Sensory discrimination by isolated feeding tentacles in *Sanderia malayensis*

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18   **Summary Statement**
19   Two separate reflexes have been distinguished isolated tentacles of the
20   scyphomedusa, *Sanderia malayensis*, a bi-directional network for withdrawal and a
21   proximally directed network for feeding.
22
23   **Abstract**

24   Despite having only a diffuse neural network, tentacles of scyphomedusae exhibit a
25   variety of distinct behavioral acts. One such behavior in tentacles is the capture and
26   subsequent transfer of prey to the mouth. Interaction with prey consists of a variety of
27   distinct stimuli—mechanical contact with the organism, chemical stimulation from the
28   prey, and once captured by the tentacle, the weight of the food particles stretching the
29   tentacle. By isolating and observing these individual stimuli in intact and isolated
30   tentacles of the scyphomedusa, *Sanderia malayensis*, two separate reflexes have been
31   distinguished. The tentacle feeding response observations suggest that the tentacle has
32   two sensing networks, a bi-directional network for withdrawal and a proximally directed
33   network for feeding.
34
35   **Introduction**
36   Jellyfish medusae perform a number of distinct behavioral acts, such as rhythmic
37   swimming and food capture (Romanes 1880, Horridge 1953, Passano 1965). Two groups
38   of jellyfish, hydromedusae and cubomedusa, those behaviors are controlled by their
39   central nervous systems and peripheral neural net. Hydromedusae have a central nervous
40   system that consists of two nerve rings to process sensory inputs and generate rhythms
41   (Horridge 1955). The important neural elements for swim control in these animals are
42   found in one of these nerve rings, the inner nerve ring. The *Aglantha digitale* produces an
43   escape swimming response, in addition to slow spontaneous swimming contraction of the
44   bell. Mackie and Meech (Mackie and Meech 1985) found that the motor giant neurons
45   are able to produce two different types of spikes for these behaviors. They related
46   different input events to discrete neural sub-systems. A comprehensive picture of the
47   neuronal circuit was constructed by studying the synaptic inputs into the giant axons
48   using extracellular recordings of the nerve rings in response to different stimuli (Mackie
49   and Meech 1995, Mackie and Meech 1995, Mackie and Meech 2000, Mackie, Marx et al.
50   2003). Cubomedusae also have a central nervous system consists of the nerve ring and
51   rhopalia those contain image forming eyes with lenses. A nerve ring connects (Garm,
52   Ekstrom et al. 2006). Box jellyfishes, *Tripedalia cystophora* and *Chiropesella bronzie*,
53   shows visually guided behavior suggesting that the CNS of these jellyfishes integrates
54   visual information and innervate motor systems (Garm, O'Connor et al. 2007).
55   Recent studies on the Hydromedusae have used calcium imaging of genetically
56   engineered Hydra to record the activity of all neurons (Dupre and Yuste 2017). They
57   established that there are three distinct functional neuronal networks that mediate at least
58   4 different behavioral acts that have been established previously (Trembley 1744, Haug
59   1933, Ewer and Munro Fox 1947, Passano and McCullough 1963, Lasker, Syron et al.
60   1982).
61   A central nervous system or nerve rings on scyphomedusae have not been
62   reported, but they exhibit swimming and feeding behavior similar to hydromedusae and
63   cubomedusae that have a CNS. Pulsation rhythms are generated by any one of the

64 rhopalia located on the bell margin (Romanes 1885). Scyphomedusae usually have 8 or
65 16 rhopalia and their pacemakers are responsible for initiating contractions. The rhythm
66 of a rhopalium pacemaker can be reset by a current injection suggesting that an active
67 pacemaker resets all others to avoid simultaneous initiations of bell contractions at
68 different locations (Pantin and Dias 1952, Horridge 1959). There is no direct central axon
69 tracts connecting rhopalia (Romanes 1885, Horridge 1954, Horridge 1956, Passano and
70 McCullough 1963, Passano 2003), so signals are presumed to be conducted through the
71 nerve net in the bell.

72   A rhopalium includes a statocyst, sensory epithelia, and ocelli (Schafer 1878,
73 Martin 2002). The pacemaker is considered to be located in inner half of the rhopalium,
74 however the exact location and circuitry of the pacemaker is unclear (Passano 1973,
75 Nakanishi, Hartenstein et al. 2009). Rhopalia are connected to two kinds of nerve nets in
76 the bell, the diffused nerve net (DNN) and the motor nerve net (MNN). Sensory inputs
77 are sent to rhopalia through the DNN and an action potential from rhopalia that initiates
78 bell contraction is transmitted to swimming muscles through the MNN (Romanes 1885,
79 Horridge 1956). The DNN interacts with the MNN primarily at a rhopalium, and the
80 DNN is also capable of enhancing MNN induced contractions (Romanes 1885, Horridge
81 1956, Passano and McCullough 1965, Passano 1973).

82   With the diffused nerve nets and rhopalia, scyphomedusae integrate and respond to
83 environmental information. Arai reported that the scyphomedusa *Aurelia aurita* are
84 attracted toward prey with which they are not in direct contact (Arai 1992). This result
85 suggests that their swimming behavior is modified to produce a taxis, however, the
86 precise network which underlies this modification has yet to be identified.

87   Capturing prey is an important event that can influence the pulsation rhythm of the
88 bell. Some medusae, such as *Aurelia aurita,* have a number of short, fine tentacles on the
89 bell margin to catch zooplankton. Other medusae, such as *Chrysaora hysoscella*, have
90 fewer, longer tentacles and they feed on both zooplankton and larger prey such as fishes
91 and other jellyfish. *Chrysaora* captures prey on a tentacle, and tangles it by the swimming
92 movement of the medusa (Larson 1986). The tentacle then rapidly contracts, pulling the
93 attached prey upward to the bell, where contact is made with an oral arm.

