone Incorporation.
- Geibel G., Thorington G. and Hessinger D. A. (1988) Control of cnida discharge II. Microbasic p-mastigophore nematocysts are regulated by two classes of chemoreceptors. *Biol. Bull* 175, 132-136
- Hand, C. (1954) The morphology of nematocyst. In *Coelenterate Biology: Reviews and New Perspectives* (Edited by Muscatine L. and Lenhoff H. M.), pp. 130-179 Academic press, New York.
- Hessinger D. A. and Ford M. I. (1988) Ultrastructure of the small cnidocyte of the Portuguese Man of War (*Physalia physalis*) tentacle. In *The Biology of Nematocysts* (Edited by Hessinger D. A. and Lenhoff H. M.), pp. 75-94. Academic Press, San Diego.
- Lenhoff H. M. and Haegy W. (1977) Aquatic invertebrate model systems for study of receptor activation and evolution of receptor proteins. *Ann Rev Pharmacol Toxicol* 17, 243-258

Lenhoff H. M. and Bovaird J. (1959) Requirement of bound Calcium for the action of surface chemoreceptors. *Science* 130, 1474-1476.

Manscal R. N., Conklin E. J. and C H Bigger (1978) The putative sensory receptors associated with the Cnidaria and cnidarians. *Scanning Electron Microsc* 2, 959-966.

Mire-Thibodeaux P. M. and Watson G. M. (1994) Morphodynamic hair bundles arising from sensory cell/supporting cell complexes frequency tune nematocyst discharge in sea anemones. *J Exp. Zool* March 15. 268(4): 282-92.

Mire-Thibodeaux P. M. and Watson G. M. (1993) Direct monitoring of intracellular calcium ions in sea anemone tentacles suggests regulation of nematocyst discharge by remote, rare epidermal cells. *Biol Bull* 185, 335-345.

Muscantine L. and Lenhoff H. M. (1974) *Coelenterate Biology: Reviews and New Perspectives* Pp. 2-3, Academic press, New York.

Ozacmak V.H., Thorington G.U., Fletcher W.H. and Hessinger D.A. (2001) N-acetylneuraminic acid (NANA) stimulates *in situ* cyclic AMP production in tentacles of sea anemone (*Aiptasia pallida*): Possible role in chemosensitization of nematocyst discharge. *J. Exp. Biol.*, in press.

Parker G. H. (1916) The effector system of actinians. *J. Exp. Biol* 21 461-484.

- Thomason J. C. (1988) The allometry of nematocysts. In *The Biology of Nematocysts* (Edited by Hessinger D. A. and Lenhoff H. M.), pp. 575-588. Academic Press, San Diego.
- Thorington G. U. and Hessinger D. A. (1996) Efferent mechanisms of discharging cnidae: 1. Measurement of intrinsic adherence of cnidae discharged from tentacle of sea anemone, *Aiptasia pallida*. *Biol Bull* 190, 131-137.
- Thorington G. U. and Hessinger D. A. (1988a). Control of cnida discharge I Evidence for two classes of chemoreceptors. *Biol Bull* 174, 163-171.
- Thorington G. U. and Hessinger D. A. (1988b) Control of cnida discharge factors affecting discharge of cnidae. In *The Biology of Nematocysts* (Edited by Hessinger D. A. and Lenhoff H. M.), pp. 233-253. Academic Press, San Diego.
- Thorington G. U. and Hessinger D. A. (1990) Control of cnida discharge: III Spirocysts are regulated by three classes of chemoreceptors. *Biol Bull* 178, 74-83.
- Thorington G. U., and Hessinger D. A. (1992) Nifedipine blocks de-sensitization of chemoreceptors regulating nematocyst discharge in sea anemones (*Aiptasia pallida*) *Molec Biol Cell* 3, 250a.
- Watson G. M. and Hessinger D. A. (1989b) Cnidocyte mechanoreceptors are tuned to the movements of swimming prey by chemoreceptors *Science*, 243, 1589-1591.