94   The tentacle of *Chrysaora* is composed of four basic layers: an outer epithelial
95 layer, a layer of muscle fiber, a noncellular layer, called the mesoglia, and an endodermal
96 layer which encloses the gastric canal (Burnett and Sutton 1969). Muscles are juxtaposed
97 to the mesoglia (Perkins, Ramsey et al. 1971). A tentacle of *Chrysaora* contracts to a
98 thirtieth of its relaxed length when prey is attached. Most of the length contraction arises
99 from muscles crumpling into the mesoglia, only a small portion stems from actual
100 shortening of the muscles themselves (Burnett and Sutton 1969, Perkins, Ramsey et al.
101 1971).

102   Anderson and co-workers (Anderson, Moosler et al. 1992) described
103 immunohistochemically a nerve net in the outer epithelial layer of a *Chrysaora* tentacle.
104 The region of nerve net stained on the subumbrella is considered a DNN (Anderson,
105 Moosler et al. 1992), and it can be assumed that the stained nerve net would have a DNN
106 like function. There might be another nerve net that is not immunoreactive to Antho-
107 RFamide and innervates the motor system, like a MNN in the bell. Another nerve net has
108 been stained in the endodermal layer; this would not innervate the motor system because
109 mesoglia located between ectodermal and endodermal layers does not contain cells

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110 (Burnett and Sutton 1969). An extremely dense immunoreactive nerve net, the tentacular
111 nerve ring, can be found in the ectoderm at their base (Anderson, Moosler et al. 1992).
112   *Sanderia malayensis*, is a tropical scyphomedusae that is classified into the same
113 family to *Chrysaora* (Hargitt 1910*)*. *Sanderia* rhythmically contracts its tentacle to keep
114 the position of captured prey until an oral arm makes contact and contracts to transport
115 this prey to the mouth. This behavior persists in isolated tentacles. This kind of rhythmic
116 contraction has not been investigated and understanding the mechanism would be helpful
117 to investigate signal processing by the neural net. Similarly to hydra (Dupre and Yuste
118 2017) it is possible to distinguish two different reflexes mediated by the neural net in
119 isolated Sanderia tentacles. Through behavioral and electrophysiological experiments, we
120 can distinguish individual sensory and motor components of these two tentacular
121 responses of *Sanderia*.
122
Materials and Methods
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Animals
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127 Polyps of *Sanderia malayensis* were obtained from the New England Aquarium (Boston,
128 MA, US). They were kept in circulating natural sea water at 22 °C. *Sanderia* polyps
129 strobilated and released ephyrae at that temperature. *Sanderia* ephyrae were fed *Artemia*
130 (Brine Shrimp Direct, UT, US) daily until their bell grew to 2 cm in diameter. Grown
131 animals were fed chopped mussel or Aurelia every other day in addition to daily feedings
132 of *Artemia*.
133
Methods
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136 All experiments were performed in ambient filtered sea water. A tentacle was isolated
137 from medusa having bell diameters that ranged from 3 cm to 10 cm. Isolated tentacles
138 were placed in a specifically designed perfusion chamber, 37 cm in height, 8 cm in width,
139 and 3.5 cm in depth (Fig. 1). To record electrical activities, two Teflon coated silver
140 wires were inserted into the gastric cavity separated axially by 4mm. We placed the tip of
141 the distal (longer) electrode about 1 cm from the cut end of the relaxed tentacle. After the
142 cut end was tied with a string, the tentacle was moved into the chamber, and was
143 suspended by these electrode wires. When we did not use electrodes, the tentacle was
144 suspended by a metal wire.
145   The recorded signals were amplified using an A-M systems differential AC
146 amplifier. Tentacle behaviors were recorded using a CCD camera (SONY, XC-77) and
147 recorded simultaneously with physiological signals on the audio tracks of a (SONY, GV-
148 HD700) to provide synchrony between the behavioral and electrophysiological
149 components. Signals were digitized as Quicktime Movies or digitized using an analog to
150 digital converter (National Instruments, NI 6221) and acquired and processed with
151 LabView software (National Instruments).
152   For chemical stimuli, we made a mussel juice by homogenizing and filtering
153 mussels. We diluted the juice with sea water in a decade series. Amino acid solutions of
154 glutamic acid, glutathione, asparagine, glycine, proline, valine, cysteine,
155 methylglutathione and glutamine were made using sea water in concentrations of 10^{-2} M.

156 Lucifer yellow (0.02 %) was dissolved in each test solution to visualize the stimulus
157 making contact with the tentacle in the video signal. For glutamic acid and glutathione
158 that were found to induce a response, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} M solutions were
159 prepared. During chemical stimulus experiments sea water was continuously fed into the
160 perfusion chamber by gravity (Fig. 1). Sea water was drained from the bottom of the
161 chamber and a vacuum aspiration was used to adjust the drain speed. Stimulus solutions
162 were introduced into the chamber by temporarily switching the continuous flow of sea
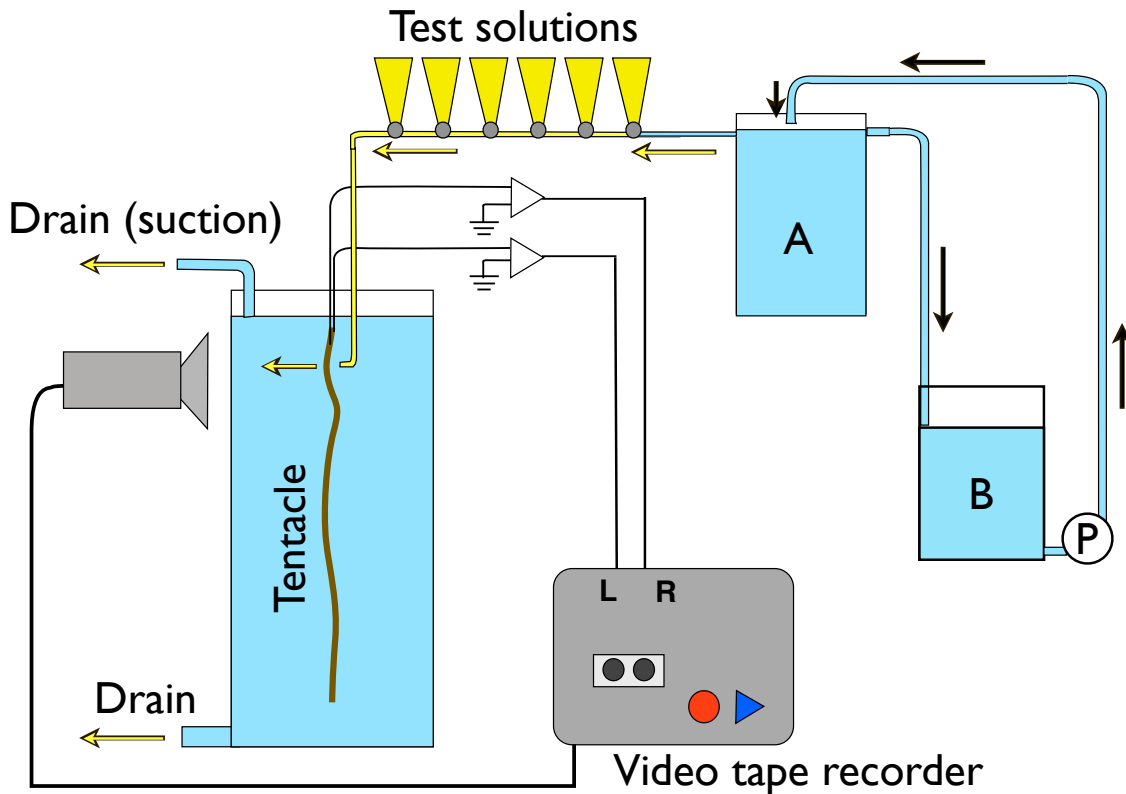163 water to a flow of the stimulus solution.
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Fig. 1. A diagram of the experimental setup. An isolated tentacle was suspended in a perfusion chamber with two metal electrodes inserted into the gastric canal. A gravity driven flow of sea water was continuously fed into the perfusion chamber (yellow arrow) from a holding tank (A) . Sea water in the chamber was drained from the bottom, and the height was adjusted with a suction aspirator from water surface. The maximum level of the holding tank (A) is set by a drain near the top, that removes water in above this level to a holding tank (B). To ensure that the level in (A) is constant, sea water is continuously pumped from (B) to (A) using a pump (P). Tubes for test solutions were connected to the perfusion tubing with three way valves. Tentacle behaviors and electrical activities were recorded using a CCD camera on a videocassette recorder.

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166 Mechanical stimulus was given using a glass rod with a heat polished tip. We
167 applied mechanical stimuli in three ways; 1) a slow touch that did not move the tentacle,
168 2) a quick touch, that resulted in tentacle motion, and 3) a rapid succession of quick
169 touches.
170 In some experiments, tentacles were stretched by applying weight to the tentacle
171 with square filter paper in sizes 2.5 mm, 5 mm, and 10 mm. The paper was introduced to

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172 the tentacle in the chamber with a tweezers and touched until the filter paper adhered to
173 the tentacle, presumably through the release and attachment of nematocysts. After
174 confirming attachment, the paper was released and allowed to stretch the tentacle.
175   Tentacle position was analyzed by tracking the position of a fiduciary mark on the
176 tentacle, either a food particle, a cluster of nematocytes, or the filter paper stimulus, using
177 digitizing tools (Hedrick 2008) in Matlab (The Math Works).

Results

179
180 **1. Feeding response in *Sanderia malayensis***
181   In the absence of stimuli, *Sanderia* medusa tentacles are in a relaxed state, freely
182 floating as the organism swims. If contact is made with a large piece of food, such as
183 mussel flesh, nematocysts are fired, capturing the prey. The tentacle then undergoes an
184 immediate and large contraction, bringing the captured food toward the bell. Smaller
185 prey, such as *Artemia,* will be captured in a similar manner, but does not induce an
186 immediate tentacular contraction, although several such small food items will sum to
187 eventually induce contraction in the tentacle. Concurrent with contraction, an oral arm
188 swings toward the tentacle to transfer this food to the mouth. The food is then transferred
189 from the tentacle to the oral arm. This process can be slow and during transfer the
190 tentacle is observed to relax slightly followed by a subsequent contraction to bring the
191 food back near the oral arm. Only once the food has been transferred does the tentacle
192 relax completely.