Watson G. M. and Hessinger D. A. (1987) Receptor-mediated endocytosis of a Chemoreceptor involved in triggering the discharge of Cnidae in a sea anemone tentacle. *Tissue Cell* 19, 747-755.

Watson G. M. and Hessinger D. A. (1994b) Evidence for calcium channels involved in regulating nematocyst discharge. *Comp Physiol Bioch* 107, 473-481.

Watson G. M. and Hessinger D. A. (1989a) Cnidocyte mechanoreceptors are tuned to the movements of swimming prey by chemoreceptors. *Science* 243, 1589-1591.

Watson G. M. and Hessinger D. A., (1989b) Cnidocytes and adjacent supporting cells form receptor-effector complexes in anemone tentacles. *Tiss Cell* 21, 17-21.

Watson G. M. and Hessinger D. A. (1991) Chemoreceptor-mediated elongation of stereocilium bundles tunes vibration-sensitive mechanoreceptors on cnidocyte-supporting cell complexes to lower frequencies. *J Cell Sci* 99, 307-316.

Watson G. M. and Hessinger D. A. (1992) Receptors for N-acetylated sugars may stimulate adenylate cyclase to sensitize and tune mechanoreceptors involved in triggering nematocyst discharge. *Exp Cell Res* 198, 8-16.

Watson G. M. and Hessinger D. A. (1994a) Antagonistic frequency tuning of hair bundles by different chemoreceptors regulate nematocyte discharge. *J Exp Biol* 187, 57-73.

- Watson G. M. and Hessinger D. A. (1989) Cnidocyte mechanoreceptors are tuned to the movements of swimming prey by chemo-receptors. *Science* 243, 1589-1591.
- Watson G. M. and Hessinger D. A. (1992) Receptors for N-acetylated sugars may stimulate adenylate cyclase to sensitize and tune mechanoreceptors involved in triggering nematocyst discharge. *Exp Cell Res* 98(1): 8-16.
- Watson G. M. and Mire P. (1999) A comparison of hair bundle mechanoreceptors in sea anemones and vertebrate system. *Current Topic Dev Biol* 43, 51-84.
- Watson G. M., Mire P. and R. R. Hudson (1998) Frequency specificity of vibration dependent discharge of nematocyst in sea anemones. *J Exp Zool* 15: 281(6) 582-93.
- Watson G. M. and Hessinger D. A. (1989a) Cnidocytes and adjacent supporting cells form receptor-effector complexes in anemone tentacles. *Tissue Cell* 21, 17-24.
- Watson G. M., Roberts J., Parakkal M. and B Kachar (1992) Chemoreceptor-mediated mobilization of actin into and out of anemone hair bundles. *Molec Biol Cell*, 3,39a.
- Watson G. M. and Hessinger D. A. (1988) Localization of a purported (E) chemoreceptor involved in triggering cnida discharge in sea anemones. In *The Biology of Nematocysts* (Edited by Hessinger D. A. and Lenhoff H. M.), pp. 255-272. Academic Press, San Diego.

Watson G. M. and Hessinger D. A. (1991) Chemoreceptor mediated elongation of stereocilium bundles tunes vibration-sensitive mechanoreceptors on cnidocyte-supporting cell complexes to lower frequencies. *J Cell Sci* 99, 307-316.

Watson G. M. and Hessinger D. A. (1991) Chemoreceptor-mediated elongation of stereocilium bundles tunes vibration-sensitive mechanoreceptors on cnidocyte-supporting cell complexes to lower frequencies. *Journal of Cell Science* 99, 307-316.

Werner, B. (1973) New investigations on systematics and evolution of the class Scyphozoa and the phylum Cnidaria. *Publ. Seto Mar. Biol. Lab.* Proceedings of the Second International Symposium on Cnidaria, 20:35-61.

Westfall J. A. (1965) Nematocysts of the sea anemones *Metridium*. *Amer zool* 5, 377-393.