193
194 **2. Feeding response of isolated tentacles**
195   A proximal repetitive contraction is observed in an isolated tentacle in response to a
196 food stimulus. Tentacles were isolated from animals, either with or without the
197 tentacular nerve ring. The tentacle was mounted in a seawater chamber and was not
198 observed to contract in the absence of external stimuli. When a piece of flesh, either from
199 a mussel or from a moon jelly, was attached to the tentacle, a proximal contraction was
200 initiated immediately from the point of contact (Fig. 2). After fully contracting,
201 subsequent contractions kept the food particle near the same, contracted position. This
202 behavior is similar to that observed in intact animals, where the tentacle moves the food
203 to an oral arm. After 45 seconds and several repeated contractions the tentacle relaxed
204 fully and the food returned to its initial position. This demonstrates that the feeding reflex
205 is endogenous to the peripheral neural net. The durations of this feeding response, from
206 the first contraction to the full relax condition, were varied between preparations ranging
207 from a minute to more than 15 min.
208   Concurrent with behavioral observations, electrophysiological measurements were
209 made during the feeding response. Two wires inserted into the gastric cavity were
210 attached at the base of the tentacle and spaced approximately 4 mm. Action potentials
211 were observed coincident with every contraction (Fig. 2). Response from the distal
212 electrode always preceded response from the proximal electrode, indicating the signal
213 was travelling up from the tentacle (Fig. 2 inset).
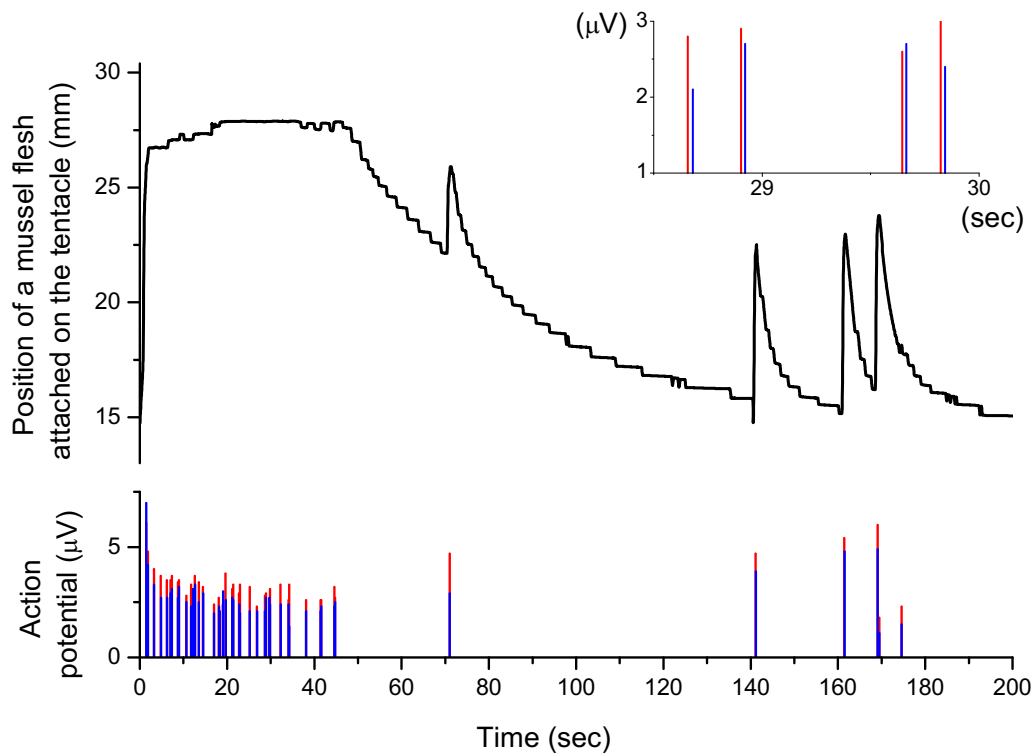
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Fig. 2. Tentacle extension and electrophysiological recording of an intact tentacle in response to a piece of mussel flesh. The tentacle extension is obtained by tracking an attached piece of mussel. Blue and red bars are two action potentials recorded with distal and proximal electrodes inserted in the gastric cavity of the tentacle. When a piece of mussel flesh was attached, at t = 0, it contracted proximally and it repeated small contractions to maintain the food position. Both action potentials followed contractions. The inset shows enlarged action potentials between 28 to 30 seconds. These contractions were initiated at the stimulated point on the tentacle and conducted proximally.

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217   A tentacular nerve ring that is located at the base of tentacle and is an extremely dense
218   nerve net has been reported in two species *Chrysaora* and *Cyanea* those are classified
219   into different families (Anderson, Moosler et al. 1992). It is assumed that *Sanderia*,
220   classified into the same family as *Chrysaora*, also has a tentacular nerve ring. The
221   feeding responses were observed on both of the tentacles prepared with and without the
222   base that is expected to contain a tentacular nerve ring. However, the response of
223   tentacles attached to the tentacular nerve net was longer in duration than those of isolated
224   tentacles. Figure 3 shows a comparison of response durations of the two preparations to
225   moon jelly and mussel stimuli. The duration was obtained by measuring the time between
226   the first contraction after the stimulus was applied and the last contraction before the
227   tentacle relaxed. Because the results were varied on preparations between 10 sec and 200
228   sec, the duration of the response an isolated tentacle was normalized with the duration of
229   that of an intact tentacle isolated from the same animal. Then, the average was obtained
230   with four experiments for each stimulus. Response durations of isolated tentacles were 25
231   % shorter than intact tentacles for moon jelly stimulus, and were 50 % shorter for mussel

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232   stimulus. This result suggests that the tentacular nerve ring may modulate the feeding
233   response but is not necessary.
234
235   **3. Feeding response to chemical stimuli**
236   A food stimulus is complicated and can elicit many possible sensory reactions.
237   Understanding the underlying sensory response requires simplifying the complicated
238   food stimulus into component parts and understanding the isolated behavior of these
239   responses. The chemical response is likely to be important for signaling the presence of
240   food. The responses to two different chemical stimuli are studied, filtered juice from a
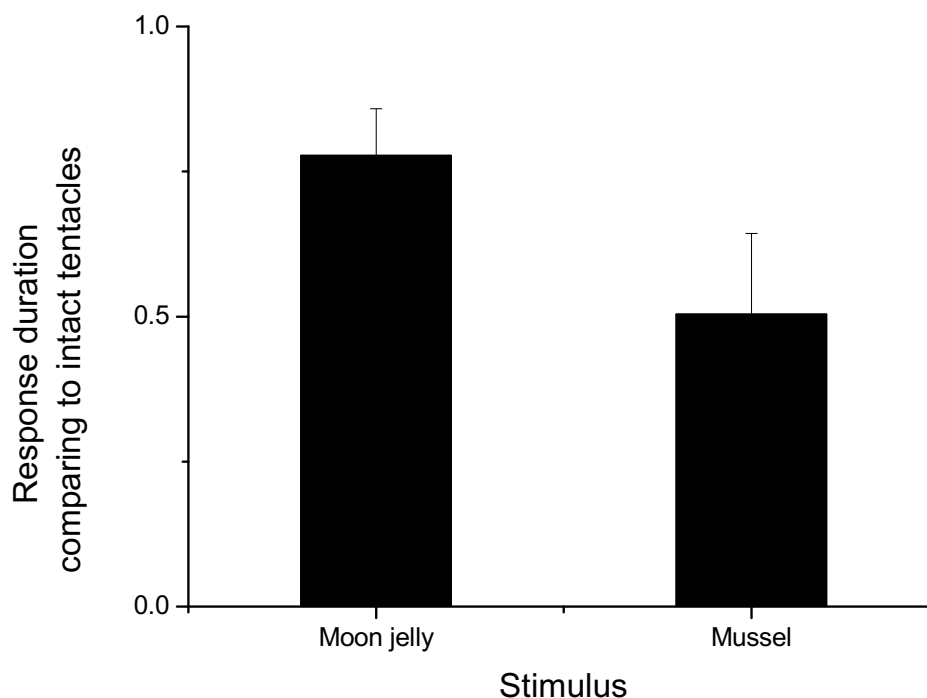241   homogenized mussel and solutions of amino acids.
242

Fig. 3.   Comparison of response durations of isolated tentacles and intact tentacles. The response durations of isolated tentacles to moon jelly and mussel flesh are normalized by those of intact tentacles containing a tentacular nerve ring obtained from the same animal. These results are obtained with four tentacles for each experiment. Response durations of isolated tentacles were 25 % shorter than intact tentacles to a moon jelly stimulus and 50 % shorter to a mussel stimulus. This indicates that tentacular nerve ring located at the base of tentacles modifies response, but is not necessary to induce a response.

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244
245   To isolate chemical effects from mechanical ones, the chemicals are introduced to
246   the tentacle through a constant stream of water flowing past the tentacle. A steady stream
247   of fresh seawater is directed toward the tentacle under observation. There is no response
248   observed to the stream of water itself. An experiment is performed by switching a valve
249   upstream of the seawater outlet to inject a controlled amount of the chemical under test
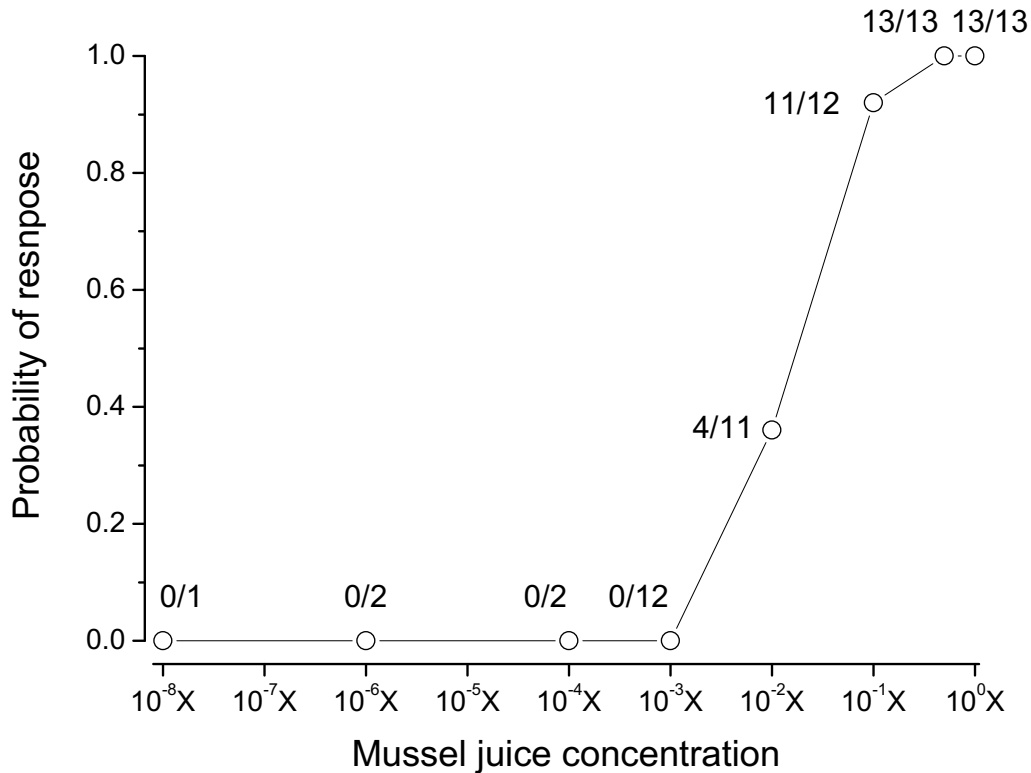250   (200 µl).

Fig. 4. Dose response curve of tentacle to mussel juice. The number over the symbol indicates actual number of tentacles that responded to mussel juice and the number of tentacle tested. No tentacles responded at or below 10^{-3} times diluted mussel juice.

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252 Tentacle response to undiluted mussel juice was the same as the response to food
253 stimuli. The tentacle immediately started repetitive proximal contraction and relaxed
254 when the perfusion solution was switched back to sea water. The probability of invoking
255 a tentacle response was decreased by decreasing the concentration. Below a dilution of
256 10^{-3} no response is observed (Fig. 4). At a dilution of 10^{-1} 11 of 12 tentacles proximally
257 contracted, but localized contraction was observed before the proximal contraction. At a
258 dilution of 10^{-2} 4 of 12 tentacles proximally contracted after a localized contraction. This
259 result suggests that chemical stimuli alone can induce a feeding response and it has a
260 threshold between 10^{-2} and 10^{-3}.
261 Comparison between the initiation of the first contraction at the stimulated point to
262 100 % mussel juice and the first measured electrical response recorded at the base of the
263 tentacle allows for measurement of the signal delay. By adjusting the distance between
264 the stimulation and observation points, the conduction velocity can be measured. The

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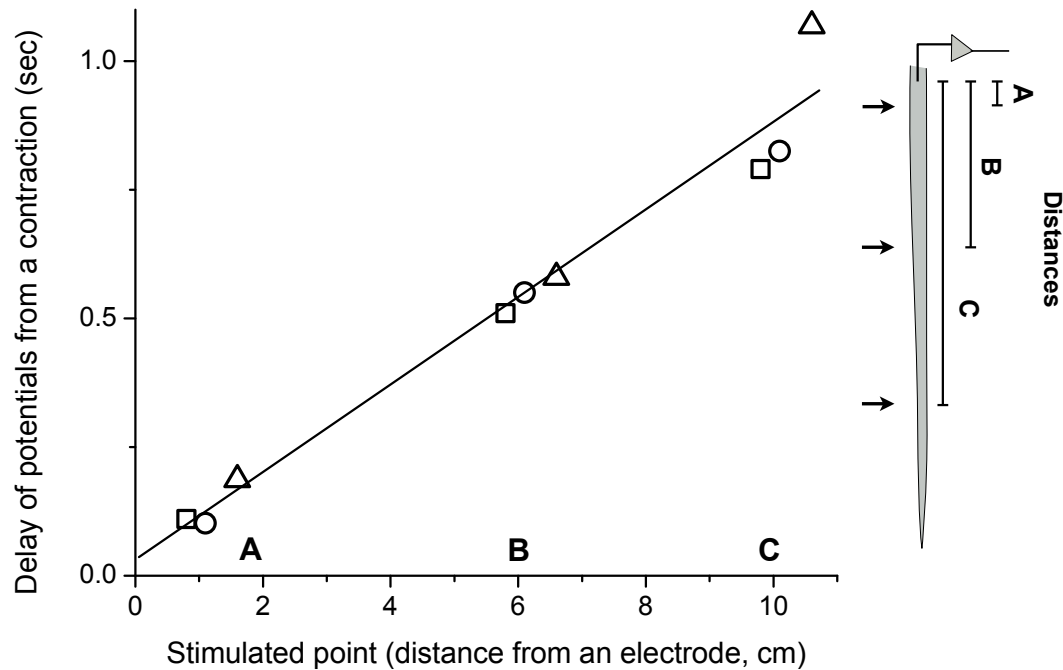
Fig. 5.   Delays of the first action potentials following the first large contraction to mussel juice applied to three points on a tentacle (right). This was obtained from three tentacles, and each symbol shows individual tentacle. The delay increased linearly by increasing the distance, and the conduction velocity was 11 cm/sec.

266 delay was increased linearly with distance, yielding a conduction velocity of 11 cm/sec (Fig. 5).
267 We also used nine amino acids, glutamic acid, glutathione, glutamine, proline, asparagine, valine,
268 methylglutathione, aspartic acid, and cysteine with concentrations of 10^{-2} M. In these amino
269 acids, glutamic acid and glutathione could induce localized contraction. To investigate the detail
270 of the responses to amino acids, we used 10^{-1} to 10^{-5} M glutamic acid and glutathione. No
271 tentacles responded to these amino acids below 10^{-3} M. Tentacles responded to these amino acids
272 at concentrations higher than 10^{-2} M with localized contraction. The response to glutathione was
273 also localized contraction, but 3 of 6 tentacles proximally contracted to 10^{-1} M solution similar to
274 the response to mussel juice. This result demonstrates that a single amino acid can excite the
275 chemical sensory system to induce the feeding response of tentacles.
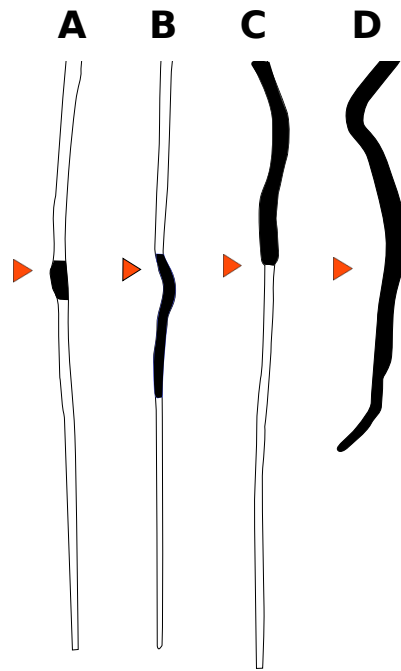276

Fig. 6. Tentacle contraction patterns: localized (A), distal (B), proximal (C) and whole contractions (D) induced by slow (A), fast (B and C) and fast and repeating touching (D) with a glass rod, respectively. Red rectangles indicate the stimulated points on tentacles and contracted areas on tentacles shown as black areas.

4. Mechanical stimuli induce a different response.

Feeding is initiated by touching prey and mechanical stimuli are expected to be important for generating feeding behavior. We used a 1mm diameter heat polished glass rod as a mechanical stimulus by careful touching the tip of the glass rod to an isolated tentacle.

Four types of response, which we termed localized, distal, proximal and full contractions, could be induced by touching (Fig. 6). All tentacles tested locally contracted in response to slow contact (such that the tentacle remained stationary) (Fig. 6A). Tentacles sometimes adhered to the glass rod suggesting that nematocytes were discharged in response to this stimulus. With stronger touching (such that the tentacle was swung away from the point of contact) or repeated touching, distal, proximal or whole contractions could be induced (Fig 6 B, C, and D). However, in no cases were repetitive proximal contractions observed, as seen during the feeding response. Compared to chemical stimuli, the mechanical stimulus duration is short, less than 1 sec, and this short duration might be the reason for the absence of a repeated contraction. Experiments were also performed with constant mechanical stimulation using a glass rod. This stimulation induced a localized contraction just after a glass rod touch, but there was immediate relaxation and no repetitive contractions were observed. These results indicate that mechanoreception is not solely responsible for generating the feeding response.

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301 **5. Stretch stimulus induced repetitive proximal contraction**
302   Repetitive tentacle contraction was not induced by a single small prey, for
303 example *Artemia*, but could be by the capture of larger prey or of multiple *Artemia* on the
304 same tentacle. Therefore, there appears to be a response to tentacle stretching that is
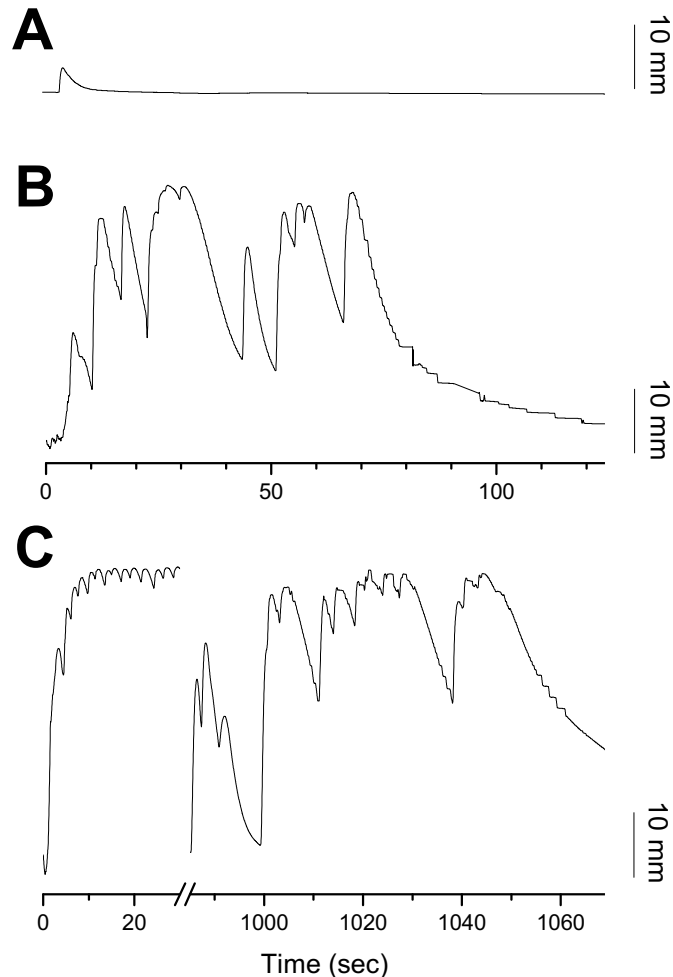305 independent of the touch response. To address this sensory mechanism, we used a piece

Fig. 7. Contraction patterns in response to paper and food stimuli on a tentacle. (A) Response to touch stimulus with a vertically suspended piece of paper. There is local contraction followed by immediate relaxation. (B) Response to a piece of paper attached directly to the tentacle. Repetitive proximal contraction was recorded. (C) Response to a piece of mussel shown in two elapsed time regions, 0 to 20 sec and 990 to 1070 sec at the same scale as in A and B. The tentacle repeated contraction with faster frequency and smaller amplitudes just after it was stimulated than the late repetitive contraction. This late response shows the same qualitative features found in attachment of paper.

306 of filter paper that is negatively buoyant and stretches the tentacle when attached, but
307 does not contain any proteins or amino acids.
308   To control for the purely tactile response, we first investigated the influence of the
309 paper by touching the tentacle at a fixed position, with the filter paper supported by a
310 thread. The response to this stimulation was a localized contraction and immediate

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311 relaxation (Fig. 7A), similar to the response to constant mechanical stimulus with a glass
312 rod.
313   With mechanical stimulation, the filter paper would often become attached to the
314 tentacle, likely through a nematocyte. To induce a stretch stimulus, the paper was
315 released after attachment, causing it to sink due to gravity. After some strain of the
316 tentacle, a repetitive proximal contraction was observed, originating from the contacted
317 point (Fig. 7B). This response could be induced by attaching filter paper to any point on
318 tentacle. Compared to the food response, the frequency of the contraction of the paper
319 response was lower and the amplitude was bigger. Figure 7C shows the beginning and
320 end of the response to a piece of mussel, with remarkable similarity between the late
321 stage response to a food particle and the response to filter paper. This suggests that the
322 food particle first stimulates the chemical and stretch responses, and only later does a
323 stretch response induce repetitive contractions.
324   Like a chemical stimulus, the stretch stimulus induces a repetitive proximal
325 contraction. The two stimuli have different regions of excitation, chemical stimuli being
326 localized on the applied point while stretching occurs from the point of contact
327 proximally to the fixed base of the tentacle. To address the directionality of this
328 response, we performed stretch experiments with a gravitationally inverted tentacle. A
329 paper stimulus placed on this inverted tentacle stretched it distally from the point of
330 contact, rather than the usual proximal stretching. Even with this distal stimulus,
331 repetitive proximal contractions were induced, suggesting that this directionality stems
332 from the organism and not from the orientation relative to gravity.

333

334 **Discussion**
335   An intact scyphomedusa, *Sanderia malayensis*, keeps swinging tentacles by bell
336 pulsations during it swims. Prey is captured on a tentacle and is brought to near the bell
337 by a tentacle proximal contraction, and the tentacle then repeats contraction to keep the
338 prey position near the bell until it transfers the prey to an oral arm. We found that isolated
339 tentacles could produce this feeding response suggesting that this behavior would be
340 generated with simple and primitive nerve nets in the tentacle. This study of *Sanderia*
341 tentacle demonstrates that the isolated tentacle nerve net can discriminate between
342 chemical and tactile stimuli and generate at least two different behavioral acts.
343   Contractions on an isolated tentacle could be initiated by mechanical, chemical and
344 stretch stimuli, but these different stimuli led to different types of response. Since prey
345 must be touched on a tentacle to induce feeding response, touching is considered to be
346 one of the triggers of the response. However, mechanical stimuli did not induce the
347 feeding response on tentacles. To a mechanical stimulus tentacle contracted locally,
348 distally, proximally or whole length (Fig. 6). After these contractions, the tentacle relaxed
349 immediately and did not repeat contraction. Horridge (Horridge 1955) reported a tentacle
350 response to mechanical stimuli on a hydromedusa, *Geryonia proboscidalis*. Tentacles on
351 an intact animal proximally contracted to a mechanical stimulus while an isolated tentacle
352 contracted locally to gentle stimulus or the whole tentacle contracted to a strong stimulus.
353 This indicates that the CNS of *Geryonia*, that consists of inner and outer nerve rings in
354 the bell, strongly innervate tentacular nervous system. However, in scyphomedusa that
355 does not have such CNS (Satterlie 2011), intact *Sanderia* exhibited variety of temporal
356 responses by mechanical stimuli those are the same to the responses of isolated tentacles.

357   This result suggests that tentacular nervous system of *Sanderia* is not strongly innervated
358   by nerve nets in the bell. This variety of temporal responses of tentacles by mechanical
359   stimulation might function to allow escape from predators or to reject non-food objects,
360   but not trigger the feeding response.
361   In cnidarians, chemical stimuli are well known as feeding response activators and a
362   specific amino acid works to induce a specific step of feeding behavior in some species.
363   In sea anemone, for example, proline triggers a food capture response while glutathione
364   triggers ingestion (Lindstedt 1971). In scyphomedusa, polyps of *Chrysaora* exhibit
365   feeding behaviors in response to various amino acids and peptides, and a specific feeding
366   step for each amino acid such as contraction or writhing was also observed in excised
367   tentacles (Loeb and Blanquet 1973). For a *Sanderia* medusa tentacle, filtered mussel juice
368   and nine amino acids were used to investigate the roles of chemosensory system on the
369   feeding response. The mussel juice induced repetitive proximal contraction, but very high
370   concentration, more than 1 % is required. Glutamic acid and gluthatione induced a
371   localized contraction to 10^{-2} M amino acids. To 10^{-1} M of glutamic acid only localized
372   contraction was produced, while 10^{-1} M of glutathione produced proximal contractions in
373   addition to a localized contraction on 3 of 6 tentacles. This low sensitivity to chemical
374   stimulus might be useful to avoid responding to a bleeding prey located too far from the
375   tentacle, but responses of the chemosensory system on a tentacle can trigger the feeding
376   response with strong stimuli.
377   Because a few *Artemia* captured on a tentacle did not evoke a tentacle contraction
378   but larger prey was able to, it is considered that tension on a tentacle would be required.
379   Our paper stimulus induced repetitive proximal contraction from the stimulated point
380   when allowed to stretch the tentacle, while touching with a fixed paper only caused a
381   temporal localized contraction. These results indicate that a stretch receptor is a trigger
382   and a modulator of the feeding response allowing it to persist until the prey is transferred
383   to the oral arm. Unlike chemical and mechanical stimuli, which are applied on a
384   stimulated point of the tentacle, tension in the tentacle is usually present proximally from
385   the point of attachment. Despite this broad area of stimulation, the response of the
386   tentacle seems to be initiated at the point of contraction and proceed proximally, even if
387   the stimulus has been applied distally by inverting the tentacle. How stretch receptors
388   located on the proximal or distal stimulated area is unclear, and to investigate the
389   mechanism more detailed experiments are required.
390   Rhythmic contraction could be evoked on any part of tentacle through the
391   application of a food, stimulus, or stretch stimuli. This rhythmic contraction arises from
392   interplay between prey weight continuously pulling the tentacle down while muscle
393   response intermittently moves the food back up. Removing the stimulus results in
394   cessation of rhythmic contraction, indicating the necessity of continuous chemical or
395   stretch stimuli to induce repeated contraction. As the tentacle habituated to the stimulus,
396   the frequency of contraction decreased, but this decreased frequency was accompanied by
397   increased amplitude to ensure the food was raised to its previous location (Fig 2 and 7C) .
398   To generate rhythmic patterns, medusa has rhopalia on the bell edge contains a
399   pacemaker that initiates a bell contraction and the rate of firing of individual rhopalial
400   pacemaker is variable and can be directly influenced by sensory inputs, which
401   presumably modify a baseline discharge rate of the pacemaker (Romanes 1885, Horridge
402   1956, Passano and McCullough 1965, Passano 1973). However, such rhoparium like

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403   organs have not been found on the tentacle, and our results indicate that the rhythmic
404   pattern is generated in the tentacle. The response duration of the preparation contained
405   tentacular nerve ring was significantly longer than preparations those did not contain the
406   tentacular nerve ring (Fig. 3), but the response pattern was the same, repetitive intrinsic
407   proximal contraction of which the tentacular nerve ring could be an extrinsic modulator.
408   The cellular nature of the pacemakers of tentacle nerve nets are not known, but the
409   sensory signals would be integrated at interneurons which must be located along the
410   tentacle, and the firing rate would be modified by sensory input levels from both
411   chemical and stretch receptors.
412   Responses to chemical and stretch stimuli signals were always conducted
413   proximally, suggesting these responses are connected to a proximally conducting
414   network. This is different from mechanical stimuli that could induce a response in both
415   directions suggesting that existence of two nervous systems in the tentacle. The bell
416   contains two nerve nets, a motor nerve net (MNN) and a diffuse nerve net (DNN), both of
417   which consist of bidirectional synapses between neurons (Horridge and Mackay 1962,
418   Horridge, Chapman et al. 1962). In analogy to the bell nervous system, there might be
419   two nerve nets on the tentacle: one for feeding response and polarized to conduct
420   proximally (feeding nerve net) and one for escape or rejection behaviors which conducts
421   either direction depending on stimulus conditions (withdrawal nerve net). The feeding
422   nerve net would be comparable to MNN that innervate muscles directly and the
423   withdrawal nerve net is similar to the DNN that modulates muscle activities (Romanes
424   1885, Horridge 1956, Passano and McCullough 1965, Passano 1973). However, the bell
425   MNN is not polarized to conduct signals (Anderson 1985, Anderson and Spencer 1989),
426   and this is a major difference between the proposed polarized feeding nerve net and the
427   MNN. This suggests that the feeding nerve net would have polarized synaptic
428   projections, however, more detailed histological and physiological information on the
429   tentacle nervous system are required.
430   From the perspective of function, it makes sense that stimuli distinctly associated
431   with food, those of chemical and stretch detection, produce different responses than touch
432   stimuli associated with both food and predation. The rapid withdrawal behavior
433   associated with full distal and proximal contraction is distinctly different than the
434   repeating and more sustained feeding behavior of raising capture prey for transfer to the
435   oral arms. On our preliminary experiments, an isolated oral arm could also be induced a
436   feeding response by a food particle suggesting that the feeding behavior of the oral am is
437   also generated by endogenous nervous activities. These results suggest that the feeding
438   response of *Sanderia* medusa consists of two endogenous processing systems located in a
439   tentacle and an oral arm system coordinated through nerve nets in the bell.
440
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445
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448   J.A.; Investigation: K.M., M.S.; Resources: J.A.; Writing - original draft: K.M., M.S; Writing -

